Oxytocin‘s involvement in the development, manifestation and treatment of posttraumatic stress disorder symptoms

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Abstract

Oxytocin is a psychologically interesting and physiologically complex neuropeptide. Due to its involvement in fear processing and social functioning, oxytocin has been discussed as a potential biomarker for posttraumatic stress disorder (PTSD).

This dissertation's first arm (**studies 1 - 5**) explored oxytocin's involvement along the pathway from traumatic event exposure to PTSD symptom development, manifestation, and remission. **Studies 1** and **2** covered the stage of PTSD symptom development and its pharmacological prevention. They are based on data from a double-blind, multicenter, randomized, placebo-controlled trial which evaluated the effects of repeated intranasal oxytocin administration early posttrauma on PTSD symptoms one and a half, three and six months later. **Study 1** demonstrated that the intervention's effects, which were beneficial in individuals with high acute PTSD symptoms, were independent of sex and hormonal contraception use. **Study 2** demonstrated that higher blood oxytocin concentrations, as assessed early posttrauma, were associated with higher PTSD symptoms at the follow-up timepoints in women using hormonal contraception. No prognostic effects were observed in men or cycling women. Interaction effects with the intervention were observed in none of the groups. **Study 3** is a systematic review which summarized studies that investigated endogenous oxytocin concentrations, oxytocin receptor gene DNA variation and methylation in trauma exposed individuals and such with manifest PTSD symptoms. Data on oxytocin receptor gene functioning were insufficient for meta-analysis. The meta-analyses on endogenous oxytocin concentrations showed that they were neither a good biomarker for traumatic event exposure, nor for PTSD symptoms. Especially the observed impact of the biochemical analysis method seems problematic when using endogenous oxytocin concentrations as potential diagnostic biomarker. **Studies 4** and **5** covered the stage of PTSD symptom remission related to psychotherapeutic treatment. They are based on data from a randomized, waitlist-controlled trial which investigated the effectiveness of an internet-based trauma-focused cognitive behavioral therapy (TF-CBT) in male German Armed Forces service members. **Study 4** showed that blood oxytocin concentrations were not influenced by trauma exposure or PTSD symptoms. Analyzing PTSD symptoms and blood oxytocin concentrations of service members who underwent the TF-CBT before, immediately and 3 months after the intervention

revealed no mean changes. However, while PTSD symptoms were stable within individuals, oxytocin concentrations were not. **Study 5** indicated that higher blood oxytocin concentrations before intervention onset were associated with more positive patient therapeutic alliance ratings during and after the intervention, even though these findings need to be interpreted cautiously due to the low temporal stability of oxytocin concentrations.

Thus, the impact of the biochemical analysis method (**study 3**) and the low temporal stability of endogenous oxytocin concentrations (**studies 4** and **5**) challenge their assumed eligibility as a PTSD-specific biomarker. Therefore, the second arm of this dissertation aimed at identifying physiological confounders of endogenous oxytocin concentrations, to increase knowledge about factors that need to be controlled for. **Studies 6** and **7** are systematic reviews and meta-analyses which summarized studies that investigated endogenous oxytocin concentrations in healthy humans. **Study 6** showed that oxytocin concentrations, as derived from extracted blood samples, were higher in samples with higher percentages of women and higher if samples were collected later in the day. **Study 7** showed that in naturally cycling women, oxytocin concentrations increased during the follicular phase, peaked at ovulation, and decreased during the luteal phase.

As a translational research project, this dissertation points out typical challenges associated with transferring findings from basic scientific studies in animals and healthy humans to the clinical context. While there is indication for beneficial effects of repeated intranasal administration after recent trauma exposure, these findings need replication and a more profound investigation of moderators. Observing that endogenous oxytocin concentrations depend on the biochemical analysis method and are highly variable within individuals, this dissertation detected some common problems related to such measurements. Controlling for relevant confounders, such as time of day, sex, and menstrual cycle phase might increase the reliability of endogenous oxytocin concentrations. However, it appears that other translation-specific challenges, such as the questionable correlation between oxytocin's brain region-specific actions and its peripheral availability, still need to be resolved. To conclude, while PTSD is a ideal disorder to implement a translational research perspective, given our current knowledge on endogenous oxytocin concentrations, they are not ideal biomarkers.

Kurzzusammenfassung (Abstract in deutscher Sprache)

Aus psychologischer Sicht ist Oxytocin ein interessantes, aus physiologischer Perspektive ein komplexes Neuropeptid. Sowohl aufgrund seiner Rolle bei der Verarbeitung von Furcht als auch aufgrund seiner sozialen Funktionen wurde Oxytocin als möglicher Biomarker für die Posttraumatische Belastungsstörung (PTBS) diskutiert.

Im ersten Teil dieser Dissertation (**Studien 1 - 5**) wurde die Rolle von Oxytocin über die verschiedenen Phasen der Verarbeitung traumatischer Ereignisse hinweg untersucht. Speziell wurden die Phasen der Entwicklung, der Manifestation und der Remission von PTBS Symptomen betrachtet. Die **Studien 1** und **2** beschäftigten sich mit der Entwicklung von PTBS Symptomen im Kontext einer pharmakologischen, präventiven Intervention. Sie basieren auf Daten einer doppelblinden, multizentrischen, randomisierten, Placebo-kontrollierten Studie. In dieser wurde die Wirksamkeit wiederholter intranasaler Oxytocinadministration kurz nach einem traumatischen Ereignis auf die Entwicklung von PTBS Symptomen einen Monat, sowie drei und sechs Monate später evaluiert. **Studie 1** zeigte, dass die Effekte der Intervention, die bei Personen mit hohen akuten PTBS Symptomen präventiv wirkte, unabhängig vom Geschlecht und der Anwendung hormoneller Verhütungsmethoden waren. **Studie 2** zeigte, dass Frauen, die hormonell verhüteten und bei welchen kurz nach dem traumatischen Ereignis höhere Oxytocinkonzentrationen im Blut gemessen wurden, höhere PTBS Symptome zu den Follow-Up Zeitpunkten aufwiesen. Weder bei Männern noch bei Frauen mit natürlichem Menstruationszyklus wurden solch prognostische Effekte beobachtet. Außerdem wurden in keiner der Gruppen Interaktionseffekte zwischen Oxytocinkonzentrationen im Blut und der Intervention beobachtet.

Studie 3 ist eine systematische Übersichtsarbeit. Sie fasste Studien zusammen, welche endogene Oxytocinkonzentrationen sowie Variationen und die Methylierung der DNS des Oxytocinrezeptorgens bei Personen mit einem erlebten traumatischen Ereignis und bei Personen mit PTBS Symptomen untersucht hatten. Die genetischen Daten waren für ein meta-analytisches Verfahren nicht ausreichend. Die Meta-Analysen zu den endogenen Oxytocinkonzentrationen zeigten, dass diese weder ein guter Biomarker für erlebte traumatische Ereignisse, noch für PTBS Symptome waren. Insbesondere der

beobachtete Einfluss der biochemischen Analysemethodik lässt die Verwendung endogener Oxytocinkonzentrationen als einen möglichen diagnostischen Biomarker problematisch erscheinen.

Die **Studien 4** und **5** beschäftigten sich mit der Remission von PTBS Symptomen im Kontext psychotherapeutischer Behandlung. Sie basieren auf Daten einer randomisierten, Wartelisten-kontrollierten Studie, welche die Wirkung einer internetbasierten, traumafokussierten, kognitiv-behavioralen Therapie (TF-KBT) bei männlichen Bundeswehrsoldaten evaluierte. **Studie 4** zeigte, dass sich deren Oxytocinkonzentrationen im Blut weder in Abhängigkeit von erlebten traumatischen Ereignissen, noch von PBTS Symptomen unterschieden. Die Analyse von PTBS Symptomen und Oxytocinkonzentrationen im Blut derjenigen Bundeswehrsoldaten, die an der TF-KBT teilnahmen, vor, direkt, und drei Monate nach der Intervention, wies im Mittel keine Veränderungen nach. Die PTBS Symptome blieben intraindividuell stabil, die Oxytocinkonzentrationen allerdings nicht. **Studie 5** lieferte Hinweise darauf, dass Patienten, die höhere Oxytocinkonzentrationen vor Interventionsbeginn aufwiesen, die therapeutische Allianz während und nach der Intervention als positiver bewerteten. Aufgrund der berichteten niedrigen zeitlichen Stabilität der Oxytocinkonzentrationen müssen diese Befunde allerdings vorsichtig interpretiert werden.

Insofern stellen sowohl der Einfluss der biochemischen Analysemethodik (**Studie 3**) als auch die niedrige zeitliche Stabilität endogener Oxytocinkonzentrationen (**Studien 4** und **5**) deren angenommene Eignung als einen PTBS-spezifischen Biomarker infrage. Deshalb zielte der zweite Teil dieser Dissertation darauf ab, physiologische, konfundierende Variablen endogener Oxytocinkonzentrationen zu identifizieren. Dadurch sollte das vorhandene Wissen über Variablen, die bei der Untersuchung endogener Oxytocinkonzentrationen kontrolliert werden müssen, erweitert werden. Die **Studien 6** und **7** sind systematische Übersichtsarbeiten und Meta-Analysen, welche Studien zusammenfassten, die endogene Oxytocinkonzentrationen bei gesunden Menschen untersucht hatten. **Studie 6** zeigte, dass endogene Oxytocinkonzentrationen, die in extrahierten Blutproben gemessen wurden, höher waren, wenn die Stichproben einen höheren Frauenanteil aufwiesen und wenn die Proben zu einem späteren Tageszeitpunkt gesammelt wurden. **Studie 7** zeigte, dass endogene Oxytocinkonzentrationen von

Frauen mit einem natürlichen Menstruationszyklus während der Follikelphase anstiegen, zum Zeitpunkt des Eisprungs ihren Höchstwert erreichten und während der folgenden Lutealphase wieder sanken.

In ihrer Gesamtheit zeigt diese Dissertation, die als translationales Forschungsprojekt angesehen werden kann, typische Herausforderungen auf, die auftreten, wenn Befunde aus der wissenschaftlichen Grundlagenforschung an Tieren und gesunden Menschen auf den klinischen Kontext übertragen werden. Die Hinweise zu den präventiven Effekten wiederholter intranasaler Oxytocinadministration bei Personen mit einem kürzlich erlebten traumatischen Ereignis müssen repliziert werden. Dabei sollte Moderatorvariablen noch mehr Aufmerksamkeit gewidmet werden. Durch die Beobachtung, dass endogene Oxytocinkonzentrationen abhängig von der biochemischen Analysemethodik und intraindividuell hoch variabel sind, veranschaulichte diese Dissertation typische Probleme solcher Messungen. Deren Reliabilität könnte durch die Kontrolle konfundierender Variablen, wie der Tageszeit, des Geschlechts und der Zyklusphase erhöht werden. Allerdings ist offenkundig, dass noch weitere translationale Herausforderungen gelöst werden müssen, etwa der ungeklärte Zusammenhang zwischen der zentralnervösen Wirkweise von Oxytocin und seiner peripheren Verfügbarkeit. Abschließend lässt sich feststellen, dass die PTBS als psychische Störung zwar ideale Voraussetzungen bietet, um eine translationale Forschungsperspektive einzunehmen, endogene Oxytocinkonzentrationen nach dem derzeitigen Wissensstand allerdings keine idealen Biomarker sind.

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CHAPTER 1

Theoretical background

1.1 The pathway from traumatic event exposure to posttraumatic stress disorder (PTSD)

1.1.1 Traumatic event exposure: A global public health challenge

As in the 1960s the Vietnam war began to escalate, the immense strain and psychological suffering of veterans who returned to the United States of America became apparent. The American Psychiatric Association was confronted with the challenge to conceptualize this form of mental distress in order to develop and provide appropriate treatment options (Andreasen, 2010). In 1980, posttraumatic stress disorder (PTSD) was, for the first time, included in the Diagnostic and Statistical Manual of Mental Disorders (DSM-III, American Psychiatric Association, 1980) by taking up and revising the former concept of a gross stress reaction (DSM-I, American Psychiatric Association, 1952). A traumatic event was defined as psychologically distressing and outside the range of usual human experience (American Psychiatric Association, 1980). Thereby, the authors presumably had in mind war catastrophes such as the Holocaust or the atomic bombings of Hiroshima and Nagasaki, which shaped historiography and media coverage at that time, but also other forms of interpersonal violence, such as torture or rape, and natural disasters, such as earthquakes or hurricanes (Friedman, 2018).

Our present understanding of a traumatic event is specified in the DSM-5 (American Psychiatric Association, 2013) which defines it as exposure to actual or threatened death, serious injury or sexual violence. Exposure may include directly experiencing the event, personally witnessing its occurrence to others, or experiencing repeated or extreme exposure to aversive details of the event. It may also include learning that a traumatic event occurred to a close family member or close friend. In this case, actual death or threats of it must have been violent or accidental. The definition underlines that traumatic event exposure in fact covers a broad range of adverse human experiences which are also prevalent outside the war context, in which they were historically predominantly described (Andreasen, 2010).

Global prevalence rates of approximately 70% indicate that most individuals experience at least one traumatic event during their lifetime (Benjet et al., 2016; Kessler et al., 2017). Notably, the average number of traumatic events experienced per capita was estimated at three (Kessler et al., 2017) and approximately 30% of individuals were exposed to at least four traumatic events during their lifetime (Benjet et al., 2016). In Europe, we are currently experiencing a historically unique period of relative

peace (Gleditsch, 1995; Mearsheimer, 2010). Nevertheless, estimates indicate that the prevalence of traumatic events is not particularly low in global comparison, which, again, emphasizes that traumatic event exposure is not limited to the context of war. In Germany, 67% of the general population indicated that they were exposed to at least one traumatic event during their lifetime (Benjet et al., 2016). Additionally, during the last years, European states increasingly accommodated refugees, many of whom were exposed to traumatic events in their homelands or during their flights (Knaevelsrud, Stammel, & Olff, 2017; Sacchetti et al., 2019). Nowadays, due to its high prevalence and its numerous devastating consequences on the global, societal, community and individual level, traumatic event exposure is regarded as a significant global public health concern (Magruder, McLaughlin, & Elmore Borbon, 2017). Consequently, preventing traumatic events and dealing with their consequences remain important and ongoing challenges for our societies (Schäfer et al., 2018).

1.1.2 Epidemiological and trajectory-oriented perspectives on PTSD

Traumatic event exposure is a prerequisite for the development of PTSD, which is characterized by symptoms that are organized in four clusters: intrusion symptoms, avoidance, negative alterations in cognitions and mood and marked alterations in arousal and reactivity. The symptoms must be associated with the traumatic event, have manifested or worsened afterwards and persisted for more than one month (American Psychiatric Association, 2013). Knowing about the high prevalence rates of traumatic event exposure, PTSD prevalence rates seem comparatively low: Globally, 3% of trauma exposed individuals suffer from PTSD in a given year and 6% during their lifetime (Koenen et al., 2017). In Germany, the 12-month prevalence of PTSD among trauma exposed individuals was estimated at 1% and the lifetime prevalence at 2% (Koenen et al., 2017).

In accordance with these epidemiological data focusing on clinically relevant PTSD, longitudinal studies, which investigated the temporal dynamics of the development, maintenance or remission of PTSD symptoms concluded that the most common response to a traumatic event is resilience. Resilient individuals often suffer from stress symptoms acutely and early posttrauma, but these symptoms are minimal or transient. In general, resilient individuals maintain stable and healthy levels of psychological and physical functioning (Bonanno, 2004). In a meta-analysis, 69% of trauma exposed individuals were

assigned to the resilience trajectory (Galatzer-Levy, Huang, & Bonanno, 2018) and in a more recent international consortium study which pooled data of *n* = 3,083 emergency department patients, 64% of patients were assigned to the low symptom severity trajectory (Lowe et al., 2020). The resilience trajectory is clearly distinguishable from the recovery trajectory, which 27% of trauma exposed individuals were assigned to in the meta-analysis (Galatzer-Levy et al., 2018). Recovery implies that individuals express threshold or subthreshold PTSD symptoms for several months and then gradually return to their pretraumatic level of functioning (Bonanno, 2004). According to the consortium study, 17% of patients showed remitting and 7% moderate symptoms during the first year posttrauma. In contrast, 10% of individuals showed chronic (Galatzer-Levy et al., 2018) or 6% high-level PTSD symptoms (Lowe et al., 2020). 6.4% (Galatzer-Levy et al., 2018) and 5% (Lowe et al., 2020) were assigned to the PTSD with delayed onset trajectory. The framework that has been used to differentiate these patterns of PTSD symptom development is graphically displayed in FIGURE 1.1.

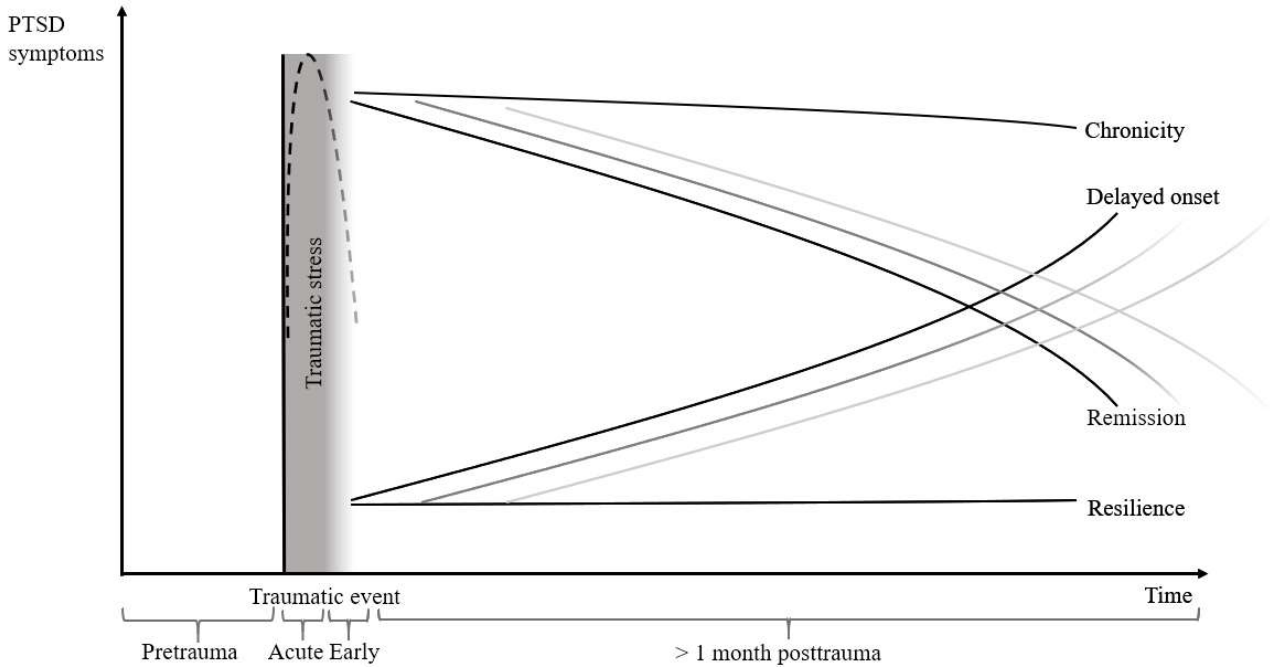

FIGURE 1.1. Theoretical framework integrating the empirically observed trajectories of posttraumatic stress disorder (PTSD) symptom development, manifestation, and remission.

A set of risk factors for PTSD has already been identified, although the exact mechanisms by which they contribute to its development are still under debate. Low sociodemographic status (Atwoli, Stein, Koenen, & McLaughlin, 2015; Brewin, Andrews, & Valentine, 2000; Koenen et al., 2017; Shalev et al., 2019), a personal or family history of mental disorders (Breslau, 2002; Brewin et al., 2000; Ozer, Best, Lipsey, & Weiss, 2003) and exposure to previous traumatic events (Ahern et al., 2004; McTeague et al., 2010; Ozer et al., 2003; Shalev et al., 2019; Wilker et al., 2015), particularly in childhood (Binder et al., 2008; Breslau, 2002; Brewin et al., 2000; Schumm, Briggs-Phillips, & Hobfoll, 2006), are well-established risk factors. Furthermore, PTSD risk is increased in individuals who were exposed to interpersonal traumatic events (Shalev et al., 2019), particularly sexual (Schumm et al., 2006; Tolin & Foa, 2006) or intimate partner violence (Forbes et al., 2014). These types of traumatic events are disproportionately more often experienced by women, but such gender-specific or structural risk factors do not fully explain their increased risk for PTSD symptom development after traumatic event exposure (Christiansen & Berke, 2020; Kornfield, Hantsoo, & Epperson, 2018; Tolin & Foa, 2006). Sex-specific neurocircuitry and neurotransmission, which is regulated by gonadal steroids, contribute to the disparity, too (Christiansen & Berke, 2020; Kornfield et al., 2018; Olff, 2017). Peritraumatic dissociation, perceived life threat and emotional responses are well-known risk factors for PTSD (Breh & Seidler, 2007; Ozer et al., 2003; Soir et al., 2015), as well as high symptoms of acute distress (Shalev et al., 2019) and lack of social support posttrauma (B. Andrews, Brewin, & Rose, 2003; Brewin et al., 2000; Ozer et al., 2003).

1.2 Neurocognitive mechanisms underlying PTSD symptom development, manifestation, and remission

Addressing the neurocognitive mechanisms underlying PTSD symptom development, manifestation and remission, complementary models focus on fear, extinction and safety learning (for overviews, see Christianson et al., 2012; Desmedt, Marighetto, & Piazza, 2015; Norrholm & Jovanovic, 2011, 2018; Parsons & Ressler, 2013; Rothbaum & Davis, 2003; Sheynin & Liberzon, 2017; Zuj & Norrholm, 2019; Zuj, Palmer, Lommen, & Felmingham, 2016). These mechanisms are accessible by experimental

paradigms in animals and humans. Relevant findings regarding PTSD are explained below and summarized in the SUPPLEMENTARY TABLES 1 -3.

1.2.1 Fear, extinction, and safety learning

In classical Pavlovian fear conditioning paradigms, a conditioned, previously neutral context or cue (CS+), simultaneously presented with an aversive, unconditioned stimulus (US), subsequently evokes a conditioned fear response. In experiments, individuals' reaction to the CS+ is compared with their reaction to another conditioned stimulus (CS-) that never co-occurs with the US and therefore remains neutral. Given a de-coupling from the US, extinction learning attenuates the conditioned fear response to the CS+ over time, resulting in fear inhibition (Sheynin & Liberzon, 2017). Vitally, extinction does not imply that the original fear memory is completely erased. Instead, it represents a distinct learning process which generates a new memory (Bouton, 2004). In experiments, the fear or extinction learning process can be studied, from acquisition to consolidation, but also its success, that is, recall.

Transferring these basic cognitive models to traumatic event processing, fear acquisition (during and immediately after the traumatic event; Parsons & Ressler, 2013) and consolidation (minutes to days afterwards; Parsons & Ressler, 2013; Schiller et al., 2010) can, in general, be regarded as adaptive responses: Individuals who have learned that a context or cue which is related to the occurrence of a traumatic event indicates threat are likely to avoid dangerous situations in the future.

Accordingly, most of the experimental evidence showed that both, healthy individuals and PTSD patients successfully acquire and consolidate conditioned fear responses. PTSD patients' fear responses neither differed from those of healthy trauma exposed individuals (Garfinkel et al., 2014; Jovanovic et al., 2009; Milad et al., 2008; Milad et al., 2009; Peri, Ben-Shakhar, Orr, & Shalev, 2000; Sijbrandij, Engelhard, Lommen, Leer, & Baas, 2013; Steiger, Nees, Wicking, Lang, & Flor, 2015; Zuj, Palmer, Hsu et al., 2016; Zuj, Palmer, Malhi, Bryant, & Felmingham, 2017), nor from those of non-trauma exposed individuals (Milad et al., 2008; Peri et al., 2000; Steiger et al., 2015; Zuj, Palmer, Hsu et al., 2016; Zuj, Palmer, Malhi et al., 2017). Only one study observed that individuals with PTSD had decreased fear responses during fear acquisition, as compared with non-trauma exposed individuals, while they did not differ from healthy trauma exposed individuals (Thome et al., 2018). However, some

studies reported increased fear responses to the CS+ (Wessa & Flor, 2007) or to both conditioned stimuli (Blechert, Michael, Vriends, Margraf, & Wilhelm, 2007; Norrholm et al., 2011) during fear acquisition in individuals with PTSD, as compared with healthy trauma exposed individuals (Norrholm et al., 2011; Wessa & Flor, 2007) and non-trauma exposed individuals (Blechert et al., 2007; Wessa & Flor, 2007).

Such PTSD-related increased fear responsiveness might be associated with PTSD-related increased conditionability. For instance, Orr et al. (2000) reported increased fear responses to the CS+, as compared with the CS-, in individuals with PTSD, as compared with healthy trauma exposed individuals. However, Wessa and Flor (2007) reported increased conditionability, as reflected in some psychological and physiological parameters, in individuals with PTSD and healthy trauma exposed individuals, as compared with non-trauma exposed individuals. Findings on the prognostic value of differential conditionability are also heterogeneous and appear to be time dependent. While increased conditionability before traumatic event exposure predicted PTSD symptom development after traumatic event exposure (Guthrie & Bryant, 2006), increased conditionability two months after deployment did not predict soldiers' symptoms nine months after deployment (Sijbrandij et al., 2013). Taken together, some evidence indicates that individuals with PTSD are more sensitive towards threat signals, even though it remains unclear whether this is specific for PTSD or for traumatic event exposure (Wessa & Flor, 2007) and how this increased sensitivity manifests over time (Guthrie & Bryant, 2006; Sijbrandij et al., 2013).

Only seemingly contradicting, some studies also detected decreased conditionability in individuals with PTSD. For instance, increased fear responses to both, the CS+ and CS- were detected in individuals with PTSD symptoms, as compared with healthy trauma exposed individuals (Grillon & Morgan, 1999; Jovanovic, Norrholm, Blanding, Phifer et al., 2010). Accordingly, Grillon and Morgan (1999) also observed impaired fear recall in individuals with PTSD. PTSD-related decreased conditionability was further confirmed when comparing individuals with PTSD symptoms with a group that included healthy individuals and such with general anxiety and depression symptoms (Acheson et al., 2015), when comparing a group that included individuals with PTSD and PTSD with comorbid major depression with a group that included individuals with major depression and healthy ones (Jovanovic, Norrholm,

Blanding, Davis et al., 2010), as well as when comparing a group that included individuals with PTSD and healthy trauma exposed individuals and a group of non-trauma exposed individuals (Zuj, Palmer, Malhi, Bryant, & Felmingham, 2018).

As mentioned, studies indicating increased versus decreased conditionability in individuals with PTSD do not necessarily contradict each other. It can be assumed that individuals with PTSD are more sensitive towards stimuli that indicate potential threat and therefore overreact to the CS+. At the same time, difficulties in distinguishing between threat-related and neutral stimuli cause increased fear responses to both, the CS+ and CS-.

It seems worth noting that most experimental paradigms which investigated fear acquisition also captured fear consolidation, as the coupling between the CS+ and US was established repeatedly over the course of several minutes. Therefore, the transitions between these two neurocognitive processes are fluid. It cannot clearly be distinguished in how far the findings on fear acquisition in PTSD also capture PTSD-specific fear consolidation. The result of fear acquisition and consolidation, that is, fear recall, was observed to be increased in individuals with PTSD, as compared with healthy trauma exposed individuals, upon successful differential conditioning in both groups (Steiger et al., 2015).

Parsons and Ressler (2013) discuss further neurocognitive processes that follow fear acquisition and consolidation and might additionally explain why neutral cues can trigger PTSD symptoms in safe contexts. According to them, individuals with PTSD underwent maladaptive reconsolidation of the traumatic memory, that is, its return into a liable state, caused by reminders of the traumatic event. Furthermore, PTSD symptoms might reinforce themselves via two processes: Firstly, the expression of fear symptoms in response to a reminder of the traumatic event might sensitize individuals who are developing PTSD, which, in turn, increases their fear symptoms. Secondly, fear can generalize to other cues or contexts which were previously not associated with the traumatic event (Parsons & Ressler, 2013). However, to date, these neurocognitive processes have rarely been addressed in experimental studies. Only one study specifically investigated generalization of fear responses in PTSD and did not detect differential responses in individuals with PTSD, healthy trauma exposed individuals and non-trauma exposed individuals (Thome et al., 2018).

Under the prerequisite that traumatic events do not occur repeatedly, adaptive traumatic event processing subsequently involves extinction learning. Learning that a context or stimulus which initially indicated threat is no longer associated with the occurrence of a traumatic event will impede a generalized threat expectancy and the development or further manifestation of PTSD symptoms. Importantly, as fear responses can spontaneously recover over time, extinction involves learning new memories rather than erasing previous ones (Quirk, 2002). Extinction acquisition, which has been related to the natural or treatment-induced remission of posttraumatic stress symptoms (Craske, Treanor, Conway, Zbozinek, & Vervliet, 2014; Zuj & Norrholm, 2019), is impaired or delayed in PTSD. In accordance with the evidence regarding fear acquisition, increased differential fear responses during extinction acquisition were observed in individuals with PTSD, as compared with healthy trauma exposed individuals (Orr et al., 2000; Wessa & Flor, 2007) and with non-trauma exposed individuals (Wessa & Flor, 2007). Moreover, indicative for impaired extinction acquisition, studies reported increased fear responses to the CS+ (Acheson et al., 2015; Blechert et al., 2007; Norrholm et al., 2011; Peri et al., 2000; Steiger et al., 2015) or to both conditioned stimuli (Norrholm et al., 2011; Orr et al., 2000; Peri et al., 2000; Zuj, Palmer, Hsu et al., 2016) in individuals with PTSD, as compared with healthy trauma exposed individuals (Acheson et al., 2015; Norrholm et al., 2011; Orr et al., 2000; Peri et al., 2000; Steiger et al., 2015; Zuj, Palmer, Hsu et al., 2016) and with non-trauma exposed individuals (Acheson et al., 2015; Blechert et al., 2007; Peri et al., 2000; Steiger et al., 2015).

It can be assumed that such PTSD-related impairments in extinction acquisition are time dependent, as, compared with healthy trauma exposed (Zuj et al., 2018; Zuj, Palmer, Gray et al., 2017; Zuj, Palmer, Malhi et al., 2017) and non-trauma exposed individuals (Zuj et al., 2018, 2017), such with PTSD showed increased fear responses to the CS+ during the early extinction phase, whereas this effect was no longer observable in the late extinction phase. Similarly, Norrholm et al. (2011) reported increased fear responses in individuals with PTSD during the early and mid-extinction phase, but during the late extinction phase, they no longer differed from healthy trauma exposed individuals. However, Zuj, Palmer, Hsu et al. (2016) observed PTSD-related impairments during late, but not during early extinction acquisition, indicating that the impact of timing on extinction acquisition in PTSD needs further clarification.

In other studies, individuals with PTSD did not show altered fear responses, but altered neuronal activity during extinction acquisition (Garfinkel et al., 2014; Milad et al., 2009), potentially causing PTSD-related impairments in subsequent extinction recall (Milad et al., 2008). Milad et al. (2009) reported increased amygdala and decreased ventromedial prefrontal cortex activation during extinction acquisition in individuals with PTSD. Their subsequent extinction recall was impaired, which, on a neurofunctional level, was associated with decreased ventromedial prefrontal cortex and hippocampus, but increased dorsal anterior cingulate cortex activation. Moreover, PTSD symptom severity was negatively correlated with extinction recall. PTSD-related impairments in extinction recall were replicated in independent samples (Garfinkel et al., 2014; Milad et al., 2008; Wicking et al., 2016), as well as their association with decreased medial and ventromedial prefrontal cortex activation (Garfinkel et al., 2014).

It seems worth noting that impairments in extinction learning are not only characteristic for manifest PTSD but may also represent a vulnerability factor for its development. This was demonstrated by Guthrie and Bryant (2006) who reported that individuals with increased differential conditioning responses during extinction acquisition before traumatic event exposure developed higher PTSD symptoms afterwards. Impaired extinction acquisition also predicted increased posttraumatic stress symptoms in a study that investigated soldiers before and after deployment (Lommen, Engelhard, Sijbrandij, van den Hout, & Hermans, 2013). However, a comparison of individuals with PTSD and their healthy trauma exposed monozygotic twins implied that impaired extinction recall results from PTSD symptom development rather than predisposing it (Milad et al., 2008). Therefore, it can be stated that PTSD is clearly associated with impaired extinction learning, but the exact underlying mechanisms need further investigation (Zuj, Palmer, Lommen et al., 2016).

An alternative approach that has been used to explain why PTSD patients experience fear symptoms in safe environments is their reduced ability to learn safety signals. Safety learning paradigms test individuals' ability to learn that a safety signal indicates the absence of a US, resulting in fear inhibition. In contrast to a CS-, which also indicates the absence of an US, but never co-occurs with a CS+, a safety signal indicates the absence of an US, even if it co-occurs with a CS+ (Eckstein et al., 2019; Foilb &

Christianson, 2018). Even though extinction learning and safety learning both result in fear inhibition, they represent two distinct neurocognitive processes (Eckstein et al., 2019; Foilb & Christianson, 2018).

As discussed before, Milad et al. (2008; 2009) and Garfinkel et al. (2014) observed impaired extinction recall in PTSD patients, but the studies' specific paradigms allow for an alternative explanation, that is, PTSD-related inability to recall safely signals. In these studies, the decoupling between the US and the CS+ was indicated by a contextual safety signal. Therefore, individuals learned that whenever the CS+ was presented in a safe context, the US would not occur. In contrast to their healthy comparison groups, individuals with PTSD continued to show increased fear responses in the presence of the safety signal. Using a conditional discrimination task, Jovanovic et al. (2009) demonstrated impaired safety signal acquisition and recall in treatment seeking individuals with high PTSD symptoms, as compared to a group of treatment seeking individuals with low PTSD symptoms and healthy individuals. PTSD-related impaired safety signal acquisition (Jovanovic, Norrholm, Blanding, Phifer et al., 2010; Sijbrandij et al., 2013) and recall (Jovanovic, Norrholm, Blanding, Phifer et al., 2010) was confirmed when comparing individuals with PTSD with healthy trauma exposed individuals (Jovanovic, Norrholm, Blanding, Phifer et al., 2010; Sijbrandij et al., 2013) and when comparing a group that included individuals with PTSD and PTSD with comorbid major depression with a group that included individuals with major depression and healthy individuals (Jovanovic, Norrholm, Blanding, Davis et al., 2010). Safety learning was further identified as a prognostic factor for PTSD symptom development, as impaired safety signal acquisition in soldiers two months after deployment predicted posttraumatic stress symptoms nine months afterwards (Sijbrandij et al., 2013).

To sum up, increased fear responsiveness during fear acquisition, consolidation and recall, the inability to discriminate between threat-related and neutral stimuli, decreased extinction acquisition, consolidation and recall, as well as the inability to acquire, consolidate and recall safety signals, are the neurocognitive processes that underly PTSD symptom development and manifestation, while inhibiting symptom remission.

1.2.2 Neurocognitive models as rationale for the prevention and treatment of PTSD

Effective PTSD prevention and treatment targets exactly those neurocognitive processes that are involved in the development, manifestation, and remission of PTSD symptoms. Generally, PTSD prevention aims at impeding the acquisition and immediate consolidation (primary prevention) or subsequent consolidation (secondary prevention) of exaggerated fear related to the traumatic event, while PTSD treatment aims at promoting fear inhibition, by promoting extinction or safety learning.

In order to interfere with fear acquisition and immediate consolidation, primary preventive interventions need to be implemented even before the exposure to a traumatic event (Parsons & Ressler, 2013). As it is extremely difficult to a-priori identify individuals who will experience a traumatic event, primary preventive interventions have predominantly been evaluated in high-risk groups for traumatic event exposure, such as firefighters (Skeffington, Rees, Mazzucchelli, & Kane, 2016), police officers (Arnetz, Nevedal, Lumley, Backman, & Lublin, 2009) or military personnel (Deahl et al., 2000). However, those psychological interventions that have been evaluated, to date, relied on techniques that are rather unspecific to the neurocognitive processes underlying PTSD symptom development, such as psychoeducation, stress management, problem solving or communication skills (see Skeffington, Rees, & Kane, 2013 for an overview). Concerning pharmacological interventions, the beta-adrenergic antagonist propranolol has been discussed as potential agent (Burbiel, 2015; Parsons & Ressler, 2013). When administered to healthy individuals prior to exposure to negatively valenced stimuli, propranolol reduced the recall of those stimuli (Lonergan, Olivera-Figueroa, Pitman, & Brunet, 2013). It has therefore been discussed whether pretraumatic propranolol administration might reduce the negative emotional content of traumatic memories and therefore prevent PTSD symptom development (Parsons & Ressler, 2013). However, this has not been investigated, yet, especially due to concerns that propranolol might not only impact emotional, but also declarative memory (Burbiel, 2015).

While for ethical and practical reasons, the evidence on primary preventive interventions is still scarce, the evidence on secondary preventive interventions is more advanced. Secondary preventive interventions that aim at disrupting fear consolidation do not need to be provided before traumatic event exposure, but within a few minutes to hours afterwards (Parsons & Ressler, 2013), as recommended in

the German guidelines for diagnosing and treating the acute consequences of traumatic events (Bengel et al., 2019). Thus, their implementation is comparatively more feasible and more targeted, addressing individuals with actual traumatic event exposure and not only such with increased risk. A range of pharmacological agents assumed to potentially prevent traumatic memory consolidation has been evaluated, including propranolol, escitalopram, imipramine, benzodiazepines, or dexamethasone (Frijling, Olff, & van Zuiden, 2019; Sijbrandij, Kleiboer, Bisson, Barbui, & Cuijpers, 2015; Wright et al., 2019). This line of research is clearly growing as these and other innovative agents are continuously being tested. Nevertheless, to date, only hydrocortisone, administered within 12 hours after traumatic event exposure, has been proven effective (Sijbrandij et al., 2015; Wright et al., 2019). Generally, evidence on secondary preventive pharmacological interventions is not yet sufficiently convincing to include them in national and international guideline recommendations (Bengel et al., 2019; International Society for Traumatic Stress Studies, 2019; National Institute of Clinical Excellence, 2018). Instead, these guidelines recommend providing trauma-focused cognitive behavioral therapies (TF-CBTs), as their effectiveness has been proven in several randomized controlled trials (Freedman, 2019; Kliem & Kröger, 2013; N. P. Roberts, Kitchiner, Kenardy, Lewis, & Bisson, 2019).

Trauma-focused psychotherapy, including TF-CBTs and Eye Movement Desensitization and Reprocessing, has further been established as the gold standard treatment for PTSD (International Society for Traumatic Stress Studies, 2019; National Institute of Clinical Excellence, 2018; Schäfer et al., 2019). The guidelines also state that these psychotherapeutic interventions are clearly superior to pharmacological ones. TF-CBTs encompass several specific programs, such as cognitive processing therapy (Forbes et al., 2012; Monson et al., 2006; Resick & Schnicke, 1993), (prolonged) exposure therapy (Foa et al., 2005; Foa, Rothbaum, Riggs, & Murdock, 1991; Lovell, Marks, Noshirvani, Thrasher, & Livanou, 2001; Marks, Lovell, Noshirvani, Livanou, & Thrasher, 1998) or narrative exposure therapy (Bichescu, Neuner, Schauer, & Elbert, 2007; Hermenau, Hecker, Schaal, Maedl, & Elbert, 2013; Neuner, Schauer, Klaschik, Karunakara, & Elbert, 2004). The central technique of these TF-CBTs is exposure, which corresponds to the neurocognitive processes underlying fear inhibition, that is, extinction and safety learning (Craske et al., 2014; Rothbaum & Davis, 2003; Smith, Doran, Sippel, & Harpaz-Rotem, 2017; Stojek, McSweeney, & Rauch, 2018). During exposure, individuals

with PTSD intentionally and repeatedly confront themselves with their traumatic memories, in a safe environment and supported by a psychotherapist. They describe their memories in detail, including their sensory perceptions, behaviors, emotions, physiological responses and cognitions, for instance by verbally describing them to the psychotherapist (Neuner et al., 2004) or by writing them down (Monson et al., 2006). Through repeatedly confronting themselves with records of this description, their initially strong and negative emotional response to the description decreases over time, finally leading to a remission of PTSD symptoms. Here, the analogy to extinction learning becomes evident. Depending on the respective TF-CBT program, exposure is preceded and followed by other psychotherapeutic techniques, such as psychoeducation (Neuner et al., 2004), autobiographical work (Hermenau et al., 2013), cognitive restructuring (Monson et al., 2006), or relaxation (Marks et al., 1998).

In recent years, classical TF-CBT has undergone several advancements. For instance, pharmacological agents, such as propranolol, hydrocortisone, D-cycloserine, allopregnanolone, or MDMA, have been investigated for their potential to augment the neurocognitive processes underlying exposure therapy (Kleine, Rothbaum, & van Minnen, 2013; Stojek et al., 2018). Moreover, internet-based TF-CBTs, which are as effective as face-to-face TF-CBTs (Kuester, Niemeyer, & Knaevelsrud, 2016), have been implemented to overcome emotional, social and practical barriers to care (C. W. Hoge et al., 2004; C. W. Hoge et al., 2014) and reach an increasing number of individuals who would otherwise not engage in psychotherapy.

To sum up, knowledge about the neurocognitive processes underlying PTSD symptom development, manifestation and remission has already led to the development of effective interventions to prevent and treat PTSD. These interventions are continuously being developed further. A better understanding of the biological mechanisms that interact with the neurocognitive processes that underly prevention and treatment response can contribute to optimizing existing techniques or inspire new, innovative interventions. Therefore, including biomarkers, that is, objectively measured indicators of normal biological processes, pathological processes, or pharmacological responses to an intervention (Biomarkers Definition Working Group, 2001), into clinical studies can further inform PTSD prevention and treatment.

1.3 Advantages of including biomarkers into PTSD research

Broadly defined as objective measure, such biomarkers can range from genetic to epigenetic, autonomic, neuronal, to endocrine markers. The latter are particularly suitable to describe healthy or pathological biological processes and responses on a broad temporal spectrum, ranging from short-term measures that reflect endocrine responses within a few minutes to long-term measures that reflect endocrine dysregulations which manifested over several months or years. Moreover, endocrine biomarkers can be measured in a comparatively time- and cost-effective, low-to non-invasive way, and are therefore suitable to reflect biological processes in individual's everyday life. Endocrine biomarkers can be useful in pursuing the following aims: predicting PTSD symptom development, improving diagnostics, as well as optimizing prevention and treatment (Fischer & Ehlert, 2019; Yehuda, Flory, Southwick, & Charney, 2006).

Concerning the predictive value of biomarkers, given the high number of individuals who remain resilient after traumatic event exposure (Bonanno, 2004; Galatzer-Levy et al., 2018; Lowe et al., 2020), it is necessary to identify individuals at increased risk for PTSD symptom development in order to provide them with targeted primary or secondary preventive interventions (Shalev & Barbano, 2019). Universally providing preventive interventions to all trauma exposed individuals would be neither acceptable for resilient individuals who would invest personal resources and risk potential negative side effects just to receive unnecessary care, nor for the society which would cover the costs of this inefficient care. However, despite the growing knowledge about PTSD risk factors (Brewin et al., 2000; Shalev et al., 2019), distinguishing resilient individuals from such who develop PTSD symptoms is a compelling and yet unresolved challenge (Zuj, Palmer, Lommen et al., 2016). Therefore, prognostic biomarkers, as assessed before or after traumatic exposure, can be used to more precisely estimate individuals' risk to develop PTSD symptoms upon traumatic event exposure. In individuals who already express PTSD symptoms, prognostic biomarkers can indicate their risk of chronification (Fournier et al., 2009).

Regarding the diagnostic value of biomarkers, as mental disorders are characterized by alterations in behaviors, cognitions and emotions, the biological systems which regulate these processes are assumed to be altered, too (Fischer & Ehlert, 2019). In PTSD, characteristic alterations in behavior, such as

avoidance of traumatic event-related places or objects, alterations in cognitions, such as rigid and negative views about the world, and alterations in emotions, such as persistent fear (American Psychiatric Association, 2013), have a biological correlate (Michopoulos, Norrholm, & Jovanovic, 2015). Therefore, diagnostic biomarkers can complement conventional assessments in order to gain a more comprehensive perspective on PTSD (Fischer & Ehlert, 2019). This is in line with the Research Domain Criteria (RDoc) approach, which has been introduced by the US American National Institute of Mental Health (Insel & Lieberman, 2013). The RDoc matrix describes mental disorders by means of different domains of psychological functioning and the corresponding biological parameters. It thereby combines psychological and biological assessments.

There are several ways in which biomarkers can be used to improve psychotherapeutic or pharmacological prevention or treatment of PTSD. Firstly, prescriptive biomarkers (Fournier et al., 2009), also referred to as moderators of intervention effects (Kraemer, Wilson, Fairburn, & Agras, 2002), can indicate whether individuals' biological states before implementation of an intervention impact its effectiveness. Thus, prescriptive biomarkers which discriminate between individuals who respond particularly well to an intervention and such who need alternative care can inform individual prevention or treatment recommendations (Fournier et al., 2009). A-priori assigning individuals to those interventions they are most likely to benefit from can ultimately increase the interventions' response rates (Lueken et al., 2016). Secondly, biomarkers can be used as an outcome measure of a preventive or therapeutic intervention. Given that a specific biomarker is a valid prognostic or diagnostic marker for a specific mental disorder and thus indicates a disorder-related dysregulation of the underlying biological system, effective prevention or treatment should re-regulate this system. Consequently, biological responses to an intervention are regarded as an objective and clinically relevant outcome (Stojek et al., 2018; Yehuda, Bierer, Pratchett, & Pelcovitz, 2010). Thirdly, biomarkers can provide insights into the mechanisms underlying prevention or treatment response (Stojek et al., 2018). As a basis for this, the assumed mechanisms of action underlying the prevention of PTSD symptom development or underlying the treatment-induced remission of PTSD symptoms must precisely be defined. Additionally, markers of those biological systems which influence these mechanisms must be investigated at a meaningful point in time during the intervention. Given these premises, monitoring

changes in biological markers during an intervention can be helpful in gaining a deeper understanding of how exactly it exerts its effects. Identifying those active ingredients of an intervention can ultimately contribute to optimizing its efficacy and effectiveness, by speeding up symptom remission and increasing response rates (Stojek et al., 2018). Lastly, biological substances which promote such active ingredients can be used as pharmacological agents, both as stand-alone interventions or augmentations of existing prevention or treatment (Bowers & Ressler, 2015; Frijling et al., 2019; Kleine et al., 2013; Sijbrandij et al., 2015; Stojek et al., 2018; Wright et al., 2019).

To sum up, identifying valid biomarkers for mental disorders in general and PTSD in particular can potentially improve prognosis, diagnosis, prevention and treatment. Importantly, the informative value of biomarkers for PTSD is timing dependent. In line with the staging approach introduced by McFarlane, Lawrence-Wood, van Hooff, Malhi, and Yehuda (2017), it is necessary to use biomarkers in their different functions at the respective corresponding stages of traumatic event processing and in correspondence with the respective neurocognitive processes underlying PTSD symptom development, manifestation and remission. This is graphically summarized in FIGURE 1.2.

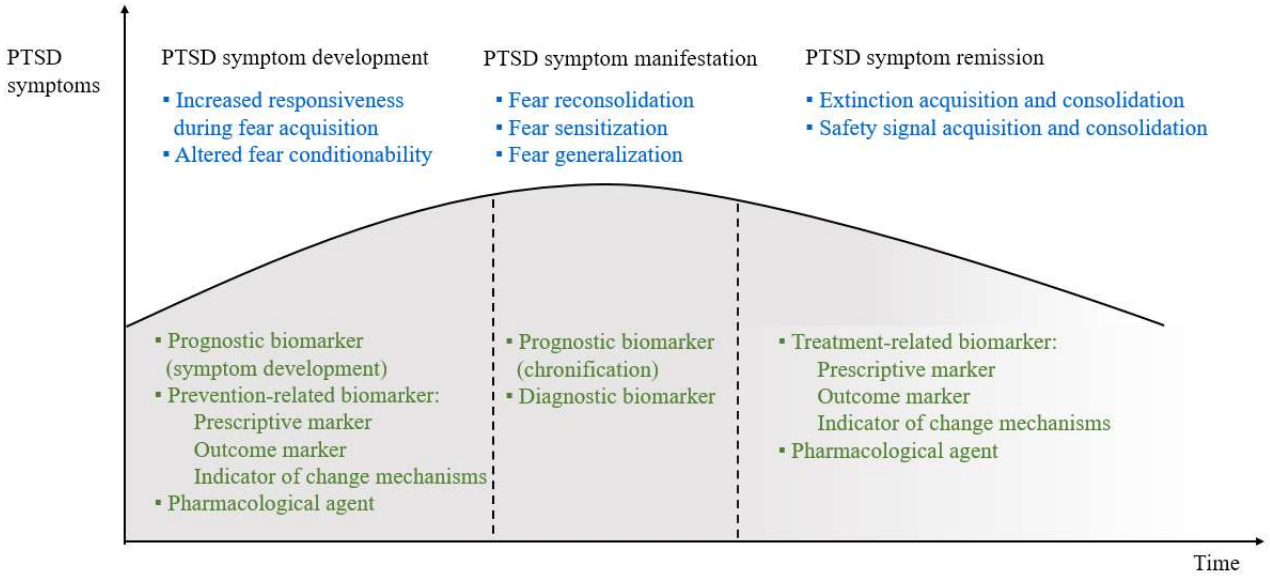

FIGURE 1.2. Theoretical framework relating the different stages of traumatic event processing (black), that is, posttraumatic stress disorder (PTSD) symptom development, expression and remission, to the corresponding neurocognitive learning processes (blue) and functions of potential biomarkers (green).

In recent years, one molecule has increasingly gained attention as a potential biomarker along those different stages of traumatic event processing, that is, oxytocin.

1.4 Oxytocin as a potential biomarker for PTSD

1.4.1 Physiological characteristics of the endogenous oxytocin system

Oxytocin is an evolutionary conserved molecule. This means that oxytocin or its precursors, as well as their receptors, are identifiable across species (Acher, Chauvet, & Chauvet, 1995; Goodson, 2008). Oxytocin is synthesized in magnocellular neurons of the supraoptic and paraventricular nuclei of the hypothalamus (Brownstein, Russell, & Gainer, 1980; Swanson & Sawchenko, 1983). These project into the posterior lobe of the pituitary gland and through action potentials, oxytocin is released from their axonal terminals into the bloodstream (Brownstein et al., 1980). This connection between the hypothalamus and neurohypophysis is an essential part of the hypothalamo-neurohypophysial system, though which oxytocin exerts its peripheral, physiological functions (Brownstein et al., 1980; Neumann, 2008). Oxytocin receptors have been identified in a variety of peripheral target organs, including the reproductive organs, mammary tissue, kidney, heart, thymus, fat cells, pancreas and adrenal gland (Gimpl & Fahrenholz, 2001; Jurek & Neumann, 2018; Song & Albers, 2018).

In addition to oxytocin's role as a hormone, it acts centrally as a neuromodulator and neurotransmitter. By binding on its central receptors, oxytocin exerts its behavioral and psychological, that is, emotion-related and cognitive, functions (Landgraf & Neumann, 2004; Neumann, 2008). Brain oxytocin release encompasses both, somatodendritic, paracrine diffusion, as well as direct axonal pathways (Landgraf & Neumann, 2004). As human oxytocin neurons are multipolar and carry extensive dendritic trees (Grinevich, Knobloch-Bollmann, Eliava, Busnelli, & Chini, 2016), oxytocin can be released in a paracrine manner. This means that oxytocin is diffusely distributed from the soma and dendrites of the magnocellular hypothalamic neurons into the extracellular fluid surrounding them (Pow & Morris, 1989). Furthermore, direct axonal projections from parvocellular neurons of the paraventricular nucleus (Gimpl & Fahrenholz, 2001) enable direct oxytocin transmission and release in a variety of extrahypothalamic brain regions, including the amygdala, hippocampus, striatum, bed nucleus of stria terminalis and brainstem (Knobloch et al., 2012; Knobloch & Grinevich, 2014; Meyer-Lindenberg,

Domes, Kirsch, & Heinrichs, 2011). Notably, oxytocin receptors have been detected in even more brain regions than those which are directly innervated by hypothalamic axons (Gimpl & Fahrenholz, 2001). It seems obvious to assume that these regions are instead targeted by paracrine diffusions. However, the short half-life of oxytocin in the brain (Mens, Witter, & van Wimersma Greidanus, 1983) makes oxytocin transport through the cerebrospinal fluid (CSF) to brain regions at greater distance from the hypothalamus seem unlikely and therefore contradicts this assumption. Therefore, the exact processes by which these receptors are targeted are still unclear (Knobloch & Grinevich, 2014).

To sum up, the pattern of oxytocin routing and targeting through both, axonal transport and somatodendritic diffusion, to both, central regions, and peripheral organs, is complex. The three main pathways of oxytocin distribution are therefore illustrated in FIGURE 1.3.

The physiological characteristics of the endogenous oxytocin system crucially determine the methods that can be applied to measure oxytocin, as well as the conclusions that can be drawn from such measurements. Research on oxytocin evolved from biology and accordingly, mainly from animal studies. In animals, the effects of increased or decreased oxytocin availability in specific brain regions on physiological processes and behaviors can be observed by performing intracerebral microperfusions, such as push-pull perfusion or microdialysis, of synthetic oxytocin or oxytocin antagonists (Neumann & Landgraf, 2019). Furthermore, gene knockout techniques can be applied in order to study animals that are unable to synthesize oxytocin (Nishimori et al., 1996).

In humans, such invasive methods cannot be applied. Applicable methodological approaches include investigating the effects of intranasal oxytocin administration as well as measuring endogenous oxytocin concentrations and oxytocin receptor gene functioning. Intranasal administration enables oxytocin to cross the blood-brain barrier (Born et al., 2002). Thereby, the effects of increased central oxytocin availability on certain behaviors and psychological processes can be estimated, albeit not in a brain region-specific manner.

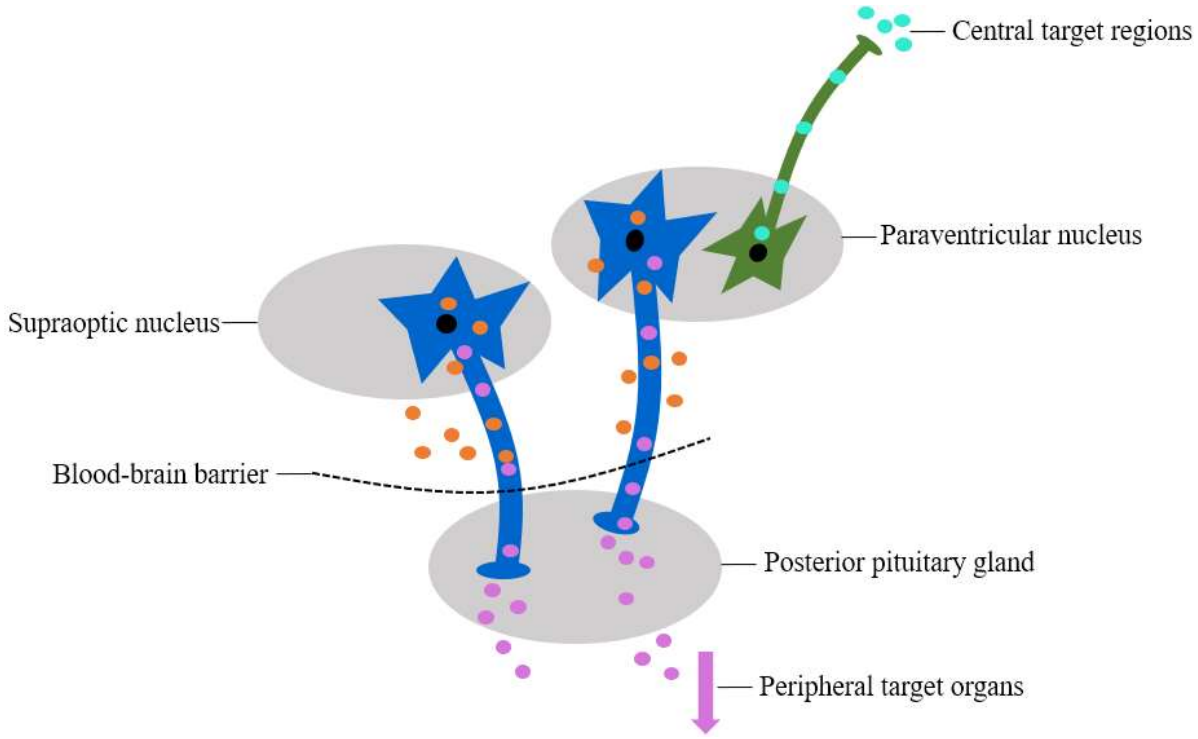

FIGURE 1.3. Schematic illustration of the three main pathways of oxytocin distribution through the human body. Oxytocin is depicted in pink, orange or turquoise, depending on its respective pathway. Firstly, axonal transport from magnocellular neurons (blue) of the hypothalamic supraoptic and paraventricular nuclei to the posterior pituitary enables oxytocin to pass the blood-brain barrier. Thereby, it can be released into the bloodstream (pink), in order to reach its peripheral target organs. Secondly, these magnocellular neurons also enable somatodenritic diffusion of oxytocin into the surrounding, extracellular fluid (orange). Thirdly, parvocellular neurons (green) in the paraventricular nucleus enable oxytocin transport to a variety of central target regions (turquoise).

The estimation is further complicated as the effects of intranasal oxytocin administration depend on individuals' baseline conditions, reflected in endogenous oxytocin concentrations and oxytocin receptor gene functioning, which can be determined by assessing variations in the oxytocin receptor gene or its DNA methylation (Bartz, Zaki, Bolger, & Ochsner, 2011). At the same time, these parameters can also give insights into oxytocin functioning in humans. Most frequently, oxytocin has been measured in central (CSF) and peripheral (blood, saliva, urine) body fluids (Crockford, Deschner, Ziegler, & Wittig, 2014; Veening, Jong, & Barendregt, 2010). While endogenous oxytocin concentrations in CSF are

suitable to reflect the effects of increased central oxytocin availability, this approach is locally unspecific, relatively invasive and effortful (Meyer-Lindenberg et al., 2011). Peripheral concentrations can be measured in a less invasive and effortful way and are therefore more suitable to reflect oxytocin functioning in everyday life (Crockford et al., 2014). However, as oxytocin is too large to cross the blood-brain barrier (McEwen, 2004), it remains unclear in how far peripheral measurements reflect central oxytocin availability. While peripheral oxytocin concentrations, as measured after stress exposure or after intranasal oxytocin administration, were positively correlated with central ones, these were uncorrelated under basal conditions (Valstad et al., 2017).

Oxytocin's physiological functions are, to a large amount, regulated by its peripheral actions as a hormone, but its behavioral and psychological functions are regulated by its actions as a neuromodulator or neurotransmitter in specific brain regions. Therefore, brain region-specific measurements of oxytocin functioning would be of great interest for biomarker research. However, to date, oxytocin's actions in specific brain regions can only be estimated from indirect measurement methods.

1.4.2 Oxytocin's physiological functions

Oxytocin is best known for its physiological functions, particularly for its role in parturition and lactation (Higuchi, Honda, Fukuoka, Negoro, & Wakabayashi, 1984; Kendrick, Keverne, Chapman, & Baldwin, 1988). It stimulates uterine contractions, which explains its use in obstetrics for the induction of labor (Alfirevic, Kelly, & Dowswell, 2009). After birth, oxytocin release is induced by suckling and oxytocin subsequently regulates milk production and release (Uvnäs-Moberg, 1996). Although they are most prominent, these are not the only reproductive functions of oxytocin. Animal studies prove that it promotes ovulation (Einspanier, Ivell, & Hodges, 1995; Einspanier, Jurdzinski, & Hodges, 1997; McCracken, Custer, & Lamsa, 1999), penile erection (Melis, Argiolas, & Gessa, 1986) and ejaculation (Filippi et al., 2003). These functions already indicate that oxytocin often exerts its effects in a sex-specific manner and interacts with gonadal steroids (for an overview, see Macdonald, 2013).

Oxytocin influences a variety of additional physiological processes, such as metabolism, nociception, or the autonomic and neuroendocrine stress responses (for overviews, see Boll, Almeida de Minas, Raftogianni, Herpertz, & Grinevich, 2018; Gimpl & Fahrenholz, 2001; Lawson, 2017; Winter & Jurek,

2019; Yang, Wang, Han, & Wang, 2013). Concerning metabolism, based on animal studies which showed that oxytocin decreased food intake (Blevins et al., 2015; Maejima et al., 2011; Maejima et al., 2015), body weight (Blevins et al., 2015; Maejima et al., 2011) and fat mass (Maejima et al., 2011), oxytocin has been discussed as potential pharmacological agent to treat obesity or diabetes (Cai & Purkayastha, 2013). Its anti-obesity effects were successfully transferred to both healthy and clinical human samples. A single dose of intranasal oxytocin administration reduced food intake in normal-weight (Lawson et al., 2015), but particularly in obese men (Thienel et al., 2016). Repeated intranasal oxytocin administration over eight weeks reduced body weight of obese men and women, again, with greater effects in more obese patients (Zhang et al., 2013).

With regard to nociception, results from numerous animal studies consistently demonstrated that oxytocin reduced the perception of acute pain (for an overview, see Rash, Aguirre-Camacho, & Campbell, 2014). However, when these findings were transferred to experimental studies in healthy humans and to clinical samples, less consistent results were yielded: While analgesic effects of intranasal oxytocin administration were observed in healthy men and women who underwent the cold pressor test (Rash & Campbell, 2014), no effects were found in healthy men who underwent an electric shock paradigm (Singer et al., 2008). Regarding clinical samples, analgesic effects of single (Wang et al., 2013) or repeated intranasal oxytocin administration over 13 weeks (Ohlsson et al., 2005) were reported in men and women suffering from headaches (Wang et al., 2013) and in women suffering from chronic constipation (Ohlsson et al., 2005). However, in a sample of women suffering from fibromyalgia, the effects of repeated intranasal oxytocin administration over two weeks were not superior to placebo (Mameli et al., 2014).

Concerning its autonomic functions, intranasal oxytocin administration increased resting high frequency heart rate variability, an indicator of predominantly parasympathetic activity (Malik, 1996), in healthy men and women (Kemp et al., 2012; Norman et al., 2011). Animal studies showed that oxytocin was centrally released in response to stressors (Nishioka, Anselmo-Franci, Li, Callahan, & Morris, 1998; Wotjak et al., 1998) and subsequently decreased the physiological stress responses (Neumann, Wigger, Torner, Holsboer, & Landgraf, 2000). These stress-decreasing effects of intranasal oxytocin

administration were documented with regard to autonomic parameters, such as blood pressure (Light et al., 2000) or alpha-amylase reactivity (Ditzen et al., 2013) in healthy women, as well as with regard to neuroendocrine parameters, such as cortisol reactivity, in healthy women and men (Ditzen et al., 2009; Heinrichs, Baumgartner, Kirschbaum, & Ehlert, 2003). Interestingly, oxytocin not only decreased cortisol output, but also subjective stress (Heinrichs et al., 2003), indicating interactions between the different levels of the human stress response.

Obviously, these physiological functions of oxytocin do not directly provide a rationale to investigate it as a biomarker for PTSD. This rationale is rather provided by oxytocin's behavioral and psychological functions. Still, it is important to keep in mind that oxytocin does not only regulate processes that are typically altered in PTSD, but instead, it influences a variety of basic physiological, behavioral and psychological functions, as these multiple functionalities might challenge its usefulness as mental disorder-specific biomarker.

1.4.3 Oxytocin's behavioral and psychological functions

It is mainly due to its social and fear processing-related functions that oxytocin has been discussed as a potential biomarker for PTSD symptom development, manifestation, and remission. In animals, these functions can be examined based on behavioral observations. In humans, they can additionally be examined in psychological investigations. Most prominently in the field of psychology, oxytocin has become known for its impact on social functioning (for overviews, see Donaldson & Young, 2008; Feldman, 2012; Heinrichs, Dawans, & Domes, 2009; Macdonald & MacDonald, 2010). Pioneering animal studies demonstrated that central injection of synthetic oxytocin increased (Pedersen & Prange, 1979), while oxytocin antagonist injection decreased maternal care (Neumann, Douglas, Pittman, Russell, & Landgraf, 1996). In line with this, endogenous oxytocin concentrations of human mothers and fathers were associated with parenting behaviors such as care, touch (Feldman et al., 2012), social engagement, affect synchrony and communication with their children (Feldman, Gordon, & Zagoory-Sharon, 2011). Oxytocin also promotes social behavior more generally, beyond the context of parenting. In fact, a whole series of studies made oxytocin gain its reputation as a prosocial hormone. For instance, it was reported that intranasally administered oxytocin increased trust in healthy men (Kosfeld,

Heinrichs, Zak, Fischbacher, & Fehr, 2005; Mikolajczak, Pinon, Lane, Timary, & Luminet, 2010) and positive communication behavior during a couple conflict paradigm in healthy women and men (Ditzen et al., 2009). It decreased interpersonal distance in healthy women, as shown in a virtual reality paradigm (Riem et al., 2019) and, in the presence of a friend, it decreased subjective anxiety and cortisol concentrations after a social stress test in healthy women (Riem, Kunst, Bekker, Fallon, & Kupper, 2020). This supports the hypothesis that oxytocin underlies the stress-reducing effects of social support (Heinrichs et al., 2003), which, in turn, is a cause of oxytocin release (Grewen, Girdler, Amico, & Light, 2005). However, oxytocin's effects on social functioning are not consistently beneficial in all individuals. For instance, intranasal oxytocin administration decreased trust and cooperative behavior in men and women with borderline personality disorder (Bartz, Simeon et al., 2011). It decreased interpersonal distance only in those healthy women who had not experienced love withdrawal as a disciplinary strategy during their childhood (Riem et al., 2019). In contrast, in combination with social support, it decreased only the stress responses of those healthy women who reported adverse experiences during childhood (Riem et al., 2020). The social salience hypothesis partially explains these seemingly contradicting findings, stating that oxytocin promotes the salience of both, positive and negative social information and that its specific effects depend on individual characteristics and context (Bartz, Zaki et al., 2011; Shamay-Tsoory & Abu-Akel, 2016). Thus, it can clearly be concluded that oxytocin is involved in social functioning, but its complex interactions with specific contextual and individual factors are not fully understood, yet (Bartz, Zaki et al., 2011; Olff et al., 2013). Given that social support is a protective factor after traumatic event exposure (Brewin et al., 2000; Ozer et al., 2003), that impaired social functioning, as expressed in symptoms such as avoidance of traumatic event-related social contacts or persistent negative expectations towards others, is characteristic for PTSD (American Psychiatric Association, 2013), and that TF-CBT occurs in a social context and is promoted by a positive therapeutic relationship (Cloitre, Stovall-McClough, Miranda, & Chemtob, 2004), oxytocin's social functions already justify its investigation as a biomarker for PTSD. Even more important for this rationale, however, are oxytocin's anxiolytic functions.

Experimental animal studies showed that central administration of synthetic oxytocin during fear conditioning decreased fear responses (Roozendaal et al., 1992) and post-conditioning central

administration of oxytocin hexapeptide fragments decreased fear recall (Stoehr, Cramer, & North, 1992). Moreover, oxytocin administration prior to fear conditioning promoted fear extinction, while the administration of an antagonist impeded it (Toth, Neumann, & Slattery, 2012). These findings support the assumption that oxytocin has anxiolytic functions, however, more recent and more elaborated studies indicate that these effects are more complex, as they are timing- (Toth et al., 2012) and brain region dependent (Campbell-Smith, Holmes, Lingawi, Panayi, & Westbrook, 2015; Zoicas, Slattery, & Neumann, 2014). Inspired by these findings, a set of placebo-controlled studies investigated the impact of intranasal oxytocin administration on fear and extinction learning in healthy humans. They are explained here and summarized in the SUPPLEMENTARY TABLE 4. Concerning oxytocin's impact on fear learning in humans, some studies indicated fear-promoting effects, while others did not replicate these observations. Healthy men who received intranasal oxytocin 30 minutes before fear learning had increased fear responses during fear acquisition, particularly to the CS+ and during the late acquisition phase (Eckstein et al., 2016). Moreover, intranasal oxytocin administration 45 minutes before fear learning led to increased subjective arousal ratings of the CS+ and the CS- during the late acquisition phase in healthy women and men (Cavalli et al., 2017). However, it neither influenced subjective arousal during the early acquisition phase, nor other physiological or psychological parameters in any acquisition phase. Furthermore, intranasal oxytocin did not impact fear recall when administered to healthy men and women immediately after fear learning on the preceding day (E. Hoge et al., 2019). Concerning oxytocin's influence on extinction learning, its effects apparently depend on the timing of administration. Intranasal oxytocin administration 45 minutes before fear learning had no impact on physiological and psychological fear responses during the subsequent extinction acquisition, as shown in healthy men and women (Cavalli et al., 2017). However, two studies consistently reported that healthy men (Eckstein et al., 2015) and women (Acheson et al., 2013) who received intranasal oxytocin 30 (Eckstein et al., 2015) or 45 minutes (Acheson et al., 2013) prior to extinction learning displayed increased physiological fear responses during the early extinction phase, but a subsequent stronger decline of the fear responses in the late extinction phase (Acheson et al., 2013; Eckstein et al., 2015), along with an increased extinction recall (Acheson et al., 2013). Other studies yielded more heterogeneous results regarding the effects of oxytocin administration after fear learning. For instance,

intranasal oxytocin administration 45 minutes before extinction learning promoted the decrease of the differential CS+ and CS- subjective valence ratings, but had no effects on the skin conductance responses of the healthy men (Petrovic, Kalisch, Singer, & Dolan, 2008). Hu, Wang, Feng, Long, and Schiller (2019) reported that the fear responses of healthy women and men who had received intranasal oxytocin or placebo one day after fear learning did not differ during extinction acquisition another day later. However, if individuals who received intranasal oxytocin were exposed to the US immediately before the intranasal administration, they showed a faster decline in their fear responses, finally resulting in deficient differential responses to the CS+ and CS-. Regarding safety learning, it has been proposed that intranasal oxytocin administration might promote the acquisition and recall of safety signals, especially social ones (Eckstein et al., 2019), but an investigation of this assumption is still pending. Such studies can certainly be awaited with great interest. However, oxytocin's apparent, yet not fully understood, impact on extinction learning already legitimates its investigation as a potential biomarker for PTSD. More specifically, as extinction learning is the neurocognitive process that is assumed to underly adaptive traumatic event processing after the initial stress response (Zuj & Norrholm, 2019) and the remission of PTSD symptoms due to TF-CBT (Craske et al., 2014; Rothbaum & Davis, 2003; Smith et al., 2017; Stojek et al., 2018), the interesting question whether oxytocin promotes adaptive traumatic event processing and PTSD symptom remission arises. Given the heterogeneity in the current state of research, it seems evident that studies attempting to answer to this question should also take contextual and individual factors, particularly sex, into account.

1.4.4 Prior investigations of oxytocin as a potential biomarker in PTSD research

A set of studies already investigated oxytocin as a potential biomarker along the pathway from traumatic event exposure to PTSD symptom development, manifestation and remission (for overviews, see Di Lorenzo et al., 2020; Giovanna et al., 2020). Below, they will be introduced in more detail and current gaps in research will be worked out.

Concerning PTSD symptom development, both endogenous oxytocin concentrations (Nishi, Hashimoto, Noguchi, Kim, & Matsuoka, 2015; Reijnen, Geuze, & Vermetten, 2017) and oxytocin receptor gene variations (Dunn et al., 2014; Feldman et al., 2012; Lucas-Thompson & Holman, 2013) have been

investigated as potential prognostic biomarkers. In male veterans, blood oxytocin concentrations, as assessed before, one and six months after deployment were not associated with PTSD symptoms up to five years after deployment (Reijnen et al., 2017). In line with this, blood oxytocin concentrations, as assessed after admittance to an intensive care unit due to a motor vehicle accident did not predict PTSD symptoms of men or women one month later (Nishi et al., 2015). Variations in the oxytocin receptor gene were not predictive for PTSD symptoms of men and women, up to four years after exposure to the Hurricane Katrina (Dunn et al., 2014). In Israelian girls and boys who were repeatedly exposed to rocket attacks and mortar shelling, a genetic risk factor that was, among others, defined by carrying two T alleles at the oxytocin receptor gene rs1042778 SNP, carrying two G alleles at its rs2254298 SNP and carrying at least one A allele at the most frequently investigated oxytocin receptor gene SNP, that is, rs53576, were unrelated to PTSD symptoms in early childhood, but predicted chronicity instead of remission of PTSD symptoms from early to middle childhood (Feldman et al., 2012). US American men and women who carried an A allele at the re53576 oxytocin receptor gene SNP reported increased PTSD symptoms two and three years after the September 11 attacks if they lived in a negative social environment. In contrast, individuals who carried two G alleles reported increased PTSD symptoms only if they additionally suffered from high economic stress (Lucas-Thompson & Holman, 2013).

Concerning oxytocin as a potential PTSD prevention-related biomarker, parameters that indicate oxytocin functioning have not yet been examined as potential prescriptive markers, outcome markers or indicators of change mechanisms that underlie effective interventions. Only one study investigated repeated intranasal oxytocin administration as a preventive pharmacological intervention (van Zuiden et al., 2017). Intranasal oxytocin, administered to moderately to severely distressed men and women within 12 days after exposure to diverse kinds of traumatic events, did not influence PTSD symptoms one month and a half, three and six months later in all individuals. However, in individuals with high acute PTSD symptoms, it did exert beneficial effects, which, again, highlights the need to precisely examine moderators.

Regarding oxytocin's potential as a diagnostic biomarker for PTSD, a large number of studies already investigated whether endogenous oxytocin concentrations (e.g. Chatzittofis, Nordström, Uvnäs-

Moberg, Asberg, & Jokinen, 2014; Frijling et al., 2015; Munro et al., 2013), oxytocin receptor gene DNA variation (e.g. Bhandari et al., 2014; Bradley, Davis, Wingo, Mercer, & Ressler, 2013) or methylation (e.g. Gouin et al., 2017; Nawijn et al., 2019; Smearman et al., 2016) were indicative for either traumatic event exposure (e.g. Bhandari et al., 2014; Bradley et al., 2013; Chatzittofis et al., 2014; Gouin et al., 2017; Smearman et al., 2016) or PTSD symptoms (e.g. Frijling et al., 2015; Munro et al., 2013; Nawijn et al., 2019). It is a current challenge to systematically summarize these studies to comprehensively and differentially analyze the impact of traumatic event exposure and PTSD symptoms on oxytocin functioning. Thereby, a valid conclusion regarding the question whether oxytocin can serve as a diagnostic biomarker for PTSD could be drawn.

Regarding PTSD treatment, just as it is the case for prevention, oxytocin has not yet been evaluated as potential prescriptive, outcome, or mechanisms of change marker. Some placebo-controlled studies investigated the effects of single intranasal oxytocin administration as a stand-alone treatment in individuals with PTSD (for an overview, see Giovanna et al., 2020). For instance, intranasal oxytocin administration reduced PTSD symptoms that were evoked by a traumatic event reminder in women with PTSD (Sack et al., 2017). It further marginally reduced cortisol reactivity, which is related to the PTSD symptom cluster of altered arousal and reactivity, in male veterans with comorbid PTSD and alcohol use disorder (Flanagan, Allan et al., 2019) and influenced the neuronal underpinnings of cognitive emotion regulation (Koch et al., 2019), emotional stimuli processing (Flanagan, Sippel et al., 2019), as well as monetary (Nawijn et al., 2016) and social reward processing (Nawijn et al., 2017) in women and men with PTSD. Even though this has frequently been suggested (Dunlop, Mansson, & Gerardi, 2012; Hurlemann, 2017; Koch et al., 2014), to date, only one study evaluated the effects oxytocin as pharmacological agent for medication-enhanced psychotherapy (Flanagan, Sippel, Wahlquist, Moran-Santa Maria, & Back, 2018). Male and female PTSD patients who received intranasal oxytocin instead of placebo before each of their 10 weekly TF-CBT sessions reported lower PTSD symptoms and a more positive therapeutic alliance. The effects were statistically not significant, which might be attributable to the small sample size (n=17) and emphasizes that this young field of research needs more studies and larger sample sizes before valid conclusions can be drawn.

To sum up, there are many cross-sectional studies that related oxytocin functioning to traumatic event exposure and PTSD symptoms. However, those have never been systematically summarized, which is a current gap in research. To examine oxytocin's impact on PTSD symptom development and remission and, related to that, its potential usefulness for PTSD prevention and treatment, longitudinal studies are needed, until now they are still scarce. Few studies have investigated oxytocin as a pharmacological agent (Flanagan et al., 2018; van Zuiden et al., 2017) and, to date, none have investigated oxytocin as a prevention- or treatment-related biomarker. It seems worthwhile to address this gap in research, because including measures of oxytocin functioning into the scientific evaluation of interventions for PTSD symptoms can inform indication or outcome of such or provide insights into their mechanisms of change. Given the remarkable degree of heterogeneity in prior investigations of oxytocin's functions in both, healthy and clinical samples, it seems almost needless to say that individual and contextual moderators need to be considered adequately in such studies. Among the many factors that could potentially be considered, this dissertation placed special emphasis on sex and gonadal steroids. Furthermore, it must be considered that the physiological complexity and multiple functionalities of the oxytocin system challenge its assumed eligibility as specific biomarker for PTSD. Biomarkers need to be measurable in a reliable way, in order to ensure that no other effects besides those of interest in PTSD research influence the findings. As oxytocin, besides the effects it exerts on social functioning and fear processing, also regulates a variety of physiological functions, it seems important to take potentially confounding physiological parameters into account. Yet, systematic knowledge on such confounding variables is still lacking and consequently, gathering such is another scientific gap this dissertation aims to address.

1.5 Scientific questions

The aim of this dissertation was two-fold: Firstly, to examine oxytocin's involvement along the pathway from traumatic event exposure to PTSD symptom development, manifestation, and remission (**studies 1** to **5**)**.** Secondly, to explore factors related to the oxytocin system's physiology in order to determine whether endogenous measurements can serve as a specific biomarker for PTSD or whether they are confounded by factors other than psychopathology (**studies 6** and **7**).

The first two studies focused on oxytocin's involvement in PTSD symptom development. They are based on empirical data from the above-mentioned double-blind, multicenter, randomized placebo-controlled trial which evaluated the effects of repeated intranasal oxytocin administration on PTSD symptom development (Frijling et al., 2014; van Zuiden et al., 2017). The follow-up analyses explored prognostic and prescriptive effects of sex, hormonal contraception use and other biomarkers. The following scientific questions specifically addressed oxytocin and are therefore relevant for this dissertation:

Study 1 (presented in CHAPTER 2) investigated the differential effectiveness of repeated intranasal oxytocin administration, as initiated early posttrauma, in preventing PTSD symptoms one month and a half, three and six months posttrauma in men, women using hormonal contraception and cycling women.

Study 2 (presented in CHAPTER 3) explored prognostic and prescriptive effects of endogenous oxytocin, as measured before treatment initiation, in men, women using hormonal contraception and cycling women.

The third study addresses oxytocin's involvement in manifest PTSD. It is the first systematic review and meta-analysis that comprehensively summarized the large number of existing empirical studies relating endogenous oxytocin concentrations, oxytocin receptor gene variation and methylation to traumatic event exposure and PTSD in men and women:

Study 3 (presented in CHAPTER 4) systematically summarized studies that investigated interactions between traumatic event exposure and PTSD symptoms on the one hand and endogenous oxytocin concentrations, oxytocin receptor gene DNA variation and methylation on the other hand.

The fourth and fifth studies aimed at studying oxytocin's involvement in PTSD remission due to psychotherapeutic treatment. They are based on empirical data from a randomized waitlist-controlled trial evaluating the feasibility, acceptability and effectiveness of an internet-based TF-CBT in male German Armed Forces service members (Niemeyer et al., 2020).

Study 4 (presented in CHAPTER 5) described trajectories of endogenous oxytocin concentrations and their correlations with PTSD symptoms before, immediately and 3 months after internet-based TF-CBT.

Study 5 (presented in CHAPTER 6) explored the impact of pre-treatment endogenous oxytocin concentrations on psychotherapy process variables, particularly the therapeutic alliance.

The sixth and seventh studies are meta-analyses addressing the impact on demographic, physiological and methodological confounders on measurements of endogenous oxytocin concentrations in healthy humans.

Study 6 (presented in CHAPTER 7) meta-analytically investigated demographical, as well as sampling- and assay-related confounders of endogenous oxytocin concentrations in healthy men and women.

Study 7 (presented in CHAPTER 8) meta-analytically described fluctuations in endogenous oxytocin concentrations during the course of the menstrual cycle in healthy women.

It is worth mentioning that some of the studies addressed additional scientific questions, but as this dissertation's focus was on oxytocin, only those which are relevant for this focus were picked out here. To sum up, the first arm of this dissertation is dedicated to oxytocin's involvement in PTSD, comprehensively covering all stages of traumatic event processing, from PTSD symptom development to its manifestation and remission related to both, pharmacological and psychotherapeutic treatment. The second arm informs about factors that might limit oxytocin's potential as biomarker for PTSD.

CHAPTER 2

Patterns of recovery from early posttraumatic stress symptoms after a preventive intervention with oxytocin: Hormonal contraception use is a prognostic factor

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Patterns of recovery from early posttraumatic stress symptoms after a preventive intervention with oxytocin: Hormonal contraception use is a prognostic factor

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In this journal, we recently reported on the efficacy of repeated intranasal oxytocin administration early after emergency department admission in preventing posttraumatic stress disorder (PTSD) symptoms, assessed 1.5, 3 and 6 months posttrauma (van Zuiden et al., 2017). In a randomized, placebo-controlled trial, we demonstrated beneficial effects in patients with high symptoms before treatment onset (Frijling, 2017; van Zuiden et al., 2017). While this indicates that oxytocin administration might be an effective preventive intervention for recently traumatized individuals with high early symptoms, the accompanying commentary to our study emphasized that it remains an ongoing challenge to identify and target individuals at risk for persistent symptoms (Garcia & Delahanty, 2017). Indeed, as trauma-related symptoms constitute a major public health issue (Magruder, Kassam-Adams, Thoresen, & Olff, 2016) and currently only few effective early interventions exist (Sijbrandij, Kleiboer, Bisson, Barbui, & Cuijpers, 2015), it is crucial to target those patients who are at increased risk to develop adverse outcomes and who are most likely to benefit from treatment.

We addressed this by means of secondary data analyses, investigating the impact of different gonadal steroids-related statuses in explaining PTSD symptom courses and treatment efficacy. This yielded interesting results that we believe are an important add-on to our previous publication.

Women experience PTSD more often, with higher severity, chronicity and comorbidity (Atwoli, Stein, Koenen, & McLaughlin, 2015; Charak et al., 2014; McLean, Asnaani, Litz, & Hofmann, 2011). This disparity can partly be explained by gender-specific factors, including socioeconomic and coping-related risks (Cromer & Smyth, 2010; Gavranidou & Rosner, 2003), but sex-specific, especially endocrine, factors also contribute (Olff, Langeland, Draijer, & Gersons, 2007). Therefore, we previously investigated sex as possible prognostic factor for different PTSD symptom courses, irrespective of treatment, and as possible prescriptive factor of treatment efficacy. Results revealed neither a prognostic nor prescriptive effect.

Etiological models explaining the development of PTSD after traumatic events comprise maladaptive neurocognitive processes, including facilitated acquisition and consolidation and impaired extinction of traumatic memories (Pitman, 1989; Wessa & Flor, 2007). These processes are also regulated by gonadal steroids, including progesterone and estrogens (Kornfield, Hantsoo, & Epperson, 2018; Li &

Graham). Estrogens, including endogenous estradiol, facilitate extinction learning: higher estradiol during extinction learning enhanced subsequent extinction recall after classical fear conditioning (Milad et al., 2010). Moreover, women with PTSD and low estradiol concentrations showed deficient extinction learning, compared with trauma-exposed controls with low and patients with high estradiol concentrations (Glover et al., 2012). The constellation of high progesterone and low estradiol concentrations characterizing the luteal menstrual cycle phase facilitates fear acquisition and consolidation: women who viewed an emotional film during the luteal phase subsequently reported more intrusions than women in the follicular phase or men (Ferree & Cahill, 2009). Moreover, intrusive memory frequency was positively correlated with progesterone concentrations (Ferree, Kamat, & Cahill, 2011). Another study reported more intrusive memories of emotional film material viewed during the mid-luteal than early follicular phase or ovulation and detected a negative association between intrusion frequency and estradiol-to-progesterone ratio (Soni, Curran, & Kamboj, 2013). Women using hormonal contraception exhibit low, but relatively stable levels of endogenous estradiol and progesterone and elevated levels of exogenous estrogens. Thus, high levels of estrogens, promoting traumatic memory extinction, and low levels of progesterone, preventing consolidation, might prevent PTSD symptom development in these women. (Kornfield et al., 2018). Preliminary evidence for this hypothesis shows that emergency and regular hormonal contraception was associated with fewer intrusive symptoms 6 months posttrauma in female sexual assault victims (Ferree, Wheeler, & Cahill, 2012).

Therefore, we investigated prognostic and prescriptive effects of different gonadal steroids-related statuses on PTSD symptom courses, reanalyzing the dataset of our previous publication. We differentiated the original intention-to-treat sample (n = 107) into three groups: n = 54 men (M = 37.94 years, SD = 13.68), n = 27 women using hormonal contraception (M = 28.74 years, SD = 9.30 years) and n = 19 naturally cycling women (M = 31.58 years, SD = 10.71). The following hormonal contraception methods were present, all delivering female gonadal steroids: oral contraceptives (n = 19), hormonal intrauterine device (n = 6), hormonal injection (n = 1) and vaginal ring (n = 1).

Six menopausal women were excluded, as their small number did not allow for valid group comparisons, and one woman was excluded because of unknown menopausal status. We implemented

the same data-analytic approach as in our previous publication (Fournier et al., 2009): a mixed effects model based on 40 multiple outcome imputed datasets, testing main and interaction effects of time, treatment condition, and participant group on square root-transformed PTSD severity scores, measured by the Clinician-Administered PTSD Scale (Blake et al., 1995; Hovens et al., 1994). We added baseline symptoms, age, and time between trauma and treatment initiation as covariates, the latter two because of significant group differences. The significant time effect indicated that overall symptom severity declined from 1.5 to 6 months posttrauma. The effect of hormonal contraception use on the intercept was non-significant, but its effect on the slope was significant: Although there were no group differences 1.5 months posttrauma, women using hormonal contraception showed significantly stronger mid- to longterm recovery (from 1.5 to 6 months posttrauma), compared with cycling women and men, independently of baseline symptoms and treatment condition (TABLE 2.1, FIGURE 2.1).

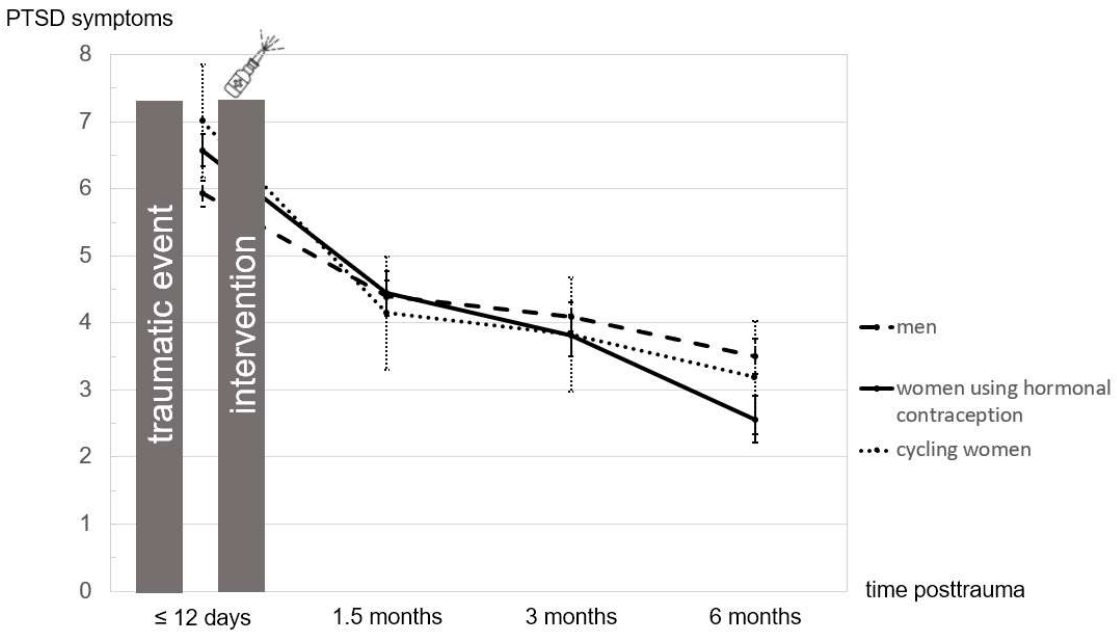

FIGURE 7.1. Predicted courses of posttraumatic stress disorder (PTSD) symptoms over follow-up timepoints, irrespective of treatment condition (intranasal oxytocin or placebo), differentiated for men (broken lines), women using hormonal contraception (full lines) and cycling women (dotted lines). PTSD symptom severity is presented as observed (≤ 12 days) and estimated (1.5, 3, 6 months) square root-transformed Clinician-Administered PTSD Scale means and standard errors.

TABLE 2.1. Exploration of possible prognostic or prescriptive effects of sex and hormonal contraception use

Predictor	β (*SEM*)	*t* (*df*)	*p*	CI
Intercept	4.35 (0.17)	25.80 (147.34; 166.36)	.00*	4.02; 4.69
Control variables				
Age	0.42 (0.16)	2.68 (97.80; 99.58)	.01*	0.11; 0.73
Time between traumatic event and treatment initiation	-0.15 (0.16)	-0.93 (97.80; 99.58)	.35	-0.46; 0.16
Baseline symptoms	1.01 (0.16)	6.30 (97.80; 99.58)	.00*	0.70; 1.33
Treatment				
Oxytocin	-0.51 (0.35)	-1.49 (146.94; 165.92)	.14	-1.19; 0.16
Time	-0.26 (0.04)	-6.39 (94.47; 110.129)	.00*	-0.34; -0.18
Treatment * Time				
Oxytocin * Time	0.08 (0.08)	0.92 (94.47; 110.129)	.36	-0.09; 0.24
Sex and hormonal contraception use				
Women using hormonal contraception	0.08 (0.31)	0.26 (139.73; 157.33)	.79	-0.52; 0.68
Cycling women	-0.21 (0.37)	-0.57 (94.47; 110.13)	.57	-0.93; 0.51
Time * Sex and hormonal contraception use				
Time * Women using hormonal contraception	-0.16 (0.07)	-2.39 (94.47; 110.129)	.02*	-0.28; -0.03
Time * Cycling women	0.05 (0.09)	0.53 (94.47; 110.129)	.59	-0.12; 0.22

Note. Final model resulting from the stepwise approach to identify prognostic and prescriptive variables according to Fournier et al. (2009). First, all possible main and interactive effects of a metric variable for time (0 = 1.5 months posttrauma, 1.5 = 3 months posttrauma, 4.5 = 6 months posttrauma), an unweighted effect coded variable for treatment (comparing oxytocin with placebo condition: -0.50 = placebo, 0.50 = oxytocin), and two weighted effect coded variables for sex and hormonal contraception use (one comparing women using hormonal contraception with men and cycling women: -0.50 = men, 0 = cycling women, 1 = women using hormonal contraception and one comparing cycling women with men and women using hormonal contraception: -0.35 = men, 0 = women using hormonal contraception, 1 = cycling women) were included to predict square root-transformed Clinician-Administered PTSD Scale scores. In the following steps, main and interactive effects were excluded according to pre-defined *p*-thresholds. The resulting final model was controlled for age, time between traumatic event and treatment initiation and baseline symptoms. The results are based on data from $n = 100$ patients. The range of degrees of freedom across 40 imputed datasets is reported. CI = confidence interval. * = effect was considered as statistically significant.

…

To conclude, while considering dichotomous sex did not divulge a prognostic effect, more detailed consideration of gonadal steroids-related statuses revealed distinct patterns of recovery from early PTSD symptoms in women using hormonal contraception. Our analyses were exploratory and hypothesis-generating rather than hypothesis-testing. Moreover, it would have been interesting to additionally evaluate effects of specific hormonal contraception methods and menstrual-cycle related fluctuations of gonadal steroids. However, that would have reduced group sizes and impeded meaningful conclusions.

Nevertheless, important implications can be derived from these results. We provide further evidence that hormonal contraception use, altering endogenous and exogenous gonadal steroid hormone concentrations, promotes recovery from traumatic stress until at least 6 months posttrauma. Interestingly, the faster recovery became apparent after the first few weeks posttrauma had passed. Our findings open up a promising line of research and should be followed up more long-term. Moreover, they clearly demonstrate that women cannot simply be merged into one participant group. Even statistically controlling for contraception use or menstrual cycle phases still impedes differential conclusions for women. We sincerely hope that our finding will not lead to further exclusion of women from psychiatric research, but instead encourages researchers to design studies and analyze data in a way that enables detection of differential effects of menstrual cycle phases and hormonal contraception use (Olff, 2016).

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Contributions

Miranda Olff, Mirjam van Zuiden, Jessie L. Frijling, Laura Nawijn, Saskia B. J. Koch and Dick J. Veltman designed the study.

Miranda Olff obtained the funding.

Mirjam van Zuiden, Jessie L. Frijling, Saskia B. J. Koch and Laura Nawijn collected the data.

Miranda Olff, J. Carel Goslings, Jan S. Luitse, Tessa H. Biesheuvel and Adriaan Honig provided administrative, technical or material support.

Sinha Engel and Mirjam van Zuiden wrote the manuscript. All authors critically revised the manuscript for important intellectual content.

Sinha Engel performed the statistical analyses, under Mirjam van Zuiden's supervision.

Miranda Olff, Dick J, Veltman and Mirjam van Zuiden supervised the study.

CHAPTER 3

Early posttraumatic autonomic and endocrine markers to predict posttraumatic stress symptoms after a preventive intervention with oxytocin

Engel, S.*, van Zuiden, M.*, Frijling, J. L., Koch, S. B. J., Nawijn, L., Yildiz, R. L. W., Schumacher, S., Knaevelsrud, C., Bosch, J. A., Veltman, D. J.. & Olff, M. (2020). Early posttraumatic autonomic and endocrine markers to predict posttraumatic stress symptoms after a preventive intervention with oxytocin. *European Journal of Psychotraumatology*, *11*(1), 1761622.

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BASIC RESEARCH ARTICLE

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Early posttraumatic autonomic and endocrine markers to predict posttraumatic stress symptoms after a preventive intervention with oxytocin

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ABSTRACT

Background: Efficient prevention of posttraumatic stress disorder (PTSD) needs to target individuals with an increased risk for adverse outcome after trauma. Prognostic or prescriptive biological markers assessed early posttrauma may inform personalized treatment recommendations.

Objective: To test prognostic and prescriptive effects of early (posttraumatic) autonomic and endocrine markers on PTSD symptom development.

Method: Autonomic and endocrine markers were assessed within 12 days posttrauma and before treatment initiation within a randomized placebo-controlled trial investigating repeated oxytocin administration as preventive intervention for PTSD. Linear mixed effects models were used to test the effects of heart rate (variability), resting cortisol, morning cortisol and cortisol awakening response (CAR), cortisol suppression by dexamethasone and resting oxytocin on PTSD symptoms 1.5, 3 and 6 months posttrauma in men (n = 54), women using hormonal contraception (n = 27) and cycling women (n = 19).

Results: We found significant prognostic effects of resting oxytocin and cortisol suppression. In women using hormonal contraception, higher oxytocin was associated with higher PTSD symptoms across follow-up. Stronger cortisol suppression by dexamethasone, reflecting increased glucocorticoid receptor feedback sensitivity, was associated with lower PTSD symptoms across follow-up in men, but with higher symptoms at 1.5 months in women using hormonal contraception. These effects were independent of treatment condition. No further significant prognostic or prescriptive effects were detected.

Conclusion: Our exploratory study indicates that resting oxytocin and glucocorticoid receptor feedback sensitivity early posttrauma are associated with subsequent PTSD symptom severity. Notably, prognostic effects depended on sex and hormonal contraception use, emphasizing the necessity to consider these factors in biomedical PTSD research.

Marcadores postraumáticos tempranos endocrinos y autonómicos para predecir los síntomas de estrés postraumático después de una intervención preventina con oxitocina

Antecedentes: La prevención eficiente del trastorno de estrés postraumático (TEPT) necesita dirigirse a personas con un mayor riesgo de consecuencias adversas después de un trauma. Los marcadores biológicos pronósticos o preceptivos evaluados tempranamente luego del trauma pueden informar recomendaciones de tratamiento personalizadas.

Objetivo: Evaluar los efectos pronósticos y preceptivos de los marcadores tempranos (postraumáticos) autonómicos y endocrinos sobre el desarrollo de síntomas de TEPT.

Método: Fueron evaluados marcadores autonómicos y endocrinos dentro de los 12 días postrauma y antes de la iniciación del tratamiento dentro de un estudio aleatorio placebo-control, investigando la administración repetida de oxitocina como intervención preventiva para TEPT. Se utilizaron modelos lineales de efectos mixtos para evaluar los efectos de la frecuencia cardiaca (variabilidad), cortisol en reposo, cortisol matutino y respuesta al despertar de cortisol (CAR por sus siglas en inglés), supresión del cortisol por dexametasona y oxitocina en reposo sobre los síntomas de TEPT a los 1.5, 3 y 6 meses postrauma en hombres (N=54), mujeres que usaban contracepción hormonal (N=27) y mujeres ciclantes (N=19).

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关键词
生物标志物; 预防; 预后; 糖皮质激素; 催产素; 心率

HIGHLIGHTS

- Biological markers can inform clinicians about an individual's PTSD risk after recent trauma.
- Biological markers predicting PTSD risk differed for men, cycling women and women on hormonal contraception.
- Biological markers did not predict the effects of nasal spray with oxytocin as preventive intervention for PTSD.

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Supplemental data for this article can be accessed here.

Resultados: Encontramos efectos pronósticos significativos de la oxitocina en reposo y de la supresión de cortisol. En las mujeres que usaban contracepción hormonal, los niveles de oxitocina más altos se asociaron con más síntomas de TEPT a lo largo del seguimiento. La supresión mayor del cortisol por dexametasona, que refleja una mayor sensibilidad a la retroalimentación del receptor de glucocorticoides, se asoció con menos síntomas de TEPT a lo largo del seguimiento en los hombres, pero con mayores síntomas a los 1.5 meses en las mujeres que usaban contracepción hormonal. Estos efectos fueron independientes de la condición de tratamiento. No se detectaron más efectos pronósticos o preceptivos significativos.
Conclusión: Nuestro estudio exploratorio indica que la oxitocina en reposo y la sensibilidad a la retroalimentación del receptor de glucocorticoides tempranamente luego del trauma se asocian con la subsecuente severidad de los síntomas de TEPT. Notablemente, los efectos pronósticos dependen del sexo y del uso de contracepción hormonal, lo que enfatiza la necesidad de considerar estos factores en la investigación biomédica en TEPT.

早期创伤后自主神经和内分泌标志物预测催产素预防性干预后的创伤后应激症状

背景: 有效预防创伤后应激障碍 (PTSD) 需要针对创伤后不良后果风险增加的个体。创伤后早期评估的预后 (prognostic) 或处方性 (prescriptive) 生物学标志物可能会为个性化治疗建议提供依据。
目的: 考查早期 (创伤后) 自主神经和内分泌标志物对PTSD症状发展的预后和处方性效应。
方法: 在一项考查重复服用催产素作为PTSD预防性干预的随机安慰剂对照试验中，于创伤后12天内和开始治疗之前评估了自主神经和内分泌标志物。使用线性混合效应模型考查心率 (变异性), 静息皮质醇, 早晨皮质醇和皮质醇唤醒反应 (CAR), 地塞米松皮质醇抑制和静息催产素对创伤后1.5, 3和6个月的男性 (n = 54), 使用激素避孕的女性 (n = 27) 和正常生理周期女性 (n = 19) 的PTSD症状的影响。
结果: 我们发现静息催产素和皮质醇抑制具有显著的预后效应。在使用激素避孕的女性中，较高的催产素与整个随访期间较高的PTSD症状相关。地塞米松对皮质醇的更强抑制作用，反映糖皮质激素受体反馈敏感性的提高，与男性整个随访期间PTSD症状的降低相关，而与使用激素避孕的女性在1.5个月时较高的症状相关。这些效应与治疗条件无关。未发现进一步的显著预后或处方效应。
结论: 我们的探索性研究表明，创伤后早期静息催产素和糖皮质激素受体的反馈敏感性与随后的PTSD症状严重程度有关。值得注意的是，预后效果取决于性别和激素避孕的使用，强调在生物医学PTSD研究中必须考虑这些因素。

1. Introduction

Traumatic events, i.e. exposure to actual or threatened death, serious injury or sexual violence (American Psychiatric Association, 2013) are experienced by over 70% of the general population (Benjet et al., 2016; Kessler et al., 2017). A considerable proportion of traumatized individuals subsequently develop posttraumatic distress disorder (PTSD). A meta-analysis showed that 27.0% of individuals develop initial PTSD symptoms but then recover, 10.3% develop chronic and 6.4% delayed PTSD (Galatzer-Levy, Huang, & Bonanno, 2018). In addition to treating PTSD, over the past years, efforts have increased to prevent its development in the first place (see Dunlop, Mansson, & Gerardi, 2012; Horn, Charney, & Feder, 2016; Sijbrandij, Kleiboer, Bisson, Barbui, & Cuijpers, 2015). Recently, we reported the results of a randomized controlled trial (RCT) evaluating the potential preventive effects of intranasal oxytocin administration early posttrauma on PTSD symptom development (Frijling et al., 2014; van Zuiden et al., 2017). Beneficial effects were found in individuals with high acute PTSD symptoms. This supports the growing notion that instead of offering preventive interventions to all traumatized individuals, targeted interventions should be offered only to those who are at increased risk for adverse outcomes and most likely to benefit from the interventions. At the same time, interventions should not interfere with the adaptive recovery observed in the majority of traumatized individuals (Shalev & Barbano, 2019). Such targeted preventive interventions could be informed by prognostic markers, predicting PTSD symptom development irrespective of treatment, and prescriptive markers, moderating an intervention's effectiveness (Garcia & Delahanty, 2017; National Institute of Clinical Excellence, 2018).

A variety of biological systems associated with perceiving, reacting to, and recovering from (traumatic) stress appear to be involved in PTSD symptom development, including the autonomic nervous system (ANS) and the hypothalamic-pituitary-adrenal (HPA) axis (Heim & Nemeroff, 2016; Olff & van Zuiden, 2017). During acute stress, the sympathetic branch of the ANS provides a rapid response, amongst others increasing heart rate (HR), peripheral vasoconstriction and energy mobilization. During recovery, the parasympathetic branch's activity increases, exerting opposing effects on the ANS's target organs (Andrews, Ali, & Pruessner, 2013; Chrousos & Gold, 1992). The interplay of sympathetic and parasympathetic activity determines heart

rate variability (HRV), i.e. the beat-to-beat variation in heart rate over time. HRV represents an index for the ANS's ability to respond appropriately to environmental challenges (Thayer, Ahs, Fredrikson, Sollers, & Wager, 2012). A meta-analysis summarizing prognostic studies reported that PTSD development was associated with high resting HR, assessed within 72 hours posttrauma (Morris, Hellman, Abelson, & Rao, 2016).

To date, three prospective studies investigated pretrauma HRV. These reported that pretrauma lower resting HRV was associated with higher posttrauma PTSD symptoms in male military personnel (Minassian et al., 2015) and a mixed-sex sample of children (Mikolajewski & Scheeringa, 2018). The third study, again in a predominantly male, military personnel sample, found this association was specific to the context of high pretrauma symptoms (Pyne et al., 2016). Further, lower 24 hours HRV 2 days posttrauma (Shaikh Al Arab et al., 2012) as well as during rapid eye movement sleep at 5 to 30 days posttrauma (Mellman, Knorr, Pigeon, Leiter, & Akay, 2004) were associated with subsequent PTSD development in mixed-sex samples. Thus, evidence indicates that autonomic dysregulation before, acutely and early after trauma is associated with increased PTSD risk.

The HPA axis response is part of the comparatively slower, neuroendocrine stress response. Acute stress recovery is crucially modulated by HPA axis's end product cortisol, via glucocorticoid receptor (GR)-induced inhibition of the HPA axis (Andrews et al., 2013; Chrousos & Gold, 1992). Most studies that tested prognostic effects of posttrauma cortisol used a single cortisol measurement and included mixed-sex samples (Bonne et al., 2003; McFarlane, Atchison, & Yehuda, 1997; Mouthaan et al., 2014; Price, Kearns, Houry, & Rothbaum, 2014; Resnick, Yehuda, Pitman, & Foy, 1995; Walsh et al., 2013). Assessment times varied from within the first hours to weeks posttrauma. Not surprisingly, given the methodological variability between studies, a meta-analysis showed that cortisol concentrations, assessed within 72 hours posttrauma, did not predict subsequent PTSD (Morris et al., 2016). Prospective studies assessing pretrauma hair cortisol concentrations in male military populations found negative associations with subsequent PTSD development when intermittent trauma exposure was adequately accounted for (Steudte-Schmiedgen et al., 2015).

High pretrauma GR function and GR-sensitivity to inhibitory effects of glucocorticoids in immune cells predicted subsequent PTSD symptoms in male, military populations (van Zuiden et al., 2012, 2011, 2012; van Zuiden, Kavelaars, Geuze, Olff, & Heijnen, 2013). One study reported a trend towards stronger cortisol suppression by the synthetic glucocorticoid dexamethasone, indicating higher GR sensitivity at the level of hypothalamus and pituitary, 2 days posttrauma in male and female emergency care patients with subsequent PTSD diagnosis (McFarlane, Barton, Yehuda, & Wittert, 2011), but the sample was rather small and findings have not been replicated yet. Thus, despite substantial methodological heterogeneity, preliminary evidence indicates that high GR feedback sensitivity and, potentially as a consequence, low long-term cumulative cortisol output might predict PTSD risk.

The neuropeptide oxytocin is a well-known modulator of the HPA axis, such that stress-induced oxytocin release dampens HPA axis reactivity (Alley, Diamond, Lipschitz, & Grewen, 2019; Engelmann, Landgraf, & Wotjak, 2004; Winter & Jurek, 2019). Moreover, oxytocin has anxiolytic effects (Jurek & Neumann, 2018), and promotes various aspects of prosocial behaviour (Heinrichs, von Dawans, & Domes, 2009) but these effects strongly depend on individual factors (e.g. secure vs. insecure attachment style; presence vs. absence of psychopathology) and on contextual factors (e.g. environment perceived as safe vs. as unsafe (Olff et al., 2013)). A recent meta-analysis showed increased expression of oxytocin pathway genes within functional brain networks associated with stress, fear, anxiety and cognitive processes relevant for social behaviours, such as reward and motivation (Quintana et al., 2019). As these processes presumably underlie vulnerability to PTSD after trauma, these findings provided the rationale to investigate intranasal oxytocin as candidate preventive intervention for PTSD (Frijling et al., 2014). They also suggest that blood oxytocin concentrations early posttrauma may impact PTSD risk or preventive treatment effectiveness. To date, two studies assessed blood oxytocin concentrations and PTSD symptoms prospectively. In a male, military sample, no impact of oxytocin concentrations, assessed before, 1 and 6 months post-deployment, on PTSD symptoms up to 5 years post-deployment was found (Reijnen, Geuze, & Vermetten, 2017). Likewise, in male and female emergency care patients, no significant association between oxytocin concentrations upon hospital admittance and PTSD symptoms 1 month later was found (Nishi, Hashimoto, Noguchi, Kim, & Matsuoka, 2015). Prescriptive effects of oxytocin concentrations have not been investigated yet.

One aspect that has largely been neglected is the impact of participants' sex and, accordingly, gonadal steroid-related status on the prognostic value of biological markers for PTSD symptom development. There is accumulating evidence that cross-sectional associations between PTSD symptoms and HRV (Kamkwalala et al., 2012), as well as diurnal cortisol

output (Nicolson & Ponnamperuma, 2019), are sex-dependent. Furthermore, HPA axis function and oxytocin concentrations differ between men and women, and, within women, are additionally influenced by hormonal contraception use and menstrual cycle phase (Engel, Klusmann, Ditzen, Knaevelsrud, & Schumacher, 2019; Kirschbaum, Kudielka, Gaab, Schommer, & Hellhammer, 1999; Stock, Karlsson, & von Schoultz, 1994). We also previously reported that hormonal contraception use promoted mid- to long-term recovery of early PTSD symptoms (Engel et al., 2019). However, to date, most published studies on biological prognostic markers for PTSD risk relied on single-sex, most commonly all-male samples. Even if studies included both men and women, sex has seldomly been taken into account adequately. Moreover, gonadal steroid-related statuses, such as menstrual cycle phase or hormonal contraception use, have rarely been considered as factors of influence in this line of research. In this context, it seems important to stress out that even statistically controlling for these factors impedes a precise estimation of the specific prognostic effects within the specific group participants (i.e. within men vs. within cycling women). This can only be achieved by stratifying analyses for sex and gonadal steroid-related statuses.

To sum up, a variety of autonomic and endocrine systems respond to traumatic events and might predict subsequent PTSD symptoms, although their predictive value may be dependent on sex and hormonal contraception use. It seems promising to concomitantly explore ANS functioning, HPA axis activity and oxytocin concentrations early posttrauma as dysregulations in these systems may be biological underpinnings of PTSD development. However, studies examining this broader range of stress response systems parameters early posttrauma are still sparse and to date no study comprehensively assessed all these systems within the same design, nor were previous analyses stratified for sex and hormonal contraception use. Moreover, biological prescriptive factors for preventive, pharmacological interventions for PTSD remain understudied to date, let alone whether these effects may be dependent on sex and hormonal contraception use. Therefore, the present study explored prognostic and prescriptive effects of ANS and HPA axis markers and oxytocin concentrations, all assessed early posttrauma, on PTSD symptom development, while stratifying for sex and hormonal contraception use. This was a secondary analysis of a trial studying the potential of oxytocin administration to prevent PTSD development (van Zuiden et al., 2017).

2. Methods and materials

This study encompasses follow-up analyses of a double-blind, placebo-controlled, multicenter RCT evaluating the effects of repeated intranasal oxytocin administration on PTSD symptom development (see (Frijling et al., 2014) for study protocol). Results of the main analyses of the RCT have been published elsewhere (van Zuiden et al., 2017).

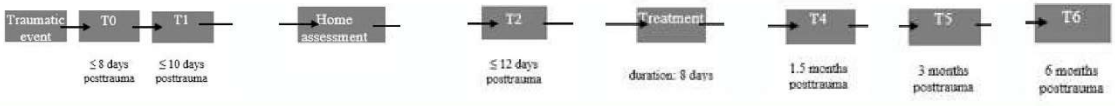
2.1. Participants

Potential participants were eligible for inclusion in the RCT if they were recently (≤8 days) traumatized (fulfiling DSM-IV PTSD A1 criterion) adults (18–65 years) with moderate to severe peritraumatic or acute posttrauma distress (defined by cut-off scores ≥5 on the Trauma Screening Questionnaire (Mouthaan, Sijbrandij, Reitsma, Gersons, & Olff, 2014; Walters, Bisson, & Shepherd, 2007) or ≥17 on the Peritraumatic Distress Inventory (Nishi et al., 2010). Exclusion criteria were current PTSD or depression, psychotic, bipolar, substance-related (either reported by participants during screening or objectified during the pre-treatment clinical interview), and personality disorder (in case of a previous formal diagnosis reported by participants), severe or chronic systemic disease, mental retardation, neurological or endocrine disorder, ongoing traumatization, use of medications potentially interfering with oxytocin administration, oxytocin allergy, persistent impaired consciousness or amnesia, pregnancy and breastfeeding at the pre-treatment assessment. For an overview of the study design, participants flow and availability of autonomic and endocrine data, see Table 1.

2.2. Study design

Within 8 days after emergency department admission due to acute exposure to various kinds of traumatic events (most frequently: motor vehicle accidents), participants were screened for eligibility for the RCT via telephone or at the bedside if still hospital admitted (T0, screening). Within 10 days posttrauma, the remaining exclusion criteria were checked and psychometric data gathered, by means of clinical interviews and questionnaires (T1, pre-treatment assessment). Subsequently, participants were enrolled in the RCT. Within 12 days posttrauma, they administered their first, randomly assigned dose of intranasal oxytocin or placebo, under experimenter supervision (T2, treatment initiation). If there was sufficient time before initiation of the intervention (within 12 days posttrauma), post-awakening cortisol output, cortisol awakening response (CAR) and cortisol suppression by dexamethasone were assessed at home at two subsequent days between T1 and T2. Furthermore, at T2, resting biological markers were collected prior to the first administered dose. T2 assessments were scheduled in the afternoon or early evening. Sixty-eight per cent of assessments took place between 12:00pm and 18:00pm. On

Table 1. Study design, participant flow and available data for psychological, endocrine and autonomic parameters of interest.



Available data	*n* (CAPS)	*n* (cortisol AUCg) *n* (CAR AUCi) *n* (DST AUCg) *n* (DST AUCi)	*n* (oxytocin) *n* (cortisol) *n* (ANS)	Condition *n* (oxytocin) *n* (placebo)	*n* (CAPS)	*n* (CAPS)	*n* (CAPS)
All participants	100	68 68 31 31	74 84 83	49 51	84	78	77
Men	54	36 35[b] 15 15	45[a] 43[b] 46	26 28	43	42	40
Women using hormonal contraception	27	20 20 11 11	17 23[c,d] 20	17 10	26	24	25
Cycling women	19	12[b] 13 5[e] 5[e]	12 18 17	6 13	15	12	12

The number of available data is based on the intention-to-treat sample. With regard to autonomic and endocrine parameters, outliers deviating more than 3 SD from the mean were removed. There were no differences in availability of heart rate, high-frequency heart rate variability (HRV) and low-frequency HRV measures; thus, these parameters are summarized as autonomous nervous system (ANS) markers. Missing CAPS (Clinician-Administered PTSD Scale) data at T4, T5 and T6 were imputed for analyses. The pre-defined maximum number of 12 days between trauma and T2 limited the number of performed dexamethasone suppression tests (DSTs). CAR = cortisol awakening response; AUCg = area under the curve with respect to the ground; AUCi = area under the curve with respect to increase. [a]One value was removed due to technical problems [b]One value was removed as an outlier [c]Two values were removed as outliers [d]One value was removed as coefficient of variation was ≥10% [e]Lacking sufficient data, cycling women were not considered in the DST linear mixed models.

average, assessments began at 14:30pm. The earliest assessment started at 9:33am and the latest at 20:51pm. Participants watched a nature documentary depicting plants for 25 minutes while HR(V) was recorded. Immediately afterwards, saliva was collected in order to measure resting cortisol and thereafter blood was drawn in order to measure resting oxytocin.

In all biological assessments, participants were asked to record their behaviour with regard to potentially confounding variables, that is, time of day, food, caffeine and alcohol intake, smoking and sports. Participants were asked to refrain from eating, drinking (except water), smoking and sporting 2 hours before T2 initiation. Compliance was inquired upon arrival at T2. The potential covariates were considered in the statistical analyses.

For the following 7 days, participants administered nasal spray twice daily at their homes. Participants were instructed to maintain approximately 12 hours between administrations. Adherence to the intervention regimen and potential adverse events were registered in a diary on a daily basis (see van Zuiden et al., (2017) for more details and related findings). At T1, as well as upon treatment completion at 1.5 (T4), 3 (T5) and 6 (T6) months posttrauma, participants' PTSD symptoms were assessed with the Clinician-Administered PTSD Scale (CAPS; Blake et al., 1995). All interviewers had at least a Bachelor's degree in psychology or medicine and received standardized training in administering and scoring the interview. There were no significant differences in CAPS total scores from T1 to T6 between interviewers ($F_{(19,\ 300)} = 1.17$, $p = .29$). For a graphical representation of the study design see Table 1. Further information concerning participants, study design, psychometric instruments and pharmacological details, including CONSORT flow diagram and checklist is reported in our previous publication (van Zuiden et al., 2017).

2.3. Assessment of biological parameters

2.3.1. Autonomic nervous system (ANS) markers

HR(V) was recorded using the Polar RS800CX (wristwatch and chest strap), during the 25 minutes resting time participants spent at T2, watching a nature documentary depicting plants. Participants were placed in a sitting position, while spontaneously breathing. Five minute HR(V) samples were obtained from the 25 minutes measurements. All samples were obtained after at least 10 minutes of recording, to ensure a minimum consistent resting period (Shaffer & Ginsberg, 2017). The 5 minutes HR(V) samples were collected approximately 40–45 minutes after start of the T2 assessment. The sampling rate of the device was 1000 Hz, which is sufficiently rapid for accurate HRV measurements (Kuusela, 2013; Shaffer, McCraty, & Zerr, 2014). Raw unfiltered interbeat intervals (RR-intervals) were processed and analysed using the process software Kubios HRV (Standard Software version 3.0.2; University of Eastern Finland). HRV indices were computed with the power spectrum analysis using an autoregressive model without factorization (Wahbeh & Oken, 2013). For statistical analyses, three different parameters were calculated. HR, defined as mean number of heartbeats per minute, was used as indicator of autonomic, predominantly sympathetic activity. Additionally, we investigated HRV, i.e. the beat-to-beat variation in heart rate over time, as index of autonomic regulation. We investigated high-frequency HRV (HF-HRV) power (0.15 to <0.40 Hz frequency band) and low-frequency HRV (LF-HRV) power (0.04 to <0.15 Hz frequency band (Malik et al., 1996; Quintana, Alvares, & Heathers, 2016)). To facilitate interpretation, we opted to report the normalized instead of absolute power. To calculate the percentage spent in each frequency band, we divided the absolute power (in ms^2/Hz) of the respective frequency band by the summed absolute power of HF, LF and very-LF bands and multiplied it by 100 (Burr, 2007). Trend components were removed using a time-varying high pass filter (smoothness priors) with a cut-off frequency of 0.018 Hz (lambda 500; Tarvainen, Ranta-Aho, & Karjalainen, 2002)

2.3.2. Hypothalamic-pituitary-adrenal (HPA) axis markers

At T2, after HR(V) recording, participants provided a single saliva sample for measuring resting cortisol concentrations. To account for cortisol's diurnal rhythm, time of day was considered as a potential covariate. Saliva was collected by Salivettes (Sarstedt, Rommelsdorf, Germany). Samples were immediately stored at −20°C until analysis. Free cortisol concentrations were determined by an enzyme immunoassay (IBL, Hamburg, Germany). Samples were analysed in duplicate, and all samples of the same participants were run in a single assay. The mean intra-assay coefficient of variation was 2%.

For measuring total morning cortisol output, CAR and its suppression by the dexamethasone suppression test (DST), participants collected saliva samples at home on two subsequent days between T1 and T2. Participants received precise instructions with regard to the sampling protocol and reported sampling protocol adherence in a diary. Collection times were immediately, 15, 30 and 60 min after awakening. At 11pm on the first day, participants took a low dose (0.25 mg) of dexamethasone, aimed to suppress cortisol production on the second day. We decided to use this low dose of dexamethasone instead of the more commonly used 0.5 mg because of previous findings of high prevalence of super-suppression early posttrauma (McFarlane

et al., 2011). Participants stored saliva samples in their refrigerators and brought them back to the laboratory for the T2 assessment, where they remained stored at −20°C until further analysis by the same assay as the resting cortisol samples. Although our data collection started prior to publication of expert consensus guidelines for CAR assessments (Stalder et al., 2016), our procedures follow the guidelines' recommendations, except that waking times were not objectively monitored. For statistical analyses, four different indicators of HPA axis activity were calculated. Area under the curve coefficients were calculated for natural (first day) and the dexamethasone suppressed (second day) morning cortisol, using the formulas provided by Pruessner, Kirschbaum, Meinlschmid, and Hellhammer (2003). As recommended, we distinguished between the area under the curve with respect to the ground (AUCg), indicating total cortisol production during the first hour after awakening, and the AUC with respect to increase (AUCi), indicating the CAR, i.e. amount of cortisol production relative to the first measurement after awakening (Stalder et al., 2016).

2.3.3. Oxytocin

Resting oxytocin concentrations were measured in blood plasma at T2 after HR(V) recording and saliva collection. Plasma was collected into 7 ml EDTA tubes, placed on ice immediately after sampling, centrifuged at 4°C and stored at −80°C until analysis. Oxytocin was quantified by a highly specific and sensitive radioimmunoassay (RIA) using 0.8 ml of plasma (RIAgnosis, Munich, Germany) as previously described (Kagerbauer et al., 2013). All samples were extracted prior to analysis and analysed simultaneously in the same assay. The sensitivity was 0.1 pg per sample and the intra- and inter-assay variability was <10%. All samples were within detection limit.

2.4. Statistical analyses

Prognostic and prescriptive effects were tested by means of linear mixed models. The baseline model included the variables treatment condition and time as well as their interactions, as predictors, and – in accordance with the main RCT publication (van Zuiden et al., 2017) – square root-transformed CAPS scores at T4, T5 and T6 as outcomes. Concerning the predictor treatment condition, we applied unweighted contrast coding (−0.5 for placebo condition and 0.5 for oxytocin condition, as done by Fournier et al. (2009)) this means, the effects of oxytocin administration were compared with the effects of placebo administration as a reference. The predictor time was metric and indicated time in months between T4, T5 and T6 (0 = T4, 1.5 = T5, 4.5 = T6). Accordingly, in addition to modelling random intercept effects and fixed slope effects, the predictors' effects on CAPS at T4 were indicated by the intercept and their effects on the change from T4 to T6 were indicated by the slope. The outcome CAPS was metric and positively skewed and therefore square root-transformed. In line with our aim to specifically consider sex and hormonal contraception use, analyses were performed separately in men, women using hormonal contraception and cycling women. In order to subsequently analyse prognostic and prescriptive effects of biological parameters early posttrauma, we followed the stepwise approach recommended by Fournier et al. (2009), as we also did in our previous paper (van Zuiden et al., 2017). Following this approach, we subsequently added the biological parameters of interest as predictors to the baseline model. A linear mixed effects model was tested per biological parameter (i.e. HR, HF-HRV, LF-HRV, resting cortisol, morning cortisol AUCg, CAR AUCi, DST AUCg, DST AUCi and resting oxytocin) and per group (men, women using hormonal contraception and cycling women). Distributions of HPA axis parameters and resting oxytocin concentrations were positively skewed. Therefore, values were log-transformed. For all biological parameters, outliers deviating more than 3 *SD*s from the mean were removed (see Table 1 for an overview on outlier removal). All biological parameters were *z*-transformed in order to yield standardized regression coefficients in the analyses, enabling comparisons of the magnitude of effects between parameters.

In a first step, main and interaction effects between the respective biological parameter, treatment condition and time were included in the model. Main effects on the intercept indicated a prognostic effect on PTSD symptoms at T4. Interactive effects of the respective biological variable with time (i.e. on the slope) indicated a prognostic effect on the change from T4 to T6. Interaction effects of the respective biological variable with treatment condition indicated a prescriptive effect at T4.

In a second, third, and fourth step, effects with *p*s <.20, <.10 and <.05, respectively, were maintained and effects with *p*-values above these thresholds were excluded. If interaction effects were maintained, the corresponding main effects were maintained too. If *p* was <.05 in the final model, the effect was considered statistically significant (Fournier et al., 2009).

The robustness of the respective final models was tested by adding relevant covariates. As such, we considered general potential confounders (age, body mass index (BMI), habitual smoking, current employment, current sick leave, recent drug use, having children, number of children, (non-)Dutch ethnicity and time since trauma at T2). For ANS parameters, resting cortisol and oxytocin, we additionally considered time of day at assessment, food, caffeine and alcohol intake, smoking and sports prior to assessment. For morning cortisol, CAR and DST parameters, we considered deviations >5 minutes from the defined sampling timepoints, smoking and caffeine intake during the sampling period, alcohol intake

on the sampling day or day before, breakfast intake at the sampling day and whether the sampling day was normal or busy, as potential confounders. In order to select confounders for the mixed effects models, we conducted one-way ANOVAS and $\chi 2$ tests to investigate whether men, women using hormonal contraception and cycling women differed with regard to general potential confounders. In case of significant group differences, Bonferroni and Scheffé posthoc tests were performed. Hereby, we ensured that any detected prognostic of prescriptive effect within a specific group was not due to group differences in potential confounders.

Furthermore, we correlated general and specific potential confounders with each biological parameter in each group (results presented in supplementary material 1). Potential confounders that yielded significant results in these analyses were statistically controlled for in the respective mixed effects model. Included metric potential confounders were also z-transformed and for dichotomous potential confounders, weighted contrast coding was applied. If case numbers of dichotomous potential confounders were very small, sensitivity analyses were performed, in order to test whether excluding the respective participants changed the results.

The statistical approach we chose was specifically designed to balance type I and type II error risks in exploratory analyses (Fournier et al., 2009). Therefore, we refrained from correcting for multiple testing and instead would like to emphasize that our approach serves for the purpose of generating, but not testing hypotheses. All mixed effects models were performed in 40 datasets with missing CAPS scores imputed, using auxiliary variables treatment, demographic, trauma, and baseline clinical characteristics as reported before (van Zuiden et al., 2017). Pooled results are reported. All analyses were performed with SPSS software (version 22; IBM Corp., Armonk, NY).

3. Results

3.1. Participants

The original intention-to-treat sample, consisting of $n = 107$ participants, was split up according to sex and hormonal contraception use. Due to their small number ($n = 6$), menopausal women were not included in the present analyses and one woman from the original sample had to be excluded because of unknown fertility status. The present analyses are thus based on $n = 100$ participants: $n = 54$ men ($M = 37.94$, $SD = 13.68$ years), $n = 27$ women using hormonal contraception ($M = 28.74$, $SD = 9.30$ years) and $n = 19$ naturally cycling women ($M = 31.58$, $SD = 10.71$ years). Concerning hormonal contraception, all used methods delivered female gonadal steroids. The following methods were applied: oral contraceptives ($n = 19$), hormonal intrauterine device ($n = 6$),

Table 2. Sample description.

				Group differences	
	Men	Women using hormonal contraception	Cycling women	F/χ2	p
General information					
Age	37.94 (13.68)	28.74 (9.30)	31.58 (10.72)	5.76	**<.01**
BMI	25.10 (3.09)	23.99 (5.24)	24.86 (5.17)	.63	.53
Time between traumatic event and T2	9.09 (1.64)	7.85 (1.96)	9.58 (1.39)	7.04	**<.01**
Habitual smoking (yes/no, %(yes))	19/35 (35.19)	3/24 (11.11)	4/15 (21.05)	5.72	.06
Current employment (yes/no, %(yes))	44/10 (81.48)	25/2 (92.59)	14/5 (73.68)	3.02	.22
Current sick leave (yes/no, %(yes))	28/26 (51.85)	12/15 (44.44)	11/8 (57.89)	.84	.66
Recent drug use (yes/no, %(yes))	13/41 (24.07)	8/19 (29.63)	2/17 (10.53)	2.37	.30
Dutch ethnicity (yes/no, %(yes))	42/12 (77.78)	23/4 (85.19)	11/8 (57.89)	4.76	.09
Children (yes/no, %(yes))	29/25 (53.70)	8/19 (29.63)	10/9 (52.63)	4.49	.11
Number of children	2.22 (1.29)	1.62 (.77)	1.88 (.78)	1.64	.20
Autonomic and endocrine parameters					
HR	68.88 (8.43)	74.10 (12.73)	71.62 (9.23)	2.08	.13
HF-HRV	20.52 (13.72)	31.39 (13.31)	30.11 (12.59)	6.08	**<.01**
LF-HRV	50.81 (13.20)	45.73 (11.06)	48.56 (12.74)	1.15	.32
Resting cortisol (ug/cl)	.38 (.23)	.38 (.16)	.33 (.22)	.62	.54
Cortisol AUCg	59.04 (29.09)	69.20 (31.72)	68.57 (33.45)	1.27	.29
CAR AUCi	.76 (1.18)	.60 (1.21)	1.18 (1.57)	.74	.48
DST AUCg	34.46 (25.47)	26.37 (22.93)	()	.86	.36
DST AUCi	.49 (.71)	.30 (.59)	()	.49	.49
Resting oxytocin (pg/ml)	1.77 (1.93)	7.15 (8.57)	2.70 (4.16)	6.19	**<.01**
PTSD symptoms over time					
CAPS T1	37.20 (18.00)	44.78 (17,18)	51.53 (21.28)	4.67	**.01**
CAPT T4	24.67 (20.57)	24.42 (21.60)	30.07 (27.01)	.11	.90
CAPS T5	17.81 (17.95)	15.92 (17.51)	24.08 (25.67)	.29	.75
CAPS T6	14.25 (15.11)	8.56 (7.91)	16.58 (18.76)	.86	.42

If not indicated differently, *M* (*SD*) is reported. Group differences were determined by means of one-way ANOVAs (for metric variables) or χ2 tests (for dichotomous variables). To facilitate interpretation, non-transformed cortisol and oxytocin concentrations, area under the curve coefficients and post-treatment (T4, T5 and T6) Clinician-Administered PTSD Scale (CAPS) values are reported, although these parameters were log- (biological parameters) or square root-transformed (CAPS scores) for the statistical analyses. Bold values indicate significant *p* values. BMI = body mass index, HR = heart rate; HF-HRV = heart rate variability, percentage of time spent in the high frequency band (.15 to <.40 Hz); LF-HRV = heart rate variability, percentage of time spent in the low frequency band (.04 to <.15 Hz); CAR = cortisol awakening response; AUCg = area under the curve with respect to the ground; AUCi = area under the curve with respect to increase; DST = dexamethasone suppression test; PTSD = posttraumatic stress disorder; () = parameter was not analysed, lacking sufficient number of participants.

hormonal injection (n = 1) and vaginal ring (n = 1). Participants information is presented in Table 2.

Men, women using hormonal contraception, and cycling women significantly differed in age ($F_{(2, 97)}$ = 5.76, p < .01). Posthoc tests indicated men were significantly older than women using hormonal contraception. Significant group differences were also present with regard to time since trauma at T2 ($F_{(2, 97)}$ = 7.04, p < .01). Posthoc tests indicated that women using hormonal contraception had a shorter time since trauma than both other groups. Moreover, the three groups significantly differed with regard to initial PTSD symptoms ($F_{(2,97)}$ = 4.67, p = .01), with posthoc tests showing that cycling women had higher initial PTSD symptoms than men. No further significant group differences were found.

3.2. Group differences and correlations

Group differences in biological variables are also presented in Table 2. A comprehensive overview of correlations of biological variables with pre-treatment PTSD symptoms is given in supplementary material 2.

HR and LF-HRV did not significantly differ between groups and these parameters were not significantly correlated with pre-treatment PTSD symptoms. Significant group differences emerged for HF-HRV ($F_{(2, 80)}$ = 6.08, p < .01). Posthoc tests showed that men had lower HF-HRV than both female groups. HF-HRV was not significantly correlated with pre-treatment PTSD symptoms.

There were no significant group differences in resting cortisol. In cycling women, higher concentrations were significantly correlated with higher pre-treatment symptoms (r = .48, p < .05). No significant group differences or correlations were detected with regard to morning cortisol AUCg, CAR AUCi, DST AUCg or DST AUCi, but significant differences were found in resting oxytocin ($F_{(2, 71)}$ = 6.19, p < .01). Posthoc tests showed that men had significantly lower concentrations than women using hormonal contraception. Oxytocin was not significantly correlated with pre-treatment PTSD symptoms.

3.3. Prognostic and prescriptive effects

3.3.1. ANS markers

An overview of all linear mixed effects models' results is provided in supplementary material 3.

No significant prognostic or prescriptive effects of ANS parameters were observed in men, women using hormonal contraception or cycling women. In women using hormonal contraception, we initially detected a significant main effect of HR on the slope. However, when we performed sensitivity analyses, excluding women who reported acute exercise, caffeine or alcohol intake prior to measurement (n = 2), the effect was no longer significant.

3.3.2. HPA axis markers

No significant prognostic or prescriptive effect of resting cortisol, morning cortisol AUCg or CAR AUCi was found.

In men, the final model for DST AUCg yielded a significant main effect on the intercept. It remained significant when controlling for age and time between trauma and T2 but was no longer significant when taking pre-treatment PTSD symptoms into account. In women using hormonal contraception, we detected significant main effects of DST AUCg on the intercept and slope, indicating that stronger morning cortisol suppression was associated with higher PTSD symptoms at 1.5 months, and subsequently, a stronger symptom decrease from 1.5 to 6 months (see Table 3 and Figure 1). These effects remained significant when controlling for age and time between trauma and T2, and when additionally controlling for pre-treatment PTSD symptoms.

In men, the final model for DST AUCi included a significant prognostic effect on the intercept, indicating that stronger CAR suppression by dexamethasone was associated with lower PTSD symptoms 1.5 months posttrauma (see Table 3 and Figure 2). The effect remained significant when controlling for age and time between trauma and T2 and also when additionally controlling for pre-treatment PTSD symptoms. In women using hormonal contraception, no significant prognostic or prescriptive effect of DST AUCi was found.

3.3.3. Oxytocin

In men and cycling women, we detected no significant prognostic or prescriptive effect of resting oxytocin. In women using hormonal contraception, a significant main effect on the intercept was found, indicating that higher oxytocin concentrations were associated with higher PTSD symptoms at 1.5 months (see Table 3 and Figure 3). The effect remained significant when controlling for age and time between trauma and T2 and when we additionally took pre-treatment PTSD symptoms into account.

4. Discussion

We investigated possible prognostic and prescriptive effects of biological parameters, measured within 12 days posttrauma, in a secondary analysis of an RCT evaluating repeated intranasal oxytocin administration as a preventive intervention for PTSD. Specifically, we investigated effects of HR(V), resting cortisol, morning cortisol and CAR, cortisol suppression by the DST, and resting oxytocin in men, women using hormonal contraception and cycling women. The analyses revealed no prescriptive effects (i.e. no significant interaction effect between the respective biological variables and treatment condition), indicating that the main effect of intranasal oxytocin administration was independent of

Table 3. Exploration of possible prognostic or prescriptive effects of autonomic and endocrine parameters on posttraumatic stress disorder (PTSD) symptoms 1.5 (intercept), 3 and 6 months posttrauma (linear slope).

	Effect on the intercept				Effect on the linear slope			
	b (SEM)	*t*	*p*	95% CI	*b (SEM)*	*t*	*p*	95% CI
Effect of DST AUCg in women using hormonal contraception (n = 11)								
Reference	4.34 (.65)	6.70	**<.01**	3.07; 5.61	−.44 (.09)	−4.75	**<.01**	−.62; −.26
Age	−.11 (.36)	−.31	.76	−.82; .60				
Days between trauma and T2	.63 (.81)	.78	.44	−.96; 2.23				
Pre-treatment PTSD symptoms	.78 (.33)	2.36	**.02**	.13; 1.43				
Treatment	1.92 (.90)	2.13	**.03**	.16; 3.68	−.35 (.18)	−1.90	.06	−.71; .01
DST AUCg	−1.17 (.28)	−4.11	**<.01**	−1.73; −.61	.19 (.09)	2.16	**.03**	.02; .37
Effect of DST AUCi in men (n = 15)								
Reference	4.15 (.33)	11.68	**<.01**	3.45; 4.84	−.16 (.08)	−2.03	**.04**	−.32; −.00
Age	−.04 (.33)	−.12	.90	−.68; .60				
Days between trauma and T2	.13 (.40)	.32	.75	−.66; .92				
Pre-treatment PTSD symptoms	.49 (.33)	1.46	.14	−.17; 1.14				
Treatment	1.04 (.70)	1.49	.14	−.33; 2.41				
DST AUCi	.75 (.34)	2.17	**.03**	.07; 1.42				
Effect of resting oxytocin in women using hormonal contraception (n = 17)								
Reference	4.63 (.35)	13.28	**<.01**	3.95; 5.32	−.43 (.10)	−4.16	**<.01**	−.63; −.23
Age	.37 (.30)	1.24	.22	−.21; .95				
Days between trauma and T2	−.10 (.30)	−.32	.75	−.68; .49				
Pre-treatment PTSD symptoms	.34 (.35)	.96	.34	.36; 1.03				
Treatment	−.26 (.79)	−.34	.74	−1.80; 1.28	−.17 (.21)	−.83	.40	−.58; .23
Resting oxytocin	.87 (.33)	2.67	**.01**	.23; 1.51				

The table summarizes the final models with significant prognostic or prescriptive effects of autonomic or endocrine parameters. Effects of morning cortisol suppression by dexamethasone (DST, area under the curve with respect to the ground, AUCg), cortisol awakening response suppression by DST (area under the curve with respect to increase AUCi) and resting endogenous oxytocin concentrations on post-traumatic stress disorder (PTSD) symptoms over follow-up time points in men and women using hormonal contraception are presented. Age, days between trauma and T2, pre-treatment PTSD symptoms and the endocrine parameters are metric and were *z*-transformed. Treatment condition, a dichotomous variable, was unweighted contrast coded (*−.5* = placebo condition and *.5* = oxytocin condition). Reference indicates the effect of all parameters set at their mean. Bold values indicate significant *p* values.

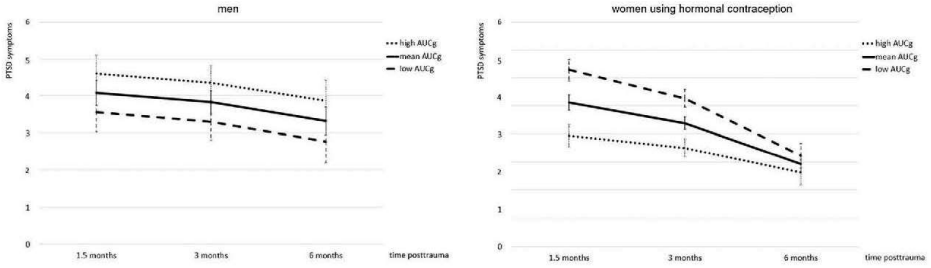


Figure 1. Effect of morning cortisol suppression by dexamethasone (area under the curve with respect to the ground, AUCg) on posttraumatic stress disorder (PTSD) symptoms over follow-up time points in men and women using hormonal contraception. Predicted *M* and *SEM* of square root-transformed total Clinician-Administered PTSD Scale (CAPS) scores at the respective measurement point are presented for participants with high (*M* + 1 *SD*), mean (*M*) and low (*M* − 1SD) AUCg. Lower AUCg values indicate stronger suppression by dexamethasone. i.e. stronger glucocorticoid feedback sensitivity. Predictions are based on the final model for women using hormonal contraception (*n* = 11), as presented in Table 3. The same model was applied to men. In this group, it did not result in significant effects. Predictions are controlled for age, time between traumatic event and T2 as well as pre-treatment PTSD symptoms.

pre-treatment biological states. Also, no prognostic effects of HR, HF-HRV, LF-HRV, resting cortisol, morning cortisol and CAR were found. Yet, the stratified analyses revealed prognostic effects of cortisol suppression and resting oxytocin on PTSD symptom severity that were sex- and hormonal contraception use-dependent. Stronger CAR suppression upon dexamethasone ingestion was associated with lower PTSD symptoms at 1.5 months in men, an effect that remained stable across follow-up. In contrast, in women using hormonal contraception, stronger morning cortisol suppression was associated with higher symptoms at 1.5 months, but also with a stronger subsequent decrease in symptoms from 1.5 to 6 months posttrauma; resulting in no difference in long-term outcome between those with high and low suppression. In women using hormonal contraception, higher oxytocin concentrations were also associated with higher PTSD symptoms at 1.5 months and also across follow-up timepoints.

Previous research has shown that autonomic dysregulation during the acute posttrauma period predicted subsequent PTSD risk (Mellman et al., 2004;

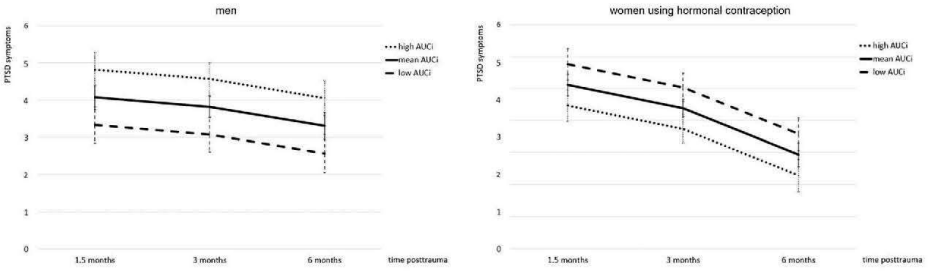


Figure 2. Effect of cortisol awakening response suppression by dexamethasone (area under the curve with respect to the ground, AUCg) on post-traumatic stress disorder (PTSD) symptoms over follow-up time points in men and women using hormonal contraception. Predicted *M* and *SEM* of square root-transformed total Clinician-Administered PTSD Scale (CAPS) scores at the respective measurement point are presented for participants with high (*M* + 1 *SD*), mean (*M*) and low (*M* − 1SD) AUCg. Lower AUCg values indicate stronger suppression by dexamethasone. i.e. stronger glucocorticoid feedback sensitivity. Predictions are based on the final model for men (n = 15), as presented in Table 3. The same model was applied to women using hormonal contraception. In this group, it did not result in significant effects. Predictions are controlled for age, time between traumatic event and T2 as well as pre-treatment PTSD symptoms.

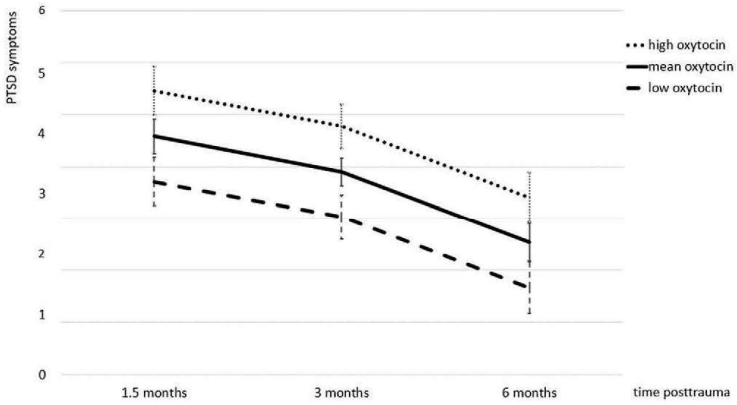


Figure 3. Effect of resting endogenous oxytocin concentrations on post-traumatic stress disorder (PTSD) symptoms over follow-up time points in women using hormonal contraception. Predicted *M* and *SEM* of square root-transformed total Clinician-Administered PTSD Scale (CAPS) scores at the respective measurement point are presented for women with high (*M* + 1 *SD*), mean (*M*) and low (*M* − 1*SD*) oxytocin concentrations. Predictions are based on the final model presented in Table 3, based on n = 17 women and controlled for age, time between traumatic event and T2 as well as pre-treatment PTSD symptoms.

Mikolajewski & Scheeringa, 2018; Minassian et al., 2015; Morris et al., 2016; Pyne et al., 2016; Shaikh Al Arab et al., 2012) We assessed HR(V) parameters during rest within the first 12 days posttrauma and did not observe any prognostic effects. The comparison with previous findings suggests that resting HR(V) measured before or acutely after trauma has prognostic value, whereas this value diminishes over time, supporting the recommendation to apply a stage-dependent approach to investigate biological mechanisms associated with PTSD (McFarlane, Lawrence-Wood, van Hooff, Malhi, & Yehuda, 2017). It also indicates that in the early period posttrauma, prospective information provided by biological assessments may vary on a day-to-day or week-to-week timescale.

We assessed HPA axis activity by means of various complementary parameters. To the best of our knowledge, this was the first study conducted in the early posttrauma period that assessed the dynamics of cortisol output in response to awakening. Concurring with our results, several prospective studies that assessed pretrauma morning cortisol and CAR did not find prognostic effects (Heinrichs et al., 2005; van Zuiden et al., 2011). Our negative finding on resting cortisol seems to be in line with the absence of a significant meta-analytic prognostic effect of cortisol, assessed within 72 hours posttrauma

(Morris et al., 2016), and with several additional studies assessing the prognostic effect of cortisol with higher time intervals since trauma (Bonne et al., 2003; Ehring, Ehlers, Cleare, & Glucksman, 2008; McFarlane et al., 1997; Price et al., 2014; Resnick et al., 1995). However, it was previously reported that while lower cortisol measured within hours posttrauma predicted higher PTSD symptoms 2 weeks and 6 months posttrauma, cortisol concentrations measured just 1 day later did not (Ehring et al., 2008). This observation is interesting as this time window within the first hours posttrauma coincides with the initial phase of acute stress recovery, during which GR-induced negative feedback on the HPA axis occurs. This fits with previously observed prognostic effects of GR sensitivity and function, as assessed pretrauma and 2 days posttrauma (McFarlane et al., 1997; van Zuiden et al., 2012, 2011, 2012, 2013). However, these studies observed that higher pretrauma GR sensitivity and function predicted subsequent PTSD symptoms in male and mixed-gender samples, while in our study, lower GR sensitivity, reflected in decreased cortisol suppression by dexamethasone, within 12 days posttrauma was predictive for higher PTSD symptoms in men. Thus, again, the comparison with previous evidence suggests that time since trauma at the assessment is crucial for prognostic effects.

With regard to the prognostic effects of GR feedback sensitivity detected in our study, one additional aspect seems particularly worth discussing, namely that the detected effects were sex- and hormonal contraception use-specific. As discussed, in men, low GR feedback sensitivity, reflected in higher AUCi after DST, was associated with PTSD symptom development. In contrast, in women using hormonal contraception, high GR feedback sensitivity, reflected in lower AUCg after DST, was a prognostic factor. In men, the prognostic effect of the AUCg was no longer significant after accounting for acute PTSD symptoms before intervention onset, which can be explained by the medium-sized correlation between the two parameters (see supplementary material 2). In women using hormonal contraception, the effect remained significant after controlling for acute symptom severity. The observed differential effects of biological prognostic markers might be explained by participants' gonadal steroid-related statuses, as these regulate the biological stress response systems (Kirschbaum et al., 1999). Specifically, interactions between testosterone, oestrogens and cortisol can be assumed (Ferree & Cahill, 2009; Glover et al., 2012; Milad et al., 2010). Hormonal contraception use is associated with low but stable levels of endogenous oestradiol and elevated levels of exogenous oestrogens, and generally decreases testosterone output (Glover et al., 2012). Based on experimental evidence (Ferree & Cahill, 2009; Ferree, Kamat, & Cahill, 2011; Glover et al., 2012; Milad et al., 2010; Soni, Curran, & Kamboj, 2013), we previously hypothesized that elevated oestrogens concentrations might promote extinction of traumatic memories and thereby prevent PTSD development in women using hormonal contraception (Engel et al., 2019). At the same time, these experimental studies investigated naturally cycling women with different concentrations of endogenous oestradiol. Another study which specifically investigated women using hormonal contraception found that their responses during extinction learning and recall were more comparable to those of naturally cycling women with low concentrations of endogenous oestradiol (Hwang et al., 2015). This implies that the effects of endogenous and exogenous oestrogens differ and it remains an interesting question whether and if so, which precise oestrogens- or testosterone-related biological mechanisms underlie the observed prognostic value of GR sensitivity for PTSD risk in men and women using hormonal contraception. Even though the impact of sex and gonadal steroids has largely been ignored in previous studies, there are indications for its relevance for understanding the biological underpinnings of PTSD development (Kornfield, Hantsoo, & Epperson, 2018). The necessity to consider these factors has also been emphasized within the current sample, as we previously observed that women using hormonal contraception had lower PTSD symptoms at 3 and 6 months posttrauma than men and cycling women, irrespective of treatment (Engel et al., 2019).

As suggested by previous evidence (Stock et al., 1994) and confirmed in our data, women using hormonal contraceptives showed higher oxytocin concentration than men and cycling women. Within this group, higher oxytocin concentrations pretreatment predicted higher symptoms across timepoints, suggesting that relatively high oxytocin concentrations in the early posttrauma period may increase subsequent PTSD risk. In line with this, one of our analyses detected adverse effects of intranasal oxytocin administration in women using hormonal contraception. The treatment effect on symptoms 1.5 months posttrauma was not significant in the main analysis within women using hormonal contraception (Engel et al., 2019). However, it became significant when considering GR feedback sensitivity. Women who used hormonal contraception and were treated with oxytocin, as compared to placebo, reported higher PTSD symptoms at 1.5, but no longer at 3 and 6 months (see Table 3). It needs to be noted that this effect only emerged under the very specific condition of controlling for GR feedback sensitivity, which incidentally was a prognostic factor for increased PTSD symptom severity at 1.5 months post-trauma but not at later time points. Thus, our

findings provide a preliminary indication that intranasal oxytocin administration should be applied cautiously to women using hormonal contraception. Moreover, it further supports the notion that this randomized controlled trial warrants replication with an even more detailed evaluation of sex-specific biological mechanisms underlying recovery from acute trauma.

4.1. Strengths and limitations

Concomitantly assessing a range of parameters reflecting three distinct biological systems involved in the acute stress response allowed us to describe and compare their respective prognostic and prescriptive effects. The time point of assessment within 12 days posttrauma provided insights into their potential role during the phase of early recovery from traumatic stress, specifically. This is important as several dysregulations in biological pathways previously associated with increased PTSD vulnerability are specifically involved in this phase (Bryant, 2003; Zuj, Palmer, Lommen, & Felmingham, 2016). Moreover, the timepoint has practical relevance for the implementation of preventive interventions since patients are often in contact with the health system acutely or early after trauma.

To date, sex and gonadal steroid use have seldomly been adequately accounted for in this field of biomedical research. Neglecting sex and hormonal contraception use can lead to imprecise results, that are valid only for a population subgroup, if at all. Considering these factors can open up options for personalized patient care (Ferretti, Santuccione-Chadha, & Hampel, 2019). Therefore, our statistical analyses were stratified for sex and hormonal contraception use. This allowed us to consider gonadal steroids as possible source of heterogeneity in previous research and responds to the call for more sex- and gender-sensitive biomedical research (Bale & Epperson, 2017; Olff, 2016).

Despite these strengths of this study, some limitations need to be mentioned, as well. The concomitant investigation of different prognostic or prescriptive markers in three different groups could only be implemented with multiple statistical tests. We chose the specific statistical approach as it was developed to balance type I and type II error risks (Fournier et al., 2009). However, due to the large number of statistical tests reported here, the risk of type I error is still increased. Therefore, all analyses we reported are exploratory. Further hypotheses-testing studies could specifically target oxytocin and GR feedback sensitivity, as well as their interactions with gonadal steroids.

In order to specifically target a high-risk group for PTSD, we only included patients that indicated high peritraumatic and immediate distress. Furthermore, in order to specifically investigate the efficacy of oxytocin in preventing PTSD development instead of a potential treatment effect on pre-existing psychiatric symptoms, we excluded participants with current PTSD, depression, psychotic, bipolar, substance-related and personality disorder at the time of traumatic event exposure. Therefore, the interpretation of prognostic effects is limited to this specific population. We cannot exclude the possibility that the biological markers we investigated would be differentially associated with PTSD development within individuals less psychologically distressed in the acute aftermath of a traumatic event or in individuals with previous psychiatric disorders. Therefore, our findings should not only be replicated within a larger, independent, but also within a more diverse sample of traumatized individuals.

All predictions we made in this study were based on methods that enable the identification of risk and protective factors on a group level. However, other methodological approaches requiring larger samples enable more precise estimation of individual risk based on unique characteristics of an individual (Schultebraucks & Galatzer-Levy, 2019). Such machine learning approaches could additionally improve personalized patient care in the future.

We did not detect any effects in cycling women, which might be attributable to their small sample size – the effects of GR feedback sensitivity could not even be tested in this group – or to the remaining heterogeneity within this group due to different cycle phases. It was a specific concern of our study to differentiate effects of sex and hormonal contraception use, in order to consider interactions with gonadal steroids that have previously been suggested (Engel et al., 2019; Kamkwalala et al., 2012; Kirschbaum et al., 1999; Nicolson & Ponnamperuma, 2019; Stock et al., 1994). However, future studies should specifically investigate cycling women and the impact of menstrual cycle phases. In addition, as their small number disabled us to conduct our analyses in menopausal women, more attention should be given to this group, too.

Additionally, total PTSD symptom severity was assessed as outcome. However, biologically mechanisms underlying development and maintenance of specific PTSD symptoms should be investigated in more detail. In this regard, as GR feedback sensitivity and oxytocin impact memory consolidation and retrieval (de Quervain, Schwabe, & Roozendaal, 2017; McEwen, 2004), it seems interesting to investigate their effects with regard to the development of intrusions specifically. However, to refrain from additional statistical testing, we decided not to pursue these analyses here.

Although we preferentially scheduled T2 assessments in the afternoon or early evening, this was

not always possible. As a result, variability in the timing of the resting cortisol and oxytocin assessments occurred. Therefore, we considered timing of the T2 assessment as potential covariate. Additionally, it remains unknown whether variability in timing of the repeated oxytocin administration between and within participants may have influenced the clinical effects of the intervention to some extent. Participants were asked to maintain approximately 12 hours between administrations, but the exact administration times were not standardized. Meta-analytic evidence supports the existence of a diurnal rhythm in endogenous oxytocin (Engel et al., 2019). However, to the best of our knowledge, it was not previously investigated whether timing of day during exogenous oxytocin administration, let alone repeated administration, influences its effects.

4.2. Conclusion

Our study is part of a promising, growing field of study in search of biological predictors of PTSD development and (preventive) treatment response. With the aim of personalizing patient care and precision medicine, one might envision that biological markers could ultimately be used to inform patients about their likelihood to develop PTSD symptoms, to provide them with the preventive treatment they are most likely to benefit from and to prevent them from receiving unnecessary or ineffective treatment. Our exploratory analyses investigated a broad range of biological parameters, enabling a comparative assessment of their use as prognostic or prescriptive markers when assessed within few days posttrauma. We did not detect prescriptive effects but found prognostic effects of GR feedback sensitivity and resting oxytocin that depended on sex and hormonal contraception use and can be followed up by future research.

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Author contributions

All authors have contributed to the manuscript and have approved the present version. Miranda Olff designed the initial concept of the RCT and obtained funding. Mirjam van Zuiden, Jessie Frijling, Saskia Koch, Laura Nawijn and Dick Veltman contributed to the design of the study. Mirjam van Zuiden coordinated data acquisition by Jessie Frijling, Saskia Koch and Laura Nawijn. Sinha Engel and Mirjam van Zuiden conceptualized the current study concerning secondary analyses of the RCT. Sinha Engel performed all data analyses. Jos Bosch oversaw the cortisol assays and aided in critical interpretation of resulting statistical findings. HR(V) data preparation was performed by Rinde Yildiz and Jessie Frijling. Sinha Engel and Mirjam van Zuiden drafted the manuscript. All authors were involved in critical interpretation of results and have approved and added to the final manuscript.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Contributions

Miranda Olff, Mirjam van Zuiden, Jessie L. Frijling, Laura Nawijn, Saskia B. Koch and Dick J. Veltman designed the study.

Miranda Olff obtained the funding.

Mirjam van Zuiden, Jessie L. Frijling, Saskia B. Koch and Laura Nawijn collected the data.

Miranda Olff, J. Carel Goslings, Jan S. Luitse, Tessa H. Biesheuvel and Adriaan Honig provided administrative, technical or material support.

Rinde L. Yilziz and Jessie L. Frijling prepared the heart rate (variability) data.

Jos A. Bosch analyzed the cortisol samples and provided assistance in analysis and interpretation of the data.

Inga D. Neumann analyzed the oxytocin samples and provided assistance in analysis and interpretation of the data.

Sinha Engel and Mirjam van Zuiden wrote the manuscript. All authors critically revised the manuscript for important intellectual content.

Sinha Engel performed the statistical analyses, under Mirjam van Zuiden's supervision.

Miranda Olff, Dick J, Veltman and Mirjam van Zuiden supervised the study.

Online supplementary material

The following supplementary material is available online:

ONLINE SUPPLEMENTARY MATERIAL 3.1. Correlations between autonomic and endocrine parameters and general potential confounders

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CHAPTER 4

Trauma exposure, posttraumatic stress disorder and oxytocin: A meta-analytic investigation of endogenous concentrations and receptor genotype

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Trauma exposure, posttraumatic stress disorder and oxytocin: A meta-analytic investigation of endogenous concentrations and receptor genotype

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Abstract

Oxytocin's stress-reducing and social functions suggest an involvement in trauma processing and posttraumatic stress disorder (PTSD).

We searched *PubMed, PubPsych, PsycINFO, PsycARTICLES, Web of Science, ProQuest* and *ClinicalTrials.gov* for studies assessing endogenous oxytocin, oxytocin receptor genotype or methylation in traumatized humans. Eligible studies (k = 66) were systematically described. We meta-analytically compared oxytocin parameters between traumatized and non-traumatized individuals (k = 17) and individuals with and without PTSD (k = 8), and correlated oxytocin with trauma exposure (k = 16) and PTSD symptoms (k = 8).

Endogenous oxytocin concentrations did not differ between PTSD patients and healthy individuals. The remaining effects on endogenous oxytocin were heterogeneous. Subgroup analyses identified sampling-related, trauma-related and demographic moderators, resulting in inconsistent or non-significant effects. Methylation data were insufficient for meta-analyses, and meta-analytic genotype results were inconsistent.

Unstimulated endogenous oxytocin was not a biomarker for trauma exposure or PTSD. Given the impact of methodology, more basic research on endogenous oxytocin measurements is needed. Future studies might consider the oxytocin stress response and investigate oxytocin longitudinally.

Keywords: Traumatic experience; trauma; oxytocin receptor gene; OXTR; adversity; maltreatment; abuse; systematic review

Registration: We published a protocol of this systematic review and meta-analysis on PROSPERO.

Title: "Traumatic experiences, posttraumatic stress disorder and the oxytocin system: a systematic review and meta-analysis protocol"

Registration number: CRD42018103036

Available online at: https://www.crd.york.ac.uk/PROSPERO/display_record.php?RecordID=103036

4.1 Introduction

4.1.1 Neuroendocrine consequences of trauma exposure and posttraumatic stress disorder (PTSD)

Trauma exposure can have long-term and potentially devastating psychological and physiological consequences. On a psychological level, it increases the risk of mental disorders such as adjustment disorders, anxiety disorders, depression, alcohol abuse and, most paradigmatically, posttraumatic stress disorder (PTSD) (Gradus, 2017). PTSD is associated with enormous individual strain and adverse societal outcomes (Wittchen et al., 2011) and develops in approximately 2 - 19% of traumatized individuals (Atwoli, Stein, Koenen, & McLaughlin, 2015). From a physiological perspective, both trauma exposure and PTSD have been linked to neuroendocrine dysregulations, most frequently with regard to the hypothalamic-pituitary-adrenal (HPA) axis. To date, three meta-analyses reported PTSD-related basal hypocortisolism, but often, effects were only detected in subgroups (Meewisse, Reitsma, Vries, Gersons, & Olff, 2007; Morris, Compas, & Garber, 2012; Schumacher et al., 2019) and were not confirmed by another meta-analysis (Klaassens, Giltay, Cuijpers, van Veen, & Zitman, 2012). This suggests that various, possibly undetected moderators influence PTSD-related HPA axis alterations.

The neuropeptide oxytocin is a well-known moderator of the HPA axis activation in response to stress. It is synthesized in magnocellular neurons of the paraventricular and supraoptic nuclei of the hypothalamus (Brownstein, Russell, & Gainer, 1980; Ma & Morris, 2002), and in smaller neurons of the paraventricular nucleus (Armstrong, Warach, Hatton, & Mcneill, 1980; Sawchenko & Swanson, 1982). Central axonal projections (Knobloch et al., 2012) and diffuse somatodendritic release (Pow & Morris, 1989) target oxytocin receptors (OXTRs) in various brain regions (Gimpl & Fahrenholz, 2001). Moreover, magnocellular hypothalamic neurons project to the posterior pituitary, which releases oxytocin into the bloodstream (Brownstein et al., 1980). The hypothalamic-neurohypophyseal system influences a variety of physical and psychological processes, mediated via peripheral OXTRs (Gimpl & Fahrenholz, 2001). Breastfeeding, which most prominently increases peripheral oxytocin release, has been associated with decreased basal cortisol output (Amico, Johnston, & Vagnucci, 1994) and decreased HPA axis reactivity to physical (Altemus, Deuster, Galliven, Carter, & Gold, 1995) and social stressors (Heinrichs et al., 2001). Central oxytocin injection in rats led to decreased corticosterone stress

responses (Windle, Shanks, Lightman, & Ingram, 1997). In healthy humans, intranasal oxytocin administration led to decreased cortisol stress responses (Ditzen et al., 2009; Heinrichs, Baumgartner, Kirschbaum, & Ehlert, 2003). Interestingly, oxytocin was found to dampen HPA axis reactivity to social stress in individuals exposed to childhood trauma, whereas the cortisol responses of non-exposed individuals were unaffected by oxytocin (Grimm et al., 2014).

As oxytocin additionally regulates a wide range of social behaviors (Feldman, 2012; Heinrichs, Dawans, & Domes, 2009; MacDonald & MacDonald, 2010), it is often referred to as pro-social hormone - even though it needs to be noted that, depending on context factors and interindividual differences, oxytocin can also exert opposite effects (Olff et al., 2013). One particularly relevant social effect of oxytocin is related to social support, a key protective factor against PTSD development after trauma exposure (Brewin, Andrews, & Valentine, 2000). Social support has been shown to evoke oxytocin release (Grewen, Girdler, Amico, & Light, 2005) and oxytocin has been discussed as underlying biological factor mediating the health promoting effects of social support. (Heinrichs et al., 2003)

Thus, it does not seem surprising that intranasal oxytocin administration has also been evaluated as therapeutic intervention for PTSD (Pitman, Orr, & Lasko, 1993; Yatzkar & Klein, 2010) and PTSD symptom development (van Zuiden et al., 2017). However, it remains unclear whether the endogenous oxytocin system itself is affected by trauma exposure or interacts with PTSD development. Although empirical studies have already addressed this question, to date, these data have not been systematically and statistically summarized. Therefore, we provide the first systematic review and meta-analysis on the interactions between trauma exposure, PTSD, and parameters of the oxytocin system currently established in human research, that is, endogenous oxytocin concentrations, OXTR genotype, and gene expression by means of OXTR DNA methylation.

4.1.2 Associations between trauma exposure, PTSD and oxytocin

Oxytocin might be involved at different stages of trauma processing. Based on the possible courses of resilience and dysfunction upon trauma exposure (Galatzer-Levy, Huang, & Bonanno, 2018), we created an overview of all interactions between trauma processing, PTSD and oxytocin, which we considered

theoretically conceivable. This overview is illustrated in FIGURE 4.1 and served as the theoretical rationale for the present study.

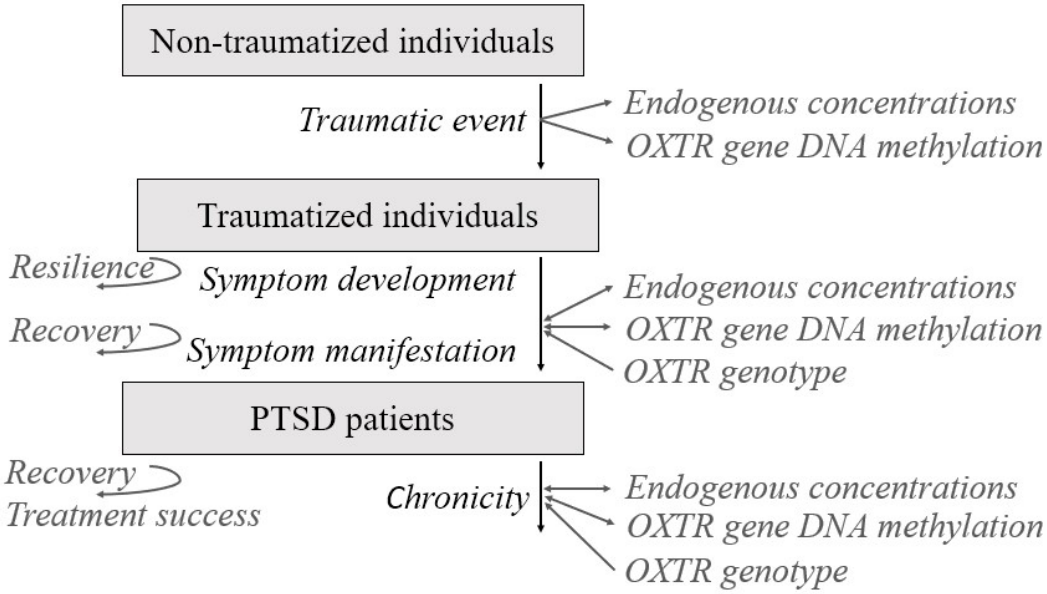

FIGURE 4.1. Possible interactions between traumatic event exposure, posttraumatic stress disorder (PTSD) and oxytocin parameters that were of interest for the present systematic review and meta-analysis. OXTR = oxytocin receptor gene.

As trauma exposure challenges biological systems regulating physiological homeostasis, such as the HPA axis (Yehuda & LeDoux, 2007), we hypothesized that it might impact endogenous oxytocin concentrations and OXTR methylation, as well.

Furthermore, increased oxytocin system activity, as reflected in endogenous oxytocin concentrations, OXTR genotype and methylation parameters, might impact PTSD pathogenesis. In this regard, two mechanisms of action have been proposed: First, an oxytocin-related reduction of fear reactivity, as oxytocin regulates the amygdala and neuroendocrine stress response and promotes extinction learning, and second, an oxytocin-related increase of social functions, reflected in reward processing on a neural and health-promoting social interactions on a behavioral level (Olff, Langeland, Witteveen, & Denys,

2010). In turn, PTSD symptom development, manifestation and chronicity might impact endogenous oxytocin concentrations and OXTR methylation over time. Dysregulations of the oxytocin system have already been documented in different mental disorders, particularly autism spectrum disorder, schizophrenia and anxiety disorders (Cochran, Fallon, Hill, & Frazier, 2013) and shared characteristics of social deficits and inadequate fear processing suggest that these findings are transferable to PTSD.

The associations between oxytocin parameters and PTSD at different stages of pathogenesis depend on different moderators, a central one being sex. Sex moderates PTSD risk, with a higher proportion of women developing PTSD after trauma exposure (Atwoli et al., 2015; Brewin et al., 2000), and PTSD remission, with a more favorable prognosis for women to respond to trauma-focused treatment (Wade et al., 2016). These differences can be explained by biological factors, especially female gonadal steroids (Kornfield, Hantsoo, & Epperson, 2018). Interactions between female gonadal steroids and oxytocin are well-documented, as oxytocin concentrations are influenced by sex (Engel et al., 2019), menstrual cycle phase (Engel, Klusmann, Ditzen, Knaevelsrud, & Schumacher, 2018) and hormonal contraception use (Stock, Karlsson, & Schoultz, 1994). Consequently, we systematically collected information on these female-specific aspects and considered sex as moderator in the present study.

4.1.3 Objectives

This systematic review and meta-analysis summarizes evidence on the associations between trauma exposure, PTSD and oxytocin. We investigated endogenous oxytocin concentrations, OXTR genotype and methylation. First, we systematically described all primary studies, focusing on their design, quality, and the timing of traumatic events and of oxytocin and psychological assessment. Second, we meta-analytically compared oxytocin parameters between traumatized and non-traumatized individuals and between individuals with and without PTSD. Third, we meta-analytically correlated oxytocin parameters with extent of trauma exposure and PTSD symptom severity.

4.2 Methods

4.2.1 Protocol and registration

This study was pre-registered on PROSPERO (Registration number: CRD42018103036) on the 27th July 2018, available online at: https://www.crd.york.ac.uk/PROSPERO/display_record.php?RecordID=103036.

4.2.2 Eligibility criteria

As recommended by the PRISMA group (Moher, Liberati, Tetzlaff, Altman, & PRISMA Group, 2009), we defined our eligibility criteria according to the PICOS framework: We included primary studies investigating (sub-)samples of traumatized humans of any age or health status. Studies were eligible if they compared traumatized individuals with non-traumatized individuals or individuals with and without PTSD, but studies without a comparison group were also considered. Individuals were classified as traumatized if they had been exposed to at least one life event described as traumatic in the primary study. We also considered broader definitions of childhood adversity; therefore, the definitions of traumatic events could range from childhood neglect to serious life threats which completely fulfill the criteria of a classification system such as DSM or ICD. Individuals were classified as non-traumatized if they had not been exposed to the respective trauma under study. This does not necessarily exclude the possibility that they might have been exposed to other traumatic events other than those investigated in the primary study. Individuals were classified as PTSD patients if they fulfilled diagnostic criteria for PTSD, but also if they reported subclinical PTSD symptoms, as indicated by questionnaire or interview data. Individuals without PTSD did not fulfill diagnostic criteria or did not indicate subclinical symptoms. As outcomes, we defined differences in endogenous oxytocin, OXTR genotype or methylation between traumatized individuals, PTSD patients and their respective comparison groups as well as correlations between oxytocin parameters and trauma exposure or PTSD symptoms. Concerning study designs, we included between- within- and single-group designs but excluded case studies and reviews. The evaluation of a pharmacological or psychological intervention was neither defined as an

inclusion, nor an exclusion criterion. Published and unpublished studies in the English and German language were eligible. There were no restrictions concerning publication year.

4.2.3 Search strategy

Our search strategy was based on the recommendations by Lipsey and Wilson (2001). We systematically searched *PubMed PubPsych, PsycINFO, PsycARTICLES, Web of Science, ProQuest* and *ClinicalTrials.gov* up to 19th November 2017. The full electronic search strategy is presented in the ONLINE SUPPLEMENTAL MATERIAL 4.1. In addition, we searched the grey literature by examining conference abstracts and contacting experts in the field. Snowball search methods were applied by screening reference lists of review articles and primary studies included in this systematic review. The literature search was performed by a trained researcher (BW) and supervised by a second researcher (SE).

4.2.4 Study Selection

First, titles and abstracts were screened, excluding duplicates and studies meeting our exclusion criteria (FIGURE 4.2). Subsequently, we screened full-text articles to select eligible studies. If abstracts or full texts were not available, we contacted the corresponding authors requesting access to the article. Since we also screened study registers, we asked the corresponding authors for accessible publications or data. All studies meeting our eligibility criteria were included in the systematic review and those providing sufficient data were also included in the meta-analytic procedures. Study selection was performed by two independent trained researchers (SI, BW) and supervised by a third researcher (SE), who made the final decision in the case of discrepancies.

4.2.5 Data extraction

Sample characteristics and sizes, sex distributions and comparison groups were extracted. Furthermore, we extracted whether the comparison groups were matched for covariates. We also extracted age at the time of the traumatic event and at the time of oxytocin measurements and psychometric assessments. Trauma characteristics, frequency and assessment scales, as well as PTSD assessment scales, symptom

severity and symptom duration were extracted. We also extracted study designs and information about possibly evaluated interventions. General study quality was rated using the Newcastle-Ottawa Quality Assessment Scale (Wells et al.) for cohort or case-control studies.

Concerning endogenous oxytocin concentrations, we extracted M, SD, unit, specimen, assay, extraction and whether stimulated or unstimulated oxytocin concentrations were assessed. We previously showed that fluctuations in female sex hormones (Engel et al., 2018) and other physiological factors (Engel et al., 2019) impact endogenous oxytocin concentrations. Therefore, we extracted whether time of day, acute smoking, habitual smoking, body mass index, and for women, fertility, hormonal contraception use and menstrual cycle phase were reported or considered as covariates in the primary studies. Concerning OXTR genotype and methylation, we extracted ethnicity, SNP and number of minor allele carriers, as well as genomic coordinates of sites and methylation frequency (M, SD). Moreover, we extracted correlations (*r*) between oxytocin parameters and trauma exposure or PTSD severity. If M and SD were not directly reported, they were estimated (Hozo, Djulbegovic, & Hozo, 2005). If data were not reported in texts or tables but were extractable from figures, we used an online plot digitizer (Rohatgi, 2015). If data necessary for the meta-analytic procedure were unavailable, we asked the corresponding authors to provide the respective information.

Information was extracted in duplicate and independently by two researchers (AS and SE), using a predefined form. In the case of extraction discrepancies, a third researcher (SSch) made the final decision. Study quality rating was performed in duplicate and independently by two researchers (HK and SL). In the case of rating discrepancies, a third independent researcher (SE) made the final decision.

4.2.6 Meta-analyses

We used Hedges' *g* to compare groups with regard to continuous parameters and risk ratios to compare frequencies (Borenstein, Hedges, Higgins, & Rothstein, 2009). Coefficient *r* was used for correlations (Borenstein et al., 2009). Heterogeneity was determined by I^2 and *Q* (Borenstein et al., 2009). Effect sizes with significant *Q* statistics ($p < .05$) and I^2 values of 25, 50, and 75 were interpreted as indicating low, moderate and high heterogeneity, respectively (Higgins, Thompson, Deeks, & Altman, 2003). In

the case of homogeneous effects based on at least six primary studies, we tested for publication bias, applying Egger's regression test (Egger, Davey Smith, Schneider, & Minder, 1997) and the trim-and-fill procedure (Duval & Tweedie, 2000). For heterogeneous effects, we performed subgroup analyses (Borenstein et al., 2009), considering the following moderators: age at traumatic event, assessment scale, use of a restrictive traumatic event criterion (actual or threatened death, serious injury or sexual violence), type of traumatic event (interpersonal vs. accidental vs. war-related), frequency of trauma exposure (single vs. repeated), age at oxytocin assessment, sex, health status (clinical for individuals with a physical or mental disorder vs. non-clinical for healthy individuals), specific physical diseases or mental disorders, recruitment setting (community vs. hospital vs. military or police), PTSD severity (clinical diagnosis vs. subclinical symptoms), PTSD control group type (traumatized vs. non-traumatized), and for endogenous oxytocin also specimen and extraction. Subgroup analyses were performed if at least two primary studies were available per subgroup. All analyses were conducted under the random-effects model, using the software Comprehensive Meta-Analysis (Biostat, 2014).

4.3 Results

4.3.1 Study selection

The results of study selection are presented in FIGURE 4.2. Sixty-six studies met the inclusion criteria for the qualitative analysis. Of these, 41 measured endogenous oxytocin, 25 assessed OXTR genotype and four assessed methylation. Within these, two studies assessed both endogenous concentrations and OXTR genotype and two studies assessed both OXTR genotype and methylation. The reference list of included studies is presented in the ONLINE SUPPLEMENTARY MATERIAL 4.2.

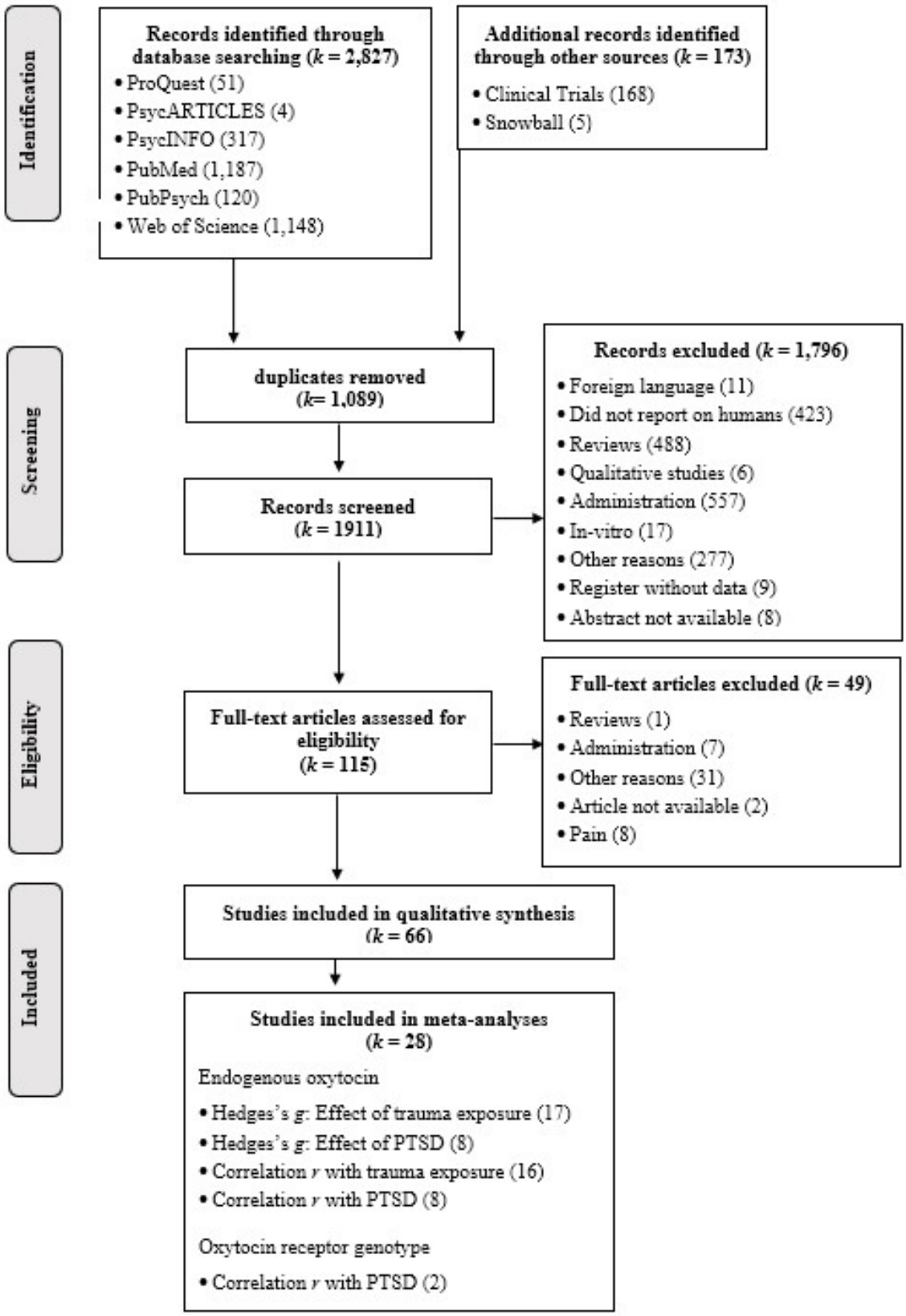

FIGURE 4.2. Flow diagram of identification and selection of primary studies. Due to overlaps, the numbers of studies included per meta-analysis do not add up to the total number of studies included in meta-analyses. PTSD = posttraumatic stress disorder.

4.3.2 Participant and study characteristics

In TABLE 4.1, studies are described with regard to participants and trauma characteristics. Numbers of eligible participants ranged from 9 (Garfield, 2012) to 907 (Reijnen, Geuze, & Vermetten, 2017) in studies investigating endogenous oxytocin concentrations, from 40 (Eidelman-Rothman et al., 2014) to 7,723 (Connelly et al., 2014) in studies investigating OXTR genotype and from 46 (Gouin et al., 2017) to 393 (Smearman et al., 2016) in studies investigating OXTR methylation. Across oxytocin parameters, k = 1 study investigated exclusively traumatized participants. Traumatized and non-traumatized individuals were compared in k = 41 studies, PTSD patients and traumatized controls were compared in k = 6 studies, and traumatized individuals, non-traumatized individuals and PTSD patients were compared in k = 18 studies. Altogether, k = 65 studies compared groups with regard to trauma exposure or PTSD. Of those, k = 57 did not match comparison groups for covariates. Accordingly, k = 8 studies matched comparison groups. Covariates that studies matched for were age (k = 8), education (k = 4), body mass index, employment, marital status and birth order (k = 2, respectively), as well as professional experience and income (k = 1, respectively). Only k = 5 studies matched comparison groups for sex or gender, but of those k = 60 studies not matching for sex or gender, k = 27 were single-sex samples, excluding the impact of sex as a covariate. Altogether, k = 21 studies investigated all-female, k = 32 mixed-sex, and k = 6 all-male samples. Sex of participants was not reported in k = 7 studies. Between, but partly also within studies, there was considerable variability with regard to the kind of traumatic event. Trauma frequency was seldomly (k = 10) and PTSD duration was never reported. In k = 11 studies, clinical PTSD, in k = 12 studies, subclinical PTSD, and in k = 1 study, clinical and subclinical PTSD were investigated. The most frequently used scale to assess trauma exposure was the Childhood Trauma Questionnaire (Bernstein et al., 2003; Bernstein & Fink, 1998). Altogether, 29 different scales were used to assess trauma exposure and in k = 15 studies, the scale was not reported. PTSD was most frequently assessed by the PTSD Checklist (Weathers et al., 2013; Weathers, Litz, Herman, Huska, & Keane, 1993), but altogether, 17 different instruments were used for PTSD assessments while in k = 5 studies, the assessment scale was not reported.

TABLE 4.2 provides an overview of timing of traumatic events and of assessments of oxytocin, trauma exposure and PTSD. Most studies investigated trauma exposure in childhood (k = 66). Some studies investigated trauma exposure in adulthood (k = 18), and a small number of studies did not report timing of trauma exposure (k = 6). Most studies assessed oxytocin (k = 55), trauma exposure (k = 55), and PTSD (k = 20) in adulthood. Consequently, trauma exposure was most frequently assessed retrospectively, and many years passed between traumatic events and assessments of oxytocin and of PTSD. In k = 10 studies, oxytocin was measured in children and k = 2 studies did not report timing of oxytocin measurement. Equally, in k = 10 studies, trauma exposure was assessed in childhood and k = 2 studies did not report timing of trauma assessment. In k = 3 studies, PTSD assessment took place in childhood and k = 1 study did not report age at PTSD assessment.

4.3.3 Endogenous oxytocin concentrations, OXTR genotype and methylation

Details on endogenous oxytocin and OXTR genotype assessments, including NOS (Wells et al.) ratings and databases for the meta-analyses, are provided in TABLES 4.3 and 4.4. Concerning those studies that investigated endogenous oxytocin concentrations, k = 35 had a cross-sectional design. In k = 6 studies, traumatic event exposure, PTSD or oxytocin were assessed longitudinally. There was only k = 1 intervention study, evaluating a group drumming intervention. In k = 25 studies, no challenge or reactivity design was applied, while k = 16 studies applied one. Most frequently, oxytocin reactivity to mother-infant interactions (k = 3) or social stress (k = 3) was investigated. As all challenge or reactivity studies reported unstimulated endogenous oxytocin concentrations at baseline, this parameter was used for the present meta-analyses. Oxytocin was measured in extracted blood (k = 8), unextracted blood (k = 3), or blood (without available information about extraction, k = 17). It was measured in extracted saliva (k = 4), or saliva (without information about extraction, k = 5). It was measured in extracted urine samples (k = 3). In k = 1 study, oxytocin was measured in urine, but it was not reported whether extraction was performed. Moreover, oxytocin was measured in unextracted cerebrospinal fluid in k = 1 study. In k = 2 studies, oxytocin was measured in cerebrospinal fluid, but information about extraction was not available. Oxytocin was most frequently analyzed by enzyme immunoassays (k = 23), followed by radioimmunoassays (k = 12). In k = 6 studies, no information about the assay type was given. With

regard to confounders, we extracted whether certain pre-defined variables of interest were considered. As there are currently no guidelines available, we decided that consideration could imply that confounders were standardized, statistically controlled for as covariates or by means of sensitivity analyses. Time of day was considered in $k = 27$ studies. Hormonal contraception was considered in $k = 15$, female fertility status in $k = 14$, and menstrual cycle phase in $k = 15$ studies. Study quality ratings based on the NOS (Wells et al.) ranged from 3 to 9, with M = 5.87 (SD = 1.73) stars awarded

TABLE 4.1. Description of traumatized individuals, posttraumatic stress disorder (PTSD) patients and comparison groups.

Study	Group	*n* (oxytocin)	% ♀ (oxytocin)	Sample description	Matching of comparison groups	Trauma characteristics	Trauma assessment scale	Trauma frequency	PTSD assessment scale	PTSD severity
Studies investigating endogenous oxytocin										
*Bertsch et al. (2013)	TE	N/A	100.0	Borderline personality disorder patients and controls with traumatic childhood experiences	Not matched	Childhood physical, sexual, and emotional abuse, physical and emotional neglect	CTQ	N/A	N/A	Clinical
	NT	N/A	100.00	Borderline personality disorder patients and controls without traumatic childhood experiences						
	PTSD	11	100.00	Borderline personality disorder patients with comorbid PTSD						
	Total	74	100.00							
*Bhandari et al. (2014)	TE	N/A	100.00	Students with childhood emotional maltreatment	Not matched	Childhood emotional maltreatment	CTQ-SF	N/A	( )	( )
	NT	N/A	100.00	Students without childhood emotional maltreatment						
	Total	102	100.00							
*#Bizik et al. (2012)	TE	N/A	N/A	Patients with depression who experienced a traumatic event	Not matched	Traumatic event	N/A	N/A	TSC-40	Subclinical
	NT	N/A	N/A	Patients with depression who did not experience a traumatic event						
	PTSD	N/A	N/A	Patients with depression and trauma-related symptoms						
	Total	69	N/A							
#+Böck et al. (2016)	TE	15	100.00	Mothers considered as positive for the experience of a childhood trauma	Matched for age and body mass index	Childhood physical, sexual, and emotional abuse, physical and emotional neglect	CTQ inter-view version	N/A	( )	( )
	NT	15	100.00	Mothers considered as negative for the experience of a childhood trauma						

Study	Group	*n* (oxytocin)	% ♀ (oxytocin)	Sample description	Matching of comparison groups	Trauma characteristics	Trauma assessment scale	Trauma frequency	PTSD assessment scale	PTSD severity
*+Böck et al. (2017)	TE	18	100.00	Mothers considered as positive for the experience of a childhood trauma	Matched for age and body mass index	Childhood physical, sexual, and emotional abuse, physical and emotional neglect	CTQ inter-view version	N/A	( )	( )
	NT	31	100.00	Mothers considered as negative for the experience of a childhood trauma						
Bomann et al. (2017)	TE	N/A	100.00	Borderline personality disorder patients and controls with traumatic childhood experiences	Not matched	Childhood physical, sexual, and emotional abuse, physical and emotional neglect	CTQ	N/A	MINI	Clinical
	NT	N/A	100.00	Borderline personality disorder patients and controls without traumatic childhood experiences						
	PTSD	3	100.00	Borderline personality disorder patients with comorbid PTSD						
	Total	38	100.00							
#Bradley (2012)	TE	N/A	N/A	Highly traumatized adults without PTSD symptoms	Not matched	Repeated interpersonal trauma, childhood maltreatment	N/A	N/A	N/A	Subclinical
	PTSD	N/A	N/A	Highly traumatized adults with PTSD symptoms						
	Total	90	N/A							
#Cao et al. (2014)	TE	N/A	0.00	Adults who suffered from deadly 2008 Wenchuan earthquake without PTSD symptoms	Not matched	Deadly 2008 Wenchuan earthquake	N/A	1.00	PCL-5	Subclinical
	PTSD	N/A	0.00	Adults who suffered from deadly 2008 Wenchuan earthquake with PTSD symptoms						
	Total	106	0.00							

Study	Group	*n* (oxytocin)	% ♀ (oxytocin)	Sample description	Matching of comparison groups	Trauma characteristics	Trauma assessment scale	Trauma frequency	PTSD assessment scale	PTSD severity
*Chatzittofis et al. (2014)	TE	8 (7-8)	62.50	Revictimized suicide attempters with higher total lifetime trauma exposure and more negative childhood emotional climate	Not matched	Exposure to violence and expressed violent behavior in childhood and during adult life, negative childhood emotional climate	KIVS, KSP socialization subscale	N/A	( )	( )
	NT	15 (13-14)	20.00	Non-revictimized suicide attempters with lower total lifetime trauma exposure and more positive childhood emotional climate						
*Crowley et al. (2015)	TE	15	100.00	Women with menstrually related mood disorder and early life sexual abuse	Not matched	Early life sexual abuse	Standard interview	N/A	MINI	Clinical
	NT	39	100.00	Women with menstrually related mood disorder and without early life sexual abuse						
	PTSD1	5	100.00	Women with menstrually related mood disorder, early life sexual abuse and PTSD history						
	PTSD2	1	100.00	Woman with menstrually related mood disorder, without early life sexual abuse and with PTSD history		Traumatic event	N/A	N/A		
#+Eidelman-Rothman et al. (2014)	TE	15	N/A	Trauma exposed war veterans	Not matched	Traumatic event	N/A	N/A	N/A	Clinical
	NT	14	N/A	Controls						
	PTSD	11	N/A	Trauma exposed war veterans with PTSD						
*+Eidelman-Rothman et al. (2015)	TE	17 (14-15)	0.00	Veterans exposed to a combat related trauma	Matched for age and education	Active battle that involved life endangerment, and witnessed injury or death of a comrade	PDS	N/A	SCID-I	Clinical
	NT	15 (14-15)	0.00	Trauma unexposed controls from non-combat units						
	PTSD	11 (9-10)	0.00	Veterans exposed to a combat related trauma with PTSD						

Study	Group	*n* (oxytocin)	% ♀ (oxytocin)	Sample description	Matching of comparison groups	Trauma characteristics	Trauma assessment scale	Trauma frequency	PTSD assessment scale	PTSD severity
*Frijling et al. (2015)	TE	40 (39)	50.00	Trauma exposed police officers without post-traumatic stress disorder	Matched for sex, age, professional experience and education	Traumatic event according to the DSMIV A1 criterion	ETISR-SF, PLES	N/A	CAPS	Clinical
	PTSD	40 (37)	47.40	Trauma exposed police officers with post-traumatic stress disorder						
*Garfield (2012)	TE	N/A	100.00	Postpartum low-income mothers without risk for PTSD	Not matched	Birth	N/A	N/A	PPQ	Subclinical
	PTSD	N/A	100.00	Postpartum low-income mothers without risk for PTSD						
	Total	9 (8)	100.00							
*Gerra et al. (2017)	TE	N/A	N/A	Abstinent heroin patients and healthy volunteers with childhood neglect	Not matched	Parental neglect, parental antipathy, parental physical abuse, sexual abuse	CECA-Q	N/A	( )	( )
	NT	N/A	N/A	Abstinent heroin patients and healthy volunteers without childhood neglect						
	Total	36	N/A							
#Gonzalez et al. (2015)	TE	N/A	100.00	Postpartum women with childhood trauma	Not matched	Childhood physical, sexual, and emotional abuse, physical and emotional neglect	CTQ	N/A	( )	( )
	NT	N/A	100.00	Postpartum women without childhood trauma						
	Total	26	100.00							
*Heim et al. (2009)	TE	14	100.00	Women with childhood trauma	Not matched	Childhood physical, sexual, and emotional abuse, physical and emotional neglect	CTQ	N/A	( )	( )
	NT	8	100.00	Women without childhood trauma						
+Jobst et al. (2014)	TE	N/A	100.00	Borderline personality disorder patients and healthy controls with childhood trauma	Not matched	Childhood physical, sexual, and emotional abuse, physical and emotional neglect	CTQ	N/A	( )	( )
	NT	N/A	100.00	Borderline personality disorder patients and healthy controls with childhood trauma						
	Total	43	100.00							

Study	Group	*n* (oxytocin)	% ♀ (oxytocin)	Sample description	Matching of comparison groups	Trauma characteristics	Trauma assessment scale	Trauma frequency	PTSD assessment scale	PTSD severity
Jobst et al. (2015)	TE	N/A	N/A	Chronic depression patients and controls with childhood trauma	Not matched	Childhood physical, sexual, and emotional abuse, physical and emotional neglect	CTQ-SF	N/A	( )	( )
	NT	N/A	N/A	Chronic depression patients and controls without childhood trauma						
	Total	42 (38)	28.57 (31.58)							
*+Jobst et al. (2016)	TE	N/A	100.00	Borderline personality disorder patients and healthy controls with childhood trauma	Not matched	Childhood physical, sexual, and emotional abuse, physical and emotional neglect	CTQ	N/A	( )	( )
	NT	N/A	100.00	Borderline personality disorder patients and healthy controls without childhood trauma						
	Total	41 (36)	100.00							
#Jokinen et al. (2013)	TE	N/A	N/A	Suicide attempters and healthy controls with exposure to interpersonal violence	Not matched	Interpersonal violence	KIVS	N/A	( )	( )
	NT	N/A	N/A	Suicide attempters and healthy controls without exposure to interpersonal violence						
	Total	47	N/A							
#Krause et al. (2017)	TE	N/A	100.00	Postpartum mothers with childhood trauma	Not matched	Childhood physical, sexual, and emotional abuse, physical and emotional neglect	CTQ	N/A	( )	( )
	NT	N/A	100.00	Postpartum mothers with childhood trauma						
	Total	44	100.00							
*#Marshall (2012)	TE	<12	N/A	Traumatized participants from heterosexual couples	Not matched	Traumatic event	N/A	N/A	CAPS	Subclinical
	PTSD	≥12	N/A	Participants from heterosexual couples with elevated PTSD symptoms						
	Total	24	50.00							

Study	Group	*n* (oxytocin)	% ♀ (oxytocin)	Sample description	Matching of comparison groups	Trauma characteristics	Trauma assessment scale	Trauma frequency	PTSD assessment scale	PTSD severity
*Mielke et al. (2018)	TE	31	100.00	Women with early life maltreatment	Not matched	Physical or sexual abuse before the age of 18 years	CECA	N/A	N/A	Clinical
	NT	25	100.00	Women without early life maltreatment						
	PTSD	2	100.00	Women with early life maltreatment and PTSD						
*Mizuki & Fujiwara (2015)	TE	46	N/A	Parents with less severe forms of childhood trauma	Not matched	Childhood physical, sexual, and emotional abuse, physical and emotional neglect	CTQ	N/A	( )	( )
	NT	34	N/A	Parents without childhood trauma						
	Total	80	61.25							
Mizushima et al. (2015)	TE	17	N/A	Maltreated children	Matched for gender and age	Childhood physical, sexual, and emotional abuse, neglect	N/A	N/A	UPID, IES-R, TSSC - interview versions	Clinical and subclinical
	NT	26	53.58	Typically developing children						
	PTSD	21	N/A	Maltreated children with mild to moderate PTSD symptoms (n = 18) or full PTSD (n = 3)						
	Total	64	51.56							
#Mohiyeddini & Opacka-Juffry (2016)	TE	N/A	0.00	Heavy drinkers and controls with childhood trauma	Not matched	Childhood physical, sexual, and emotional abuse, physical and emotional neglect	CTQ	N/A	( )	( )
	NT	N/A	0.00	Heavy drinkers and controls without childhood trauma						
	Total	54	0.00							
*Munro et al. (2013)	TE	4	100.00	Childless, nonpregnant college students with a history of child abuse	Not matched	Childhood physical neglect, emotional, physical and sexual abuse, adulthood trauma, e.g. abortion, reported accident, sudden death of a loved one	LSC	2.90	PTSD-PCL	Subclinical
	NT	11	100.00	Childless, nonpregnant college students without history of child abuse						
	Non-PTSD	11		Childless, nonpregnant college students without probable PTSD						
	PTSD	4	100.00	Childless, nonpregnant college students with probable PTSD						

Study	Group	*n* (oxytocin)	% ♀ (oxytocin)	Sample description	Matching of comparison groups	Trauma characteristics	Trauma assessment scale	Trauma frequency	PTSD assessment scale	PTSD severity
*Nishi et al. (2015)	TE	N/A (121)	N/A	Motor vehicle accident survivors without PTSD symptoms	Not matched	Motor vehicle-related severe physical injury causing a life-threatening or critical condition	Emergency department admission	1.00	IES-R	Subclinical
	PTSD	N/A (7)	N/A	Motor vehicle accident survivors with PTSD symptoms						
	Total	235 (128-156)	21.70							
Opacka-Juffry & Mohiyeddini (2012)	TE	N/A	0.00	Adults with stressful experiences in childhood	Not matched	Stressful life experiences considered as uncontrollable or fateful	ELSI	N/A	( )	( )
	NT	N/A	0.00	Adults without stressful experiences in childhood						
	Total	90	0.00							
*+Pierrehumbert et al. (2010)	TE1	26	100.00	Women with sexual abuse in childhood or adolescence	Not matched	Sexual abuse in childhood	N/A	N/A	( )	( )
	TE2	25	52.00	Adult survivors of cancer in childhood		Cancer in childhood				
	NT	29	55.17	Adults without sexual abuse or cancer in childhood						
+Pierrehumbert et al. (2012)	TE1	23	100.00	Women with sexual abuse in childhood	Not matched	Sexual abuse in childhood or adolescence	N/A	N/A	( )	( )
	TE2	23	52.17	Adult survivors of cancer in childhood		Cancer in childhood				
	NT	28	57.14	Adults without sexual abuse or cancer in childhood						

Study	Group	*n* (oxytocin)	% ♀ (oxytocin)	Sample description	Matching of comparison groups	Trauma characteristics	Trauma assessment scale	Trauma frequency	PTSD assessment scale	PTSD severity
*Reijnen et al. (2017)	TE1	675	0.00	Soldiers with childhood trauma	Not matched	Early life trauma	ETISR-SF	N/A	SRIP	Subclinical
	TE2	549	0.00	Soldiers who reported combat-related stressors 1 month post deployment		Combat-related stressor, related to 4 months Afghanistan deployment	DES			
	NT1	114	0.00	Soldiers without childhood trauma						
	NT2	62	0.00	Soldiers who reported no combat-related stressors 1 month post deployment						
	TENT	567	0.00	Soldiers with low or without PTSD symptoms 6 months post deployment						
	PTSD	57	0.00	Soldiers with high PTSD symptoms 6 months post deployment						
	Total	907	0.00							
*Riem et al. (2017)	TE	20	0.00	Adults with high childhood emotional maltreatment	Not matched	Childhood emotional maltreatment, composite score of emotional abuse and emotional neglect	CTQ-SF	N/A	( )	( )
	NT	29	0.00	Adults with low childhood emotional maltreatment						
	Total	49	0.00							
*Scott (2017)	TE1	38	N/A	Undergraduate students with childhood trauma	Not matched	Childhood physical, sexual, and emotional abuse, physical and emotional neglect	CTQ	N/A	N/A	Clinical
	TE2	36	N/A	Offenders with childhood trauma						
	NT1	5	N/A	Undergraduate students without childhood trauma						
	NT2	15	N/A	Offenders without childhood trauma						
	PTSD	2	N/A	Offenders with PTSD						
	Total	101 (96)	38.61							
*Seltzer et al. (2014)	TE	37	56.76	Children with verified physical abuse	Not matched	Physical abuse	CTS, court and Child Protective Service records	N/A	( )	( )
	NT	36	50.00	Typically-developing children						

Study	Group	*n* (oxytocin)	% ♀ (oxytocin)	Sample description	Matching of comparison groups	Trauma characteristics	Trauma assessment scale	Trauma frequency	PTSD assessment scale	PTSD severity
*Seng et al. (2013)	TE	7	100.00	Pregnant women with childhood maltreatment	Not matched	Childhood abuse, adulthood abuse, lifetime trauma exposure	LSC	N/A	National Women's Study PTSD Module	Clinical
	NT	18	100.00	Pregnant women without childhood maltreatment						
	Non-PTSD	15	100.00	Pregnant women with or without childhood trauma, without PTSD						
	PTSD	10	100.00	Pregnant women with or without childhood trauma, with PTSD						
*Ulmer-Yaniv et al. (2017)	TE1	66	N/A	Children exposed to continuous wartime trauma	Matched for age, gender, birth order, parental education, maternal employment and marital status	Unpredictable, continuous rocket attacks	N/A	Mulitple	DAWBA	Clinical
	TE2	101	100.00	Mothers exposed to continuous wartime trauma						
	NT1	76	N/A	Non-exposed children from comparable towns						
	NT2	76	100.00	Non-exposed mothers from comparable towns						
	PTSD1	35	N/A	Children with PTSD						
	Total1	177	52.00	Children						
*Wismer Fries et al. (2005)	TE	18	66.67	Children who had resided in orphanages for 7–42 months immediately after birth	Not matched	Early neglect	N/A	N/A	( )	( )
	NT	21	57.14	Children reared by their biological parents in a typical home environment						

Study	Group	*n* (oxytocin)	% ♀ (oxytocin)	Sample description	Matching of comparison groups	Trauma characteristics	Trauma assessment scale	Trauma frequency	PTSD assessment scale	PTSD severity
*You et al. (2017)	TE1	17	N/A	Migraine patients with adverse childhood experiences	Not matched	Family dysfunction, child abuse, child neglect prior to age 18	ACE	N/A	( )	( )
	TE2	7		Healthy controls with adverse childhood experiences						
	NT1	13	N/A	Migraine patients without adverse childhood experiences						
	NT2	19		Healthy controls without adverse childhood experiences						
	Total	58 (56)	84.48							
Yuhi et al. (2017)	TE	27	17.86	Children with mild emotional disturbance due to maltreatment and neglect	( )	Maltreatment and neglect	N/A	N/A	( )	( )
Studies investigating OXTR genotype										
+Bhandari et al. (2014)	TE	N/A	100.00	Students with childhood emotional maltreatment	Not matched	Childhood emotional maltreatment	CTQ-SF	N/A	( )	( )
	NT	N/A	100.00	Students without childhood emotional maltreatment						
	Total	102	100.00							

Study	Group	*n* (oxytocin)	% ♀ (oxytocin)	Sample description	Matching of comparison groups	Trauma characteristics	Trauma assessment scale	Trauma frequency	PTSD assessment scale	PTSD severity
+Bradley et al. (2011)	TE1	320	N/A	Visitors of publicly funded, nonprofit healthcare system clinics with two or more types of childhood maltreatment	Not matched	Childhood physical, sexual, and emotional abuse, physical and emotional neglect	CTQ	N/A	( )	( )
	TE2	N/A	N/A	Visitors of publicly funded, nonprofit healthcare system clinics with moderate or high lifetime trauma exposure		Lifetime exposure to natural disaster, serious accident or injury, sudden life-threatening illness, military combat, physical assault, and sexual assault	TEI			
	NT1	883	N/A	Visitors of publicly funded, nonprofit healthcare system clinics with two or more types of childhood maltreatment						
	NT2	N/A	N/A	Visitors of publicly funded, nonprofit healthcare system clinics with none or mild lifetime trauma exposure						
	Total	1632 (1203)	63.30							

Study	Group	*n* (oxytocin)	% ♀ (oxytocin)	Sample description	Matching of comparison groups	Trauma characteristics	Trauma assessment scale	Trauma frequency	PTSD assessment scale	PTSD severity
Bradley et al. (2013)	TE1	N/A	N/A	Visitors of publicly funded, nonprofit healthcare system clinics with moderate or severe childhood trauma	Not matched	Childhood physical, sexual, and emotional abuse, physical and emotional neglect	CTQ	N/A	MPSS	Subclinical
	TE1	N/A	N/A	Visitors of publicly funded, nonprofit healthcare system clinics with moderate or high lifetime trauma exposure		Lifetime exposure to natural disaster, serious accident or injury, sudden life-threatening illness, military combat, physical assault and sexual assault	TEI	N/A		
	NT1	N/A	N/A	Visitors of publicly funded, nonprofit healthcare system clinics with none or mild childhood trauma						
	NT2	N/A	N/A	Visitors of publicly funded, nonprofit healthcare system clinics with none or mild lifetime trauma exposure						
	PTSD	N/A	N/A	Visitors of publicly funded, nonprofit healthcare system clinics with PTSD symptoms						
	Total	971	69.72							
Cicchetti & Rogosch (2012)	TE	313	N/A	Maltreated low-income children	Not matched	Childhood neglect, emotional maltreatment, physical abuse and sexual abuse	County Department of Human Services records, MCS, MMCI	N/A	( )	( )
	NT	282	N/A	Non-maltreated low-income children						
	Total	595	46.55							
Cicchetti et al. (2014)	TE	562	N/A	Maltreated low-income children	Not matched	Childhood neglect, emotional maltreatment, physical abuse and sexual abuse	County Department of Human Services records, MCS, MMCI	N/A	( )	( )
	NT	489	N/A	Non-maltreated low-income children						
	Total	1051 (1045)	50.24							

Study	Group	*n* (oxytocin)	% ♀ (oxytocin)	Sample description	Matching of comparison groups	Trauma characteristics	Trauma assessment scale	Trauma frequency	PTSD assessment scale	PTSD severity
Connelly et al. (2014)	TE	1241	100.00	Pregnant women with history of childhood abuse	Not matched	Physical, sexual and emotional abuse in childhood	N/A	N/A	( )	( )
	NT	6482	100.00	Pregnant women without history of childhood abuse						
Cristóbal-Narváez et al. (2017)	TE	N/A	N/A	Early psychosis and non-clinical individuals with childhood trauma	Not matched	Childhood physical, sexual, and emotional abuse, physical and emotional neglect	CTQ	N/A	( )	( )
	NT	N/A	N/A	Early psychosis and non-clinical individuals with childhood trauma						
	Total	338 (319-329)	61.83							
Dannlowski et al. (2016)	TE	N/A	N/A	Participants with childhood trauma	Not matched	Childhood physical, sexual, and emotional abuse, physical and emotional neglect	CTQ	N/A	( )	( )
	NT	N/A	N/A	Participants without childhood trauma						
	Total	309	55.34							
*Dunn et al. (2014)	TE	144	95.83	Low-income parents with ≥2 trauma exposures related to Hurricane Katrina	Not matched	No fresh water to drink, no food to eat, felt their life was in danger, no necessary medicine, no necessary medical care, family member without necessary medical care, no knowledge of safety of children and no knowledge of safety of other family members	Specifically developed scale	1	IES-R	Subclinical
	NT	61	96.72	Low-income parents with ≤1 trauma exposure related to Hurricane Katrina						
	PTSD	N/A	N/A	Low-income parents with posttraumatic symptoms						
#Eidelman-Rothman et al. (2014)	TE	15	N/A	Trauma exposed war veterans	Not matched	Traumatic event	N/A	N/A	N/A	Clinical
	NT	14	N/A	Controls						
	PTSD	11	N/A	Trauma exposed war veterans with PTSD						

Study	Group	*n* (oxytocin)	% ♀ (oxytocin)	Sample description	Matching of comparison groups	Trauma characteristics	Trauma assessment scale	Trauma frequency	PTSD assessment scale	PTSD severity
Feldman et al. (2014)	TE	92	N/A	Children living in zones of continuous war	Matched for age, gender, birth order, parental education, maternal employment and marital status	Continuous war experiences, repeated rocket attacks	N/A	Multiple	Zero-to-Three, CPTSS, DWBA	Clinical
	NT	84	N/A	Controls from comparable non-exposed towns						
	PTSD	56	N/A	Children living in zones of continuous war with PTSD						
	Total	232	52.59							
Gedaly (2015)	TE	N/A	100.00	Mothers who experienced loss or trauma	Not matched	Loss or trauma	AAI	N/A	( )	( )
	NT	N/A	100.00	Mothers who did not experience loss or trauma						
	Total	182 (180)	100.00							
#Heim et al. (2011)	TE	N/A	N/A	Low-income individuals recruited from primary care clinics of an urban, public hospital with childhood maltreatment	Not matched	Childhood maltreatment	N/A	N/A	( )	( )
	NT	N/A	N/A	Low-income individuals recruited from primary care clinics of an urban, public hospital without childhood maltreatment						
Hostinar et al. (2014)	TE	263	N/A	Adolescents with objectively documented maltreatment histories	Matched for age, sex and family income	Childhood physical neglect, emotional maltreatment, physical abuse and sexual abuse	County Department of Human Services records, MCS, MMCI	Multiple ≥70.70%	( )	( )
	NT	162	N/A	Non-maltreated comparison adolescents						
Jonas et al. (2013)	TE	N/A	100.00	Mothers from a cohort study with childhood trauma	Not matched	Childhood physical, sexual, and emotional abuse, physical and emotional neglect	CTQ	N/A	( )	( )
	NT	N/A	100.00	Mothers from a cohort study with childhood trauma						

Study	Group	*n* (oxytocin)	% ♀ (oxytocin)	Sample description	Matching of comparison groups	Trauma characteristics	Trauma assessment scale	Trauma frequency	PTSD assessment scale	PTSD severity
Kimmel et al. (2016)	TE	19	100.00	Women with a previously diagnosed mood disorder with childhood sexual abuse	Not matched	Childhood sexual abuse	Single question	N/A	( )	( )
	NT	29	100.00	Women with a previously diagnosed mood disorder with childhood sexual abuse						
*Lucas-Thompson et al. (2013)	TE	N/A	N/A	Nationally representative sample of Americans who reported recent stressors between 2001 and 2004	Not matched	Negative events (e.g., serious illness or injury, natural disaster)	DIS trauma section, modified version	N/A	PTSD-PCL	Subclinical
	NT			Nationally representative sample of Americans who reported recent stressors between 2001 and 2004						
	PTSD			Nationally representative sample of Americans with posttraumatic stress (PTS) symptoms in September 2003 or 2004						
	Total	704	52.27							
Marusak et al. (2015)	TE	≥53	60,38 - 64,15	Urban, low-income, minority youths who experienced early life stress	Not matched	Potential stressors experienced as child (e.g. assault, witnessing violence, family members arrested)	TESI	2.53	( )	( )
	NT	≤2	0.00-100.00	Urban, low-income, minority youths who did not experience early life stress						
	Total	55	61.82							
McQuaid et al. (2013)	TE	N/A	N/A	University students who experienced childhood trauma	Not matched	Childhood physical, sexual, and emotional abuse, physical and emotional neglect	CTQ-SF	N/A	( )	( )
	NT	N/A	N/A							
	Total	288 (280)	73.96	University students who did not experience childhood trauma						

Study	Group	*n* (oxytocin)	% ♀ (oxytocin)	Sample description	Matching of comparison groups	Trauma characteristics	Trauma assessment scale	Trauma frequency	PTSD assessment scale	PTSD severity
McQuaid et al. (2016)	TE	N/A	N/A	University students who experienced traumatic events	Not matched	Traumatic events (e.g., natural disasters, assaults, death of a loved one)	TLEQ	≤1: 15% 2-9: 60% ≥10: 25%	( )	( )
	NT	N/A	N/A	University students who did not experience traumatic events						
	Total	243 (241)	63.37							
Mileva (2012)	TE	N/A	100.00	Mothers from a cohort study with childhood trauma	Not matched	Childhood physical, sexual, and emotional abuse, physical and emotional neglect	CTQ	N/A	( )	( )
	NT	N/A	100.00	Mothers from a cohort study without childhood trauma						
	Total	187 (157-162)	100.00							
Myers et al. (2014)	TE	N/A	N/A	Participants exposed to early life stress	Not matched	Early life stress	ELSQ	N/A	( )	( )
	NT	N/A	N/A	Participants without early life stress						
	Total	653	47.01							
Sippel et al. (2017)	TE1	1804	N/A	Veterans who experienced a potentially traumatic event	Not matched	Potentially traumatic event	THS	3.09	PCL, PCL-5	Subclinical
	TE2	1706	N/A	Traumatized participants recruited for a study on substance abuse		Trauma	N/A	N/A	SSADDA PTSD module	
	NT1	206	N/A	Veterans who did not experience a potentially traumatic event						
	PTSD1	153	15.69	Veterans with PTSD				6.47		
	PTSD2	509	N/A	Participants recruited for a study on substance abuse with PTSD						
	Total1	2163 (2025)	4.85							
	Total 2	2215	41.72							

Study	Group	*n* (oxytocin)	% ♀ (oxytocin)	Sample description	Matching of comparison groups	Trauma characteristics	Trauma assessment scale	Trauma frequency	PTSD assessment scale	PTSD severity
+Smearman et al. (2016)	TE1	189	76.72	Visitors of publicly funded, nonprofit healthcare system clinics with moderate or severe childhood trauma	Not matched	Childhood physical, sexual, and emotional abuse, physical and emotional neglect	CTQ	N/A	( )	( )
	TE2	Majority	N/A	Visitors of publicly funded, nonprofit healthcare system clinics with major lifetime trauma		Lifetime exposure to trauma such as natural disaster, serious accident or injury, and physical or sexual assault	TEI			
	NT1	200	65.00	Visitors of publicly funded, nonprofit healthcare system clinics with none or mild childhood trauma						
	NT2	Minority	N/A	Visitors of publicly funded, nonprofit healthcare system clinics without major lifetime trauma						
	Total	393 (101)	70.74							
Tollenaar et al. (2017)	TE	1182	N/A	Patients with current and/or past depressive and/or anxiety disorder and healthy controls with childhood trauma	Not matched	Childhood physical, sexual, and emotional abuse, physical and emotional neglect	CTQ, Interview from Netherlands Mental Health Survey and Incidence study	N/A	( )	( )
	NT	1376	N/A	Patients with current and/or past depressive and/or anxiety disorder and healthy controls without childhood trauma						
	Total	2567	66.30							
Studies investigating OXTR methylation										
Gouin et al. (2017)	TE	24	50.00	Adults with high abuse and low socioeconomic status	Not matched	(Very) severe physical abuse, unwanted sexual acts before the age of 18	CTS, ACE, SVC	N/A	( )	( )
	NT	22	50.00	Adults with low abuse and high socioeconomic status						

Study	Group	*n* (oxytocin)	% ♀ (oxytocin)	Sample description	Matching of comparison groups	Trauma characteristics	Trauma assessment scale	Trauma frequency	PTSD assessment scale	PTSD severity
Kimmel et al. (2016)	TE	19	100.00	Women with a previously diagnosed mood disorder with childhood sexual abuse	Not matched	Childhood sexual abuse	Single question	N/A	( )	( )
	NT	29	100.00	Women with a previously diagnosed mood disorder with childhood sexual abuse						
Simons et al. (2017)	TE	69	100.00	Primary caregivers with childhood trauma	Not matched	Exposure to various stressful events (e.g., parental divorce or family violence) when growing up	Five retrospective items	N/A	( )	( )
	NT	31	100.00	Primary caregivers with childhood trauma						
+Smearman et al. (2016)	TE1	189	76.72	Visitors of publicly funded, nonprofit healthcare system clinics with moderate or severe childhood trauma	Not matched	Childhood physical, sexual, and emotional abuse, physical and emotional neglect	CTQ	N/A	( )	( )
	TE2	Majority	N/A	Visitors of publicly funded, nonprofit healthcare system clinics with major lifetime trauma		Lifetime exposure to trauma such as natural disaster, serious accident or injury, and physical or sexual assault	TEI			
	NT1	200	65.00	Visitors of publicly funded, nonprofit healthcare system clinics with none or mild childhood trauma						
	NT2	Minority	N/A	Visitors of publicly funded, nonprofit healthcare system clinics without major lifetime trauma						
	Total	393 (101)	70.74							

Note. * indicates that study was included in meta-analysis. # indicates that study is an abstract (providing less information that journal articles or dissertations). + indicates sample overlaps. N/A indicates that information was not accessible. ( ) indicates that column was not applicable. TE = traumatized individuals, NT = non-traumatized individuals, OXTR = oxytocin receptor gene. Concerning the information on covariates considered by matching, the table only states whether TE and NT or PTSD vs. Non-PTSD-groups were matched for any covariates, respectively. The statement "not matched" exclusively indicates that TE and NT or PTSD and Non-PTSD groups were not matched. It does not indicate whether other groups within the studies were matched (for instance, psychiatric patients suffering from depression or borderline personality disorder and healthy controls). PTSD duration is not reported in the table, as it was not reported by any study. A full description of scales for traumatic event exposure and PTSD assessment is presented in the ONLINE SUPPLEMENTARY MATERIAL 4.3. Most traumatic events descriptions referred to as childhood trauma include traumatic events experienced in

adolescence, as well. PTSD severity was considered as clinical if primary study reported that participants were diagnosed with PTSD and as subclinical if PTSD symptoms were indicated by means of questionnaire or interview data but PTSD was not explicitly diagnosed.

TABLE 4.2. Age and time periods between traumatic event and assessments of oxytocin, trauma exposure and posttraumatic stress disorder (PTSD).

Study	Traumatic event[a]	Oxytocin assessment[a]	Traumatic event assessment[a]	PTSD assessment[a]
Studies investigating endogenous oxytocin				
*Bertsch et al. (2013)	Childhood	24.40	24.40	24.40
*Bhandari et al. (2014)	Childhood	19.86	19.86	( )
*#Bizik et al. (2012)	N/A	N/A	N/A	N/A
#+Böck et al. (2016)	Childhood	Adulthood	Adulthood	( )
*+Böck et al. (2017)	Childhood	33.69	33.44	( )
Bomann et al. (2017)	Childhood	29.59	29.59	29.59
#Bradley (2012)	Childhood, adulthood	Adulthood	Adulthood	Adulthood
#Cao et al. (2014)	Adulthood	Adulthood	Adulthood	Adulthood
*Chatzittofis et al. (2014)	Childhood, adulthood	42.93	42.93	( )
*Crowley et al. (2015)	<17.00	34.10	34.10	34.10
#+Eidelman-Rothman et al. (2014)	Adulthood	Adulthood	Adulthood	Adulthood
*+Eidelman-Rothman et al. (2015)	19.68- 24.68	27.68	27.68	27.68
*Frijling et al. (2015)	Childhood, adulthood	40.36	40.36	40.36
*Garfield (2012)	>17.00	18.00-43.00	18.00-43.00	18.00-43.00
*Gerra et al. (2017)	Childhood	Adulthood	Adulthood	Adulthood
#Gonzalez et al. (2015)	Childhood	Adulthood	Adulthood	( )
*Heim et al. (2009)	Childhood	31.30	31.30	( )
+Jobst et al. (2014)	Childhood	Adulthood	Adulthood	( )
Jobst et al. (2015)	Childhood	46.11	46.11	( )
*+Jobst et al. (2016)	Childhood	30.13	30.13	( )
#Jokinen et al. (2013)	N/A	N/A	N/A	( )
#Krause et al. (2017)	Childhood	Adulthood	Adulthood	( )
*#Marshall (2012)	N/A	Adulthood	Adulthood	Adulthood
*Mielke et al. (2018)	4.90	39.03	39.03	39.03
*Mizuki & Fujiwara (2015)	Childhood	36.20	36.20	( )
Mizushima et al. (2015)	Childhood	12.58	<12.58	12.58
#Mohiyeddini & Opacka-Juffry (2016)	Childhood	Adulthood	Adulthood	( )
*Munro et al. (2013)	Childhood	21.70	21.70	21.70
*Nishi et al. (2015)	36.97	36.99	36.97	37.11
*Opacka-Juffry & Mohiyeddini (2012)	Childhood, adulthood	27.70	27.70	( )
*+Pierrehumbert et al. (2010)	Childhood	29.61	Adulthood	( )
+Pierrehumbert et al. (2012)	8.33 (TE1) N/A (TE2)	29.54	Adulthood	( )
*Reijnen et al. (2017)	Childhood (TE1) 28.46 - 28.79 (TE2)	28.38, 28.88, 29.30	28.38 (TE1) 28.88 (TE2)	28.38, 28.88, 29.30
*Riem et al. (2017)	Childhood	22.39	22.39	( )
*Scott (2017)	Childhood	22.03	22.03	22.03

Study	Traumatic event[a]	Oxytocin assessment[a]	Traumatic event assessment[a]	PTSD assessment[a]
*Seltzer et al. (2014)	Childhood	9.20	9.20	( )
*Seng et al. (2013)	Childhood, adulthood	Adulthood	Adulthood	Adulthood
*Ulmer-Yaniv et al. (2017)	Childhood (TE1 and PTSD) Adulthood (TE2)	9.30 (TE1, NT1, PTSD) Adulthood (TE2, NT2)	9.30 (TE1, NT1, PTSD) Adulthood (TE2, NT2)	9.30 (TE1, NT1, PTSD) ( )
*Wismer Fries et al. (2005)	0.00 - 1.38	4.50	4.50	( )
*You et al. (2017)	Childhood	18.70	18.70	( )
Yuhi et al. (2017)	Childhood	12.07	12.07	( )
Studies investigating OXTR genotype				
Bhandari et al. (2014)	Childhood	19.86	19.86	( )
+Bradley et al. (2011)	Childhood (TE1) Childhood, adulthood (TE2)	39.36	39.36	( )
+Bradley et al. (2013)	Childhood (TE1) Childhood, adulthood (TE2, PTSD)	34.84	34.84	34.84
Cicchetti & Rogosch (2012)	Childhood	9.81	9.81	( )
Cicchetti et al. (2014)	Childhood	10.37	10.37	( )
Connelly et al. (2014)	Childhood	Adulthood	Adulthood	( )
Cristóbal-Narváez et al. (2017)	Childhood	21.67	21.67	( )
Dannlowski et al. (2016)	Childhood	34.30	34.30	( )
*Dunn et al. (2014)	26.82	30.82	27.82	27.82
#+Eidelman-Rothman et al. (2014)	Adulthood	Adulthood	Adulthood	Adulthood
Feldman et al. (2014)	1.50-5.00, 7.00-8.00	1.50-5.00	1.50-5.00, 7.00-8.00	1.50-5.00, 7.00-8.00
Gedaly (2015)	N/A	28.10	25.64	( )
#Heim et al. (2011)	Childhood	Adulthood	Adulthood	( )
Hostinar et al. (2014)	Childhood	13.78	13.78	( )
Jonas et al. (2013)	Childhood	Adulthood	Adulthood	( )
Kimmel et al. (2016)	Childhood	30.68	30.68	( )
*Lucas-Thompson et al. (2013)	43.05-44.05, 43.55-44.55, 44.05 - 45.05, 45.05-46.05	49.80	44.05, 44.55, 45.05, 46.05	45.05, 46.05
Marusak et al. (2015)	Childhood	11.71	11.71	( )
McQuaid et al. (2013)	Childhood	19.99	19.99	( )
McQuaid et al. (2016)	Childhood, adulthood	19.17	19.17	( )
Mileva (2012)	Childhood	Adulthood	31.20	( )
Myers et al. (2014)	Childhood	37.00	37.00	( )
Sippel et al. (2017)	Childhood, adulthood (TE1, PTSD1) N/A (TE2, PTSD2)	62.93 (TE1, NT1, PTSD1) 39.20 (TE2, NT2, PTSD2)	62.93 (TE1, NT1, PTSD1) 39.20 (TE2, NT2, PTSD2)	62.93 (TE1, NT1, PTSD1) 39.20 (TE2, NT2, PTSD2)
+Smearman et al. (2016)	Childhood (TE1) Childhood, adulthood (TE2)	41.00	41.00	( )

Study	Traumatic event[a]	Oxytocin assessment[a]	Traumatic event assessment[a]	PTSD assessment[a]
Tollenaar et al. (2017)	Childhood	42.18	42.18, 46.18	( )
Studies investigating OXTR methylation				
Gouin et al. (2017)	Childhood	27.00	27.00	( )
Kimmel et al. (2016)	Childhood	30.68	30.68	( )
Simons et al. (2017)	Childhood	48.52	38.52	( )
+Smearman et al. (2016)	Childhood (TE1) Childhood, adulthood (TE2)	41.00	41.00	( )

Note. [a] numbers represent mean age scaled in years. * indicates that study was included in meta-analysis. # indicates that study is an abstract (providing less information that journal articles or dissertations). + indicates sample overlaps. N/A indicates that information was not accessible. ( ) indicates that column was not applicable. TE = traumatized individuals, NT = non-traumatized individuals, PTSD= posttraumatic stress disorder (sample definitions see TABLE 4.1), OXTR = oxytocin receptor gene. Values represent mean or range of age. If not indicated differently, values represent information for total sample. Values separated by commas indicate that several assessments were conducted.

4.3.3 Endogenous oxytocin concentrations, OXTR genotype and methylation

Details on endogenous oxytocin and OXTR genotype assessments, including NOS (Wells et al.) ratings and databases for the meta-analyses, are provided in TABLES 4.3 and 4.4. Concerning those studies that investigated endogenous oxytocin concentrations, k = 35 had a cross-sectional design. In k = 6 studies, traumatic event exposure, PTSD or oxytocin were assessed longitudinally. There was only k = 1 intervention study, evaluating a group drumming intervention. In k = 25 studies, no challenge or reactivity design was applied, while k = 16 studies applied one. Most frequently, oxytocin reactivity to mother-infant interactions (k = 3) or social stress (k = 3) was investigated. As all challenge or reactivity studies reported unstimulated endogenous oxytocin concentrations at baseline, this parameter was used for the present meta-analyses. Oxytocin was measured in extracted blood (k = 8), unextracted blood (k = 3), or blood (without available information about extraction, k = 17). It was measured in extracted saliva (k = 4), or saliva (without information about extraction, k = 5). It was measured in extracted urine samples (k = 3). In k = 1 study, oxytocin was measured in urine, but it was not reported whether extraction was performed. Moreover, oxytocin was measured in unextracted cerebrospinal fluid in k = 1 study. In k = 2 studies, oxytocin was measured in cerebrospinal fluid, but information about extraction was not available. Oxytocin was most frequently analyzed by enzyme immunoassays (k = 23), followed by radioimmunoassays (k = 12). In k = 6 studies, no information about the assay type was given. With regard to confounders, we extracted whether certain pre-defined variables of interest were considered. As there are currently no guidelines available, we decided that consideration could imply that confounders were standardized, statistically controlled for as covariates or by means of sensitivity analyses. Time of day was considered in k = 27 studies. Hormonal contraception was considered in k = 15, female fertility status in k = 14, and menstrual cycle phase in k = 15 studies. Study quality ratings based on the NOS (Wells et al.) ranged from 3 to 9, with M = 5.87 (SD = 1.73) stars awarded.

OXTR genotype studies were most frequently cross-sectional (k = 18), while k = 7 studies had a longitudinal design. The most frequently assessed SNP was rs53576 (k = 21). There was considerable variation with regard to sample ethnicity. NOS (Wells et al.) ratings ranged from 3 to 9, M = 6.32 (SD = 1.74).

TABLE 4.3. Assessment of endogenous oxytocin in traumatized individuals, posttraumatic stress disorder (PTSD) patients and comparison groups

Study	Design	Intervention	Reactivity	Group	Oxytocin	*r*	Reported correlation	Specimen	Assay	Extraction	Confounders considered	NOS
*Bertsch et al. (2013)	Cross-sectional	No	No	Total	22.98 (N/A) pg/ml	-.44	CTQ and oxytocin	Blood	RIA	Yes	HC, F, MCP	5
*Bhandari et al. (2014)	Cross-sectional	No	No	Total	2.65 (1.32) pg/ml	.25	CTQ-SF emotional maltreatment and oxytocin	Saliva	EIA	Yes	Time, HC, MCP, AS, HS	6
*#Bizik et al. (2012)	Cross-sectional	No	No	Total, patients with severe depression (n = 34)	N/A	-.55	TSC-40 and oxytocin	Blood	RIA	N/A	None	3
#+Böck et al. (2016)	Longitudinal	No	No	Total	N/A	N/A	( )	Blood	RIA	Yes	Time, HC, MCP, HS, BMI	7
*+Böck et al. (2017)	Longitudinal	No	No	TE	0.89 (0.35) pg/ml	N/A	( )	Blood	RIA	Yes	Time, HC, MCP, HS, BMI	7
				NT	0.74 (0.41) pg/ml	N/A	( )					
				Total	0.79 (0.39) pg/ml	.23	CTQ and oxytocin					
Bomann et al. (2017)	Cross-sectional	No	No	Total	452.68 (N/A) pg/ml	N/A	( )	Blood	EIA	N/A	None	6
#Bradley (2012)	Cross-sectional	No	No	Total	N/A	N/A	( )	Blood	N/A	N/A	None	3
#Cao et al. (2014)	Cross-sectional	No	No	Total	101.59 (55.89) pg/ml	N/A	( )	Blood	EIA	N/A	None	5
*Chatzittofis et al. (2014)	Cross-sectional	No	No	TE (n = 7)	4.60 (1.70) fmol/ml	N/A	( )	Blood	RIA	N/A	Time	3
				TE (n = 8)	11.00 (4.00) fmol/ml	N/A	( )	CSF				
				NT (n = 14)	7.20 (3.70) fmol/ml	N/A	( )	Blood				
				NT (n = 13)	10.90 (3.00) fmol/ml	N/A	( )	CSF				
				Total	6.33 (N/A) fmol/ml	-.30	KIVS (adulthood) and oxytocin	Blood				
				Total	10.94 (N/A) fmol/ml	.16	KIVS (childhood) and oxytocin	CSF				

Study	Design	Intervention	Reactivity	Group	Oxytocin	*r*	Reported correlation	Specimen	Assay	Extraction	Confounders considered	NOS
*Crowley et al. (2015)	Cross-sectional	No	No	TE	5.39 (2.40) pg/ml	N/A	( )	Blood	RIA	Yes	Time, HC, MCP, F	7
				NT	4.36 (1.10) pg/ml	N/A	( )					
#+Eidelman-Rothman et al. (2014)	Cross-sectional	No	Single intanasal oxytocin or placebo administration	Total	N/A	N/A	( )	Blood	EIA	N/A	Time	9
*+Eidelman-Rothman et al. (2015)	Cross-sectional	No	Single intanasal oxytocin or placebo administration	TE (*n* = 14)	411.99 (121.43) pg/ml	-.06	PDS and basal oxytocin	Blood	EIA	N/A	Time	9
				TE (*n* = 15)	10.04 (4.60) pg/ml	.11	PDS and basal oxytocin	Saliva				
				NT (*n* = 14)	455.98 (151.15) pg/ml	N/A	( )	Blood				
				NT (*n* = 15)	11.12 (7.74) pg/ml	N/A	( )	Saliva				
				PTSD (*n* = 9)	395.17 (76.04) pg/ml	.16	PDS and basal oxytocin	Blood				
				PTSD (*n* = 10)	10.57 (7.59) pg/ml	.15	PDS and basal oxytocin	Saliva				
*Frijling et al. (2015)	Cross-sectional	No	No	TE, women (*n* = 20)	2.42 (3.77) pg/ml	N/A	( )	Saliva	RIA	Yes	Time, HC, AS, HS, BMI	7
				TE, men (*n* = 20)	3.00 (3.54) pg/ml	N/A	( )					
				TE (*n* = 39)	N/A	.12, -.13	ETISR-SF and oxytocin, CAPS and oxytocin					
				PTSD, women (*n* =19)	2.10 (2.31) pg/ml	N/A	( )					
				PTSD, men (*n* = 21)	1.34 (1.71) pg/ml	N/A	( )					
				PTSD (*n* = 37)	N/A	.03, .05	ETISR-SF and oxytocin, CAPS and oxytocin					
				Total (*n* = 76)	2.21 (N/A) pg/ml	.04, -.17	ETISR-SF and oxytocin, CAPS and oxytocin					
*Garfield (2012)	Cross-sectional	No	20 min mother-infant interaction	Total	854.30 (1115.89) pg/ml	-.77	PPQ an basal oxytocin	Blood	EIA	N/A	F, AS	5
*Gerra et al. (2017)	Cross-sectional	No	No	Total	389.90 (259,40) pg/ml	.40	CECA-G[a] mother neglect subscale and oxytocin	Blood	EIA	N/A	Time, AS, HS,	8

Study	Design	Intervention	Reactivity	Group	Oxytocin	*r*	Reported correlation	Specimen	Assay	Extraction	Confounders considered	NOS
#Gonzalez et al. (2015)	Cross-sectional	No	15 min mother-infant interaction	Total	N/A	N/A	( )	Blood, saliva	N/A	N/A	F	5
*Heim et al. (2009)	Cross-sectional	No	No	TE	11.06 (3.74) pg/ml	N/A	( )	CSF	EIA	No	Time, F, MCP	7
				NT	14.35 (3.17) pg/ml	N/A	( )					
				Total	12.26 (N/A) pg/ml	-.54	CTQ and oxytocin					
+Jobst et al. (2014)	Cross-sectional	No	3 min social exclusion paradigm	Total	432.31 (N/A) pg/ml (baseline)	-.45, -.46	CTQ emotional abuse and oxytocin reactivity, CTQ physical abuse and oxytocin reactivity	Blood	EIA	No	Time, HC, MCP	7
Jobst et al. (2015)	Cross-sectional	No	3 min social exclusion paradigm	Total	511.94 (341.39) pg/ml (baseline)	N/A	( )	Blood	EIA	N/A	Time, HC, F, MCP	8
*+Jobst et al. (2016)	Cross-sectional	No	3 min social exclusion paradigm	Total	435.58 (N/A) pg/ml (baseline)	-.07	CTQ emotional abuse and basal oxytocin	Blood	EIA	No	Time, HC, MCP	7
#Jokinen et al. (2013)	Longitudinal	No	No	Total	N/A	N/A	( )	Blood, CSF	N/A	N/A	Time	3
#Krause et al. (2017)	Cross-sectional	No	No	Total	N/A	N/A	( )	Blood	RIA	N/A	F	4
*#Marshall (2012)	Cross-sectional	No	20 min video-based discussion about positive relationship aspects	Total	8.36 (6.75) pg/mg creatinine (baseline)	-.01	CAPS and basal oxytocin	Urine	N/A	N/A	None	3
*Mielke et al. (2018)	Cross-sectional	No	No	TE	4.01 (2.24) pg/ml	-.01	CECA[a] and oxytocin	Blood	RIA	N/A	Time, HC, F, MCP, HS	7
				NT	3.56 (2.44) pg/ml	N/A	( )					
				PTSD	3.12 (3.52) pg/ml	N/A	( )					
				Total	3.79 (N/A) pg/ml	.07	CECA and oxytocin					
*Mizuki & Fujiwara (2015)	Cross-sectional	No	No	TE	112.06 (44.41)	N/A	( )	Urine	RIA	Yes	Time, F	6
				NT	106.17 (30.73)	N/A	( )					

Study	Design	Intervention	Reactivity	Group	Oxytocin	*r*	Reported correlation	Specimen	Assay	Extraction	Confounders considered	NOS
Mizushima et al. (2015)	Cross-sectional	No	No	TE	32.27 (N/A) pg/ml (awakening), 60.43 (N/A) pg/ml (bedtime)	N/A	( )	Saliva	EIA	N/A	Time	8
				NT	57.21 (N/A) pg/ml (awakening), 37.93 (N/A) pg/ml (bedtime)	N/A	( )					
#Mohiyeddini & Opacka-Juffry (2016)	Cross-sectional	No	No	Total	N/A	N/A	( )	Blood	N/A	N/A	None	4
*Munro et al. (2013)	Cross-sectional	No	30 min affiliation stress film paradigm	TE	5.18 (0.22) ln (pg/ml)	N/A	( )	Blood	EIA	N/A	HC, F, MCP, AS, HS	6
				NT	5.11 (0.48) ln (pg/ml)	N/A	( )					
				NoPTSD	5.07 (0.47) ln (pg/ml)	N/A	( )					
				PTSD	5.30 (0.15) ln (pg/ml	N/A	( )					
				Total	183.60 (77.50) pg/ml	.12, .09	LSC childhood abuse and basal oxytocin, PTSD-PCL and basal oxytocin					
*Nishi et al. (2015)	Longitudinal	No	No	TE	61.30 (21.10) pg/ml	N/A	( )	Blood	EIA	Yes	Time	6
				PTSD	63.20 (21.60) pg/ml	N/A	( )					
				Total (*n* = 156, *n* = 154)	N/A	.04, -.04	IES-R (baseline) and oxytocin (baseline), IES-R (1 month posttrauma) and oxytocin (baseline)					
*Opacka-Juffry & Mohiyeddini (2012)	Cross-sectional	No	No	Total	377.60 (23.90) pg/ml	-.34, .02	ELSI childhood and oxytocin, ELSI adolescence and oxytocin	Blood	EIA	No	Time, AS, HS	5

Study	Design	Intervention	Reactivity	Group	Oxytocin	*r*	Reported correlation	Specimen	Assay	Extraction	Confounders considered	NOS
*+Pierrehumbert et al. (2010)	Cross-sectional	No	10 min Trier Social Stress Test	TE1	0.15 (1.37) residual z-score (baseline)	N/A	( )	Blood	RIA	Yes	Time, HC, F, MCP	6
				TE2	0.13 (0.94) residual z-score (baseline)	N/A	( )					
				NT	-0.29 (0.76) residual z-score (baseline)	N/A	( )					
+Pierrehumbert et al. (2012)	Cross-sectional	No	10 min Trier Social Stress Test	Total	N/A	N/A	( )	Blood	RIA	Yes	Time, HC, F, MCP	6
*Reijnen et al. (2017)	Longitudinal	No	No	TE1	13.33 (5.81) pg/ml (pre deployment)	N/A	( )	Blood	EIA	Yes	Time. HS, BMI	8
				TE2	12.18 (5.52) pg/ml (1 month post deployment)	N/A	( )					
				NT1	13.49 (5.48) pg/ml (pre deployment)	N/A	( )					
				NT2	12.21 (4.53) pg/ml (1 month post deployment)	N/A	( )					
				TE, NT	12.20 (5.03) pg/ml (6 months post deployment)	N/A	( )					
				PTSD	13.27 (5.56) pg/ml (6 months post deployment)	N/A	( )					
				Total (*n* = 789, *n* = 611, *n* = 624)	N/A	-.00, .03, .03	ETI-SF and oxytocin (pre deployment), DES and oxytocin (1 month post deployment), SRIP and oxytocin (6 months post deployment)					

Study	Design	Intervention	Reactivity	Group	Oxytocin	*r*	Reported correlation	Specimen	Assay	Extraction	Confounders considered	NOS
*Riem et al. (2017)	Cross-sectional	No	15 min mechanically-delivered massage	TE	11.92 (4.29) pg/ml (baseline)	N/A	( )	Saliva	EIA	Yes	Time	5
				NT	16.31 (6.75) pg/ml (baseline)	N/A	( )					
				Total (*n* = 46)	13.64 (3.87) pg/ml (baseline)	-.42	CTQ-SF composite of emotional abuse and emotional neglect and basal oxytocin					
*Scott (2017)	Cross-sectional	No	No	TE1	21.63 (9.24) pg/ml	N/A	( )	Saliva	EIA	Yes	Time, HC, MCP	3
				TE2	13.79 (5.02) pg/ml	N/A	( )					
				NT1	23.48 (11.60) pg/ml	N/A	( )					
				NT2	14.12 (5.89) pg/ml	N/A	( )					
				PTSD	19.34 (0.69) pg/ml	N/A	( )					
				TE2+PTSD	14.09 (5.05) pg/ml	N/A	( )					
				Total (*n* = 95)	17.57 (8.31) pg/ml	-.13	CTQ and oxytocin					
*Seltzer et al. (2014)	Cross-sectional	No	10 min Trier Social Stress Test for children	TE	36.10 (8.54) pg/ml creatinine (baseline girls), 13.29 (4.83) pg/ml creatinine (baseline boys)	N/A	( )	Urine	EIA	Yes	Time, F	7
				NT	12,37 (12.37) pg/ml creatinine (baseline girls), 12.53 (4.35) pg/ml creatinine (baseline boys)	N/A	( )					

Study	Design	Intervention	Reactivity	Group	Oxytocin	*r*	Reported correlation	Specimen	Assay	Extraction	Confounders considered	NOS
*Seng et al. (2013)	Cross-sectional	No	No	TE	4.90 (1.30) ln (pg/ml)	N/A	( )	Blood	EIA	N/A	Time, HC, F, MCP, AS, HS	5
				NT	4.59 (1.04) ln (pg/ml)	N/A	( )					
				NoPTSD	4.44 (0.97) ln (pg/ml)	N/A	( )					
				PTSD	5.03 (1.24) ln (pg/ml)	N/A	( )					
				Total	130.90 (N/A) pg/ml	0.06, 0.31	LSC childhood maltreatment and oxytocin, PTSD score and oxytocin					
*Ulmer-Yaniv et al. (2017)	Cross-sectional	No	14 min mother-child interactions (positive and conflict)	TE1+PTSD1	1419.72 (520.30) N/A	N/A	( )	Saliva	EIA	N/A	Time	8
				TE2	1634.71 (606.85) N/A	N/A	( )					
				NT1	1449.69 (379.10) N/A	N/A	( )					
				NT2	1878.06 (607.22) N/A	N/A	( )					
*Wismer Fries et al. (2005)	Cross-sectional	No	30 min interactive computer game on lap of mother or experimenter	TE	12.12 (10.61) µg/mg creatinine (baseline)	N/A	( )	Urine	N/A	Yes	None	7
				NT	18.99 (20.96) µg/mg creatinine (baseline)	N/A	( )					
*You et al. (2017)	Cross-sectional	No	No	TE1	335.12 (370.42) pg/ml^{-1}	N/A	( )	Blood	EIA	Yes	AS, HS	4
				TE2	188.36 (80.48) pg/ml^{-1}	N/A	( )					
				NT1	179.23 (104.67) pg/ml^{-1}	N/A	( )					
				NT2	170.50 (124.89) pg/ml^{-1}	N/A	( )					
				Total 1	N/A	.31	ACE and oxytocin					
				Total 2	N/A	.01	ACE and oxytocin					
				Total	N/A	.27	ACE and oxytocin					

Study	Design	Intervention	Reactivity	Group	Oxytocin	*r*	Reported correlation	Specimen	Assay	Extraction	Confounders considered	NOS
Yuhi et al. (2017)	Longitudinal	Group drumming	No	TE	213,19 (N/A) (baseline before free activity)	N/A	( )	Saliva	EIA	N/A	None	( )

Note. [a] higher scores indicate less maltreatment * indicates that study was included in meta-analysis. # indicates that study is an abstract (providing less information that journal articles or dissertations). + indicates sample overlaps. N/A indicates that information was not accessible. ( ) indicates that column was not applicable. TE = traumatized individuals, NT = non-traumatized individuals (sample definitions see TABLE 4.1, here, *n* is only reported if deviating), CSF = cerebrospinal fluid, EIA = enzyme immunoassay, RIA = radioimmunoassay, time = time of day, HC = hormonal contraception use, F = Fertility status, MCP = menstrual cycle phase, AC = acute smoking, HS = habitual smoking, BMI = body-mass-index, NOS = Newcastle-Ottawa Quality Assessment Scale. Design was considered as longitudinal only if traumatic event exposure, PTSD and oxytocin parameters were assessed longitudinally. Confounders were described as considered if they were reported or taken into account as covariates in the primary studies. Only those samples introduced in TABLE 4.1 with available oxytocin mean and standard deviation or correlation coefficient *r* are reported. A full description of scales for traumatic event exposure and PTSD assessment is presented in the ONLINE SUPPLEMENTARY MATERIAL 4.3. The detailed results of the NOS ratings are presented in the ONLINE SUPPLEMENTARY MATERIAL 4.4.

TABLE 4.4. Assessment of oxytocin receptor (OXTR) genotype in traumatized individuals, posttraumatic stress disorder (PTSD) patients and comparison groups

Study	Design	Intervention	SNP	Group	Allele carriers *n* (%)	*r*	Parameters	Ethnicity	NOS
Bhandari et al. (2014)	Cross-sectional	No	rs53576	Total	GG: 51 (54.84) AG: 36 (38.71) AA: 6 (6.45)	.05	CTQ-SF -emotional maltreatment and GG vs. AG/AA	Predominantly Caucasian	6
+Bradley et al. (2011)	Cross-sectional	No	rs53576	TE1	GG: 206 (17.12) AG: 104 (8.65) AA: 10 (0.01)	N/A	( )	African American	6
				NT1	GG: 550 (45.72) AG: 284 (23.61) AA: 49 (4.07)	N/A	( )		
			rs2254298	Total	N/A	N/A	( )		
+Bradley et al. (2013)	Cross-sectional	No	rs53567	Total	GG: 602 (62.00) AG: 320 (32.96) AA: 51 (5.25)	N/A	( )	African American	6
Cicchetti & Rogosch (2012)	Cross-sectional	No	rs53576	Total	N/A	N/A	( )	African American (62.00%), White (19.00%), Hispanic (17.00%), Other (2.00%)	8
Cicchetti et al. (2014)	Cross-sectional	No	rs53576	Total	AA: 79 (7.56) AG: 390 (37.32) GG: 576 (55.12)	N/A	( )	African American (61.20%), Hispanic (19.7%), European American (10.40%), Other/multiethnic/multiracial (8.70%)	9
Connelly et al. (2014)	Cross-sectional	No	rs53576	Total	GG: 3832 (46.00) AG: 3657 (43.90) AA: 841 (10.10)	N/A	( )	White (99.90%)	6
			rs2254298		GG: 6614 (79.30) AG: 1610 (419.30) AA: 117 (1.40)	N/A	( )		
Cristóbal-Narváez et al. (2017)	Cross-sectional	No	rs53576	Total	GG: 136 (42.63) AG: 146 (45.77) AA: 37 (11.60)	N/A	( )	Residence: Spain	5
			rs2254298	Total	GG: 227 (67.00) AG: 88 (26.75) AA: 14 (4.26)	N/A	( )		
Dannlowski et al. (2016)	Cross-sectional	No	rs53576	Total	AA: 28 (9.06) AG: 142 (45.95) GG: 139 (44.98)	N/A	( )	European	8

Study	Design	Intervention	SNP	Group	Allele carriers *n* (%)	*r*	Parameters	Ethnicity	NOS
*Dunn et al. (2014)	Longitudinal	No	rs53576	Total	N/A	-.05	IES-R and GG vs. AG/AA	Non-Hispanic Black	8
			rs2254298	Total	N/A	-.01	IES-R and GG vs. AG/AA		
#+Eidelman-Rothman et al. (2014)	Cross-sectional	No	rs2254298	Total	N/A	N/A	( )	Residence: Israel	9
Feldman et al. (2014)	Longitudinal	No	rs53576	Total	GG: 129 (55.60) AG/AA: 103 (44.40)	N/A	( )	Residence: Israel	8
			rs2254298	Total	GG: 99 (42.67) AG/AA: 133 (57.33)	N/A	( )		
			rs1042778	Total	TT: 27 (11.64) GG/GT: 205 (88.36)	N/A	( )		
Gedaly (2015)	Longitudinal	No	rs53576	Total	AG/AA: 99 (55.00) GG: 81 (45.00)	-.01	AAI trauma and loss and GG vs. AG/AA	European American (50.00%), African American (50.00%)	5
#Heim et al. (2011)	Cross-sectional	No	rs53576	Total	N/A	N/A	( )	African American	3
Hostinar et al. (2014)	Cross-sectional	No	rs53576	TE	GG: 139 (52.85) AG/AA: 124 (47.15)	N/A	( )	Black (61.41%), White (26.82%), Hispanic (11.76%)	7
				NT	GG: 97 (59.88) AG/AA: 65 (40.12)	N/A	( )		
Jonas et al. (2013)	Longitudinal	No	rs237885	Total	GG: 78 (24.84.) GC: 140 (44.59) CC: 96 (30.57)	N/A	( )	Caucasian (78.30%), Other (9.85%), Unreported (8.48%), Mixed Caucasian (3.39%)	6
Kimmel et al. (2016)	Cross-sectional	No	rs53576	Total	N/A	N/A	( )	Caucasian (70.00%)	4
*Lucas-Thompson et al. (2013)	Longitudinal	No	rs53576	Total	GG: 361 (51.28) AG: 284 (40.34) AA: 59 (8.38)	-.01, -.08, -.01	DIS, PTSD-PCL 2003, PTSD-PCL 2004 and GG vs. AG/AA	White, Non-Hispanic (74.90), Black, Non-Hispanic (8,92%), Hispanic (8,92%), Other, Non-Hispanic (7.19%)	8
Marusak et al. (2015)	Cross-sectional	No	rs2254298	Total	GG: 34 (61.82) AG: 17 (30.91) AA: 4 (7.27)	N/A	( )	African American (41.82%), Caucasian (32.73%), Mixed (12.73%), Hispanic (3.64%)	7

Study	Design	Intervention	SNP	Group	Allele carriers *n* (%)	*r*	Parameters	Ethnicity	NOS
McQuaid et al. (2013)	Cross-sectional	No	rs53576	Total	GG: 118 (42.14) AG: 119 (42.50) AA: 43 (15.36)	N/A	( )	White (58.00%), Black (11.80%), Asian (8.00%), Others (5.90%), Arab (5.70%), South Asian (5.60%), South East Asian (2.10%), Latin American (1.40%), Aboriginal (1.40%)	3
McQuaid et al. (2016)	Cross-sectional	No	rs53576	Total	GG: 109 (45.23) AG: 106 (43.98) AA: 26 (10.79)	N/A	( )	White	6
Mileva (2012)	Longitudinal	No	rs4813627	Total	GG: 44 (27.16) GA: 73 (45.06) AA: 45 (27.78)	N/A	( )	Caucasian	7
			rs2740210	Total	CC: 77 (48.73) CA: 63 (39.87) AA: 18 (11.39)	N/A	( )		
			rs237885	Total	TT: 43 (27.39) TG: 72 (45.86) GG: 44 (26.75)	N/A	( )		
Myers et al. (2014)	Cross-sectional	No	rs6770632, rs237885, rs11706648, rs237887, rs9840864, rs4686301, rs2268492, rs237889, rs11131149	Total	N/A	N/A	( )	Caucasian	3
Sippel et al. (2017)	Cross-sectional	No	rs53576	TE1	AA: 155 (10.66) AG: 645 (44.36) GG: 654 (44.98)	N/A	( )	European American	6
				PTSD1	AA: 16 (13.01) AG: 59 (47.97) GG: 48 (39.02)	N/A	( )		
			rs53576, rs2300549	Total 2	N/A	N/A	( )	N/A	

Study	Design	Intervention	SNP	Group	Allele carriers *n* (%)	*r*	Parameters	Ethnicity	NOS
+Smearman et al. (2016)	Cross-sectional	No	rs53576	Total	GG: 72 (71.29) AG: 24 (23.76) AA: 5 (4.95)	N/A	( )	African American	6
			rs237837, rs9817913, rs3901926, rs237852, rs7628723, rs6791619, rs17365093, rs6793234, rs237925, rs9860869, rs75775, rs6443206, rs7632031, rs180789, rs6777726, rs2301261, rs237911, rs237902, rs237899, rs237897, rs2268495, rs237895, rs11131149, rs237889, rs2254298, rs2268491, rs4686301, rs918316, rs237888, rs2268490, rs237887, rs11706648, rs237885, rs2139184, rs1042778, rs6770632, rs237884, rs2324728, rs11720238, rs17049507, rs7629329, rs11476, rs13093809	Total	N/A	N/A	( )		
Tollenaar et al. (2017)	Longitudinal	No	rs53576	Total	AA: 304 (11.81) AG: 1197 (46.63) GG: 1065 (41.49)	N/A	( )	North-European descent	8
			rs2254298	Total	AA: 13 (0.05) AG: 289 (11.26) GG: 2264 (88.23)	N/A	( )		
			rs2268498	Total	CC: 523 (20.37) CT: 1300 (50.64) TT: 744 (28.98)	N/A	( )		

Note. * indicates that study was included in meta-analysis. # indicates that study is an abstract (providing less information that journal articles or dissertations). + indicates sample overlaps. N/A indicates that information was not accessible. ( ) indicates that column was not applicable. SNP = single-nucleotide polymorphism, TE = traumatized individuals, NT = non-traumatized individuals (sample definitions see TABLE 4.1, here, *n* is only reported if deviating), NOS = Newcastle-Ottawa Quality Assessment Scale. Design was considered as longitudinal only if traumatic event exposure, PTSD and oxytocin parameters were assessed longitudinally. A full description of scales for traumatic event exposure and PTSD assessment is presented in the ONLINE SUPPLEMENTARY MATERIAL 4.3. Ethnicity was extracted as it was reported in primary studies. The detailed results of the NOS ratings are presented in the ONLINE SUPPLEMENTARY MATERIAL 4.4.

Studies on OXTR methylation were insufficient in number and methodologically too diverse to calculate meta-analytic effect sizes. Three studies had a cross-sectional (Gouin et al., 2017; Kimmel et al., 2016; Smearman et al., 2016) and one had a longitudinal design (Simons, Lei, Beach, Cutrona, & Philibert, 2017), although without any intervention. Sample ethnicities were 100% African American (Simons et al., 2017; Smearman et al., 2016), 70% Caucasian (Kimmel et al., 2016) and 100% Caucasian (Gouin et al., 2017), respectively. Studies were assigned with four (Kimmel et al., 2016), five (Smearman et al., 2016) and seven (Gouin et al., 2017; Simons et al., 2017) stars according to the NOS (Wells et al.). Various genomic sites were investigated (ONLINE SUPPLEMENTARY MATERIAL 4.4).

4.3.4 Meta-analyses

4.3.4.1 Endogenous oxytocin concentrations and trauma exposure

4.3.4.1.1 Group comparisons

Comparisons (k = 17) between traumatized (n = 1,236) and non-traumatized individuals (n = 628, FIGURE 4.3) yielded a non-significant effect (g = 0.06 [-0.15; 0.28], p = .57, Q = 66.67; p < .01; I^2 = 70.00).

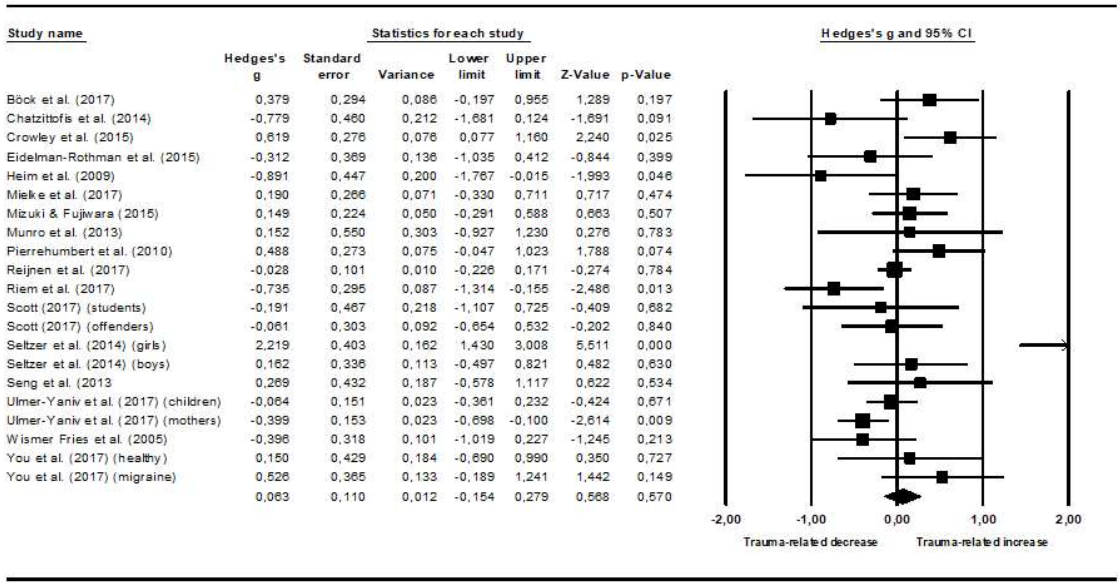

FIGURE 4.3. Comparisons of endogenous oxytocin concentrations between traumatized and non-traumatized individuals. Negative values indicate that oxytocin concentrations were lower in traumatized than in non-traumatized individuals.

TABLE 4.5. Results of the subgroup analyses - comparison of endogenous oxytocin concentrations between traumatized and non-traumatized individuals

(Sub)group	Number of studies	Number of traumatized individuals	Number of non-traumatized individuals	Hedges' *g*[a]	*p*	Confidence interval[a]	Q	*p*	I^2
All studies	17	1,236	628	0.05	.57	-0.15; 0.28	66.67	<.01	70.00
Details of oxytocin assessment									
Specimen									
Blood	10	825	328	0.17	.11	-0.04; 0.39	14.57	.15	31.37
Saliva	4	311	216	-0.26	<.01	-0.46; -0.06	5.61	.35	10.86
CSF	2	22	21	-0.42	.36	-1.32; 0.48	2.19	.14	54.34
Urine	3	101	91	0.50	.29	-0.43; 1.44	28.12	<.01	89.33
Extraction									
Yes	11	961	397	0.22	.13	-0.07; 0.51	48.24	<.01	73.05
Combinations of specimens and extraction									
Blood, extracted	6	766	257	0.27	.03	0.02; 0.53	9.16	.16	34.50
Saliva, extracted	3	94	49	-0.36	.11	-0.81; 0.09	2.71	.26	26.16
Details of traumatic event exposure									
Age at traumatic event									
Childhood	15	1,114	524	0.15	.20	-0.08; 0.38	54.51	<.01	68.81
Adulthood	3	664	152	-0.21	.17	-0.50; 0.09	3.88	.14	48.51
Assessment scale									
CTQ	5	172	122	-0.17	.39	-0.56; 0.22	11.67	.04	57.17
Restrictive traumatic event criterion									
Actual or threatened death, serious injury or sexual violence	5	811	297	0.01	.95	-0.26; 0.28	14.48	.01	65.48
Type									
Interpersonal	13	1,010	433	0.12	.40	-0.16; 0.39	56.03	<.01	73.23
War-related	3	765	228	-0.16	.12	-0.36; 0.04	4.34	.23	30.92
Frequency									
Repeated exposure to traumatic events	2	206	163	-0.21	.14	-0.48; 0.07	2.89	.23	30.89
Sample details									
Age at oxytocin assessment									
Childhood	3	156	133	0.44	.34	-0.46; 1.34	31.31	<.01	90.42
Adulthood	15	1,080	495	-0.01	.95	-0.20; 0.19	34.06	<.01	53.03
Sex									
Female	7	195	209	0.06	.78	-0.34; 0.45	18.26	<.01	67.24
Male	3	709	157	-0.30	.21	-0.76; 0.16	5.42	.07	63.12
Health status									
Clinical	4	69	96	0.30	.25	-0.21; 0.81	7.40	.06	59.44
Non-clinical	14	1,150	528	0.03	.82	-0.21; 0.27	54.91	<.01	72.68

(Sub)group	Number of studies	Number of traumatized individuals	Number of non-traumatized individuals	Hedges' *g*[a]	*p*	Confidence interval[a]	Q	*p*	I^2
Recruitment setting									
Community	12	480	412	0.11	.47	-0.19; 0.41	59.91	<.01	76.63
Hospital	4	57	92	0.18	.47	-0.31; 0.66	5.95	.11	49.56
Military	2	689	128	-0.05	.63	-0.24; 0.14	0.55	.46	0.00
Covariate control									
Matched comparison groups	3	234	197	-0.13	.41	-0.44; 0.18	6.37	.09	52.94
Non-matched comparison groups	14	1,002	431	0.11	.40	-0.15; 0.38	56.03	<.01	71.44

Note. [a] negative values indicate that oxytocin concentrations were lower in traumatized individuals than in non-traumatized individuals. Significant *Q* coefficients and I^2 scores of 25-49 indicate mild, 50-74 moderate, and ≥75 high heterogeneity. Analyses were conducted if a minimum of two studies were available per group. Accordingly, subgroup analyses for unextracted samples across specimens (k = 1), unextracted blood, (k = 0), unextracted saliva (k = 0), unextracted cerebrospinal fluid (CSF, k = 1) or unextracted urine samples (k = 0), extracted CSF samples (k = 0), other assessment scales that CTQ (childhood trauma questionnaire, k = 1, respectively), accidental traumatic events (k = 0), or exposure to a single traumatic event (k = 1) were not conducted. All urine samples were extracted (k = 3), impeding the differential investigation of extraction in urine samples. Samples were considered as clinical if they consisted of individuals with a physical or mental disorder and samples were considered as non-clinical if they consisted of healthy individuals. Clinical samples were either suffering from various psychiatric disorders (k = 1), menstrually related mood disorder (k = 1), cancer (k = 1) or migraine (k = 1), impeding the differential investigation of specific physical or mental disorders.

As the main effect was heterogeneous, we performed subgroup analyses. The detailed results are shown in TABLE 4.5. Assessment scale, use of a restrictive traumatic event criterion, and age at oxytocin assessment did not reduce heterogeneity, but specimen, extraction, type of traumatic event, frequency of traumatic event exposure, age at trauma exposure, sex, health status, recruitment setting and covariate control by means of matching did reduce heterogeneity.

The effect within saliva samples (irrespective of extraction, $k = 4$) indicated that oxytocin concentrations were lower in traumatized than in non-traumatized individuals. Within extracted saliva samples ($k = 3$) and within blood samples (irrespective of extraction, $k = 10$), no difference between traumatized and non-traumatized individuals was detected. With regard to the effect within blood samples, Egger's regression test (Egger et al., 1997) indicated no publication bias and the trim-and-fill procedure (Duval & Tweedie, 2000) imputed no missing studies. Within extracted blood samples ($k = 6$), oxytocin concentrations were higher in traumatized than in non-traumatized individuals. Egger's regression test (Egger et al., 1997) indicated no publication bias, but the trim-and-fill procedure (Duval & Tweedie, 2000) imputed four studies with lower oxytocin concentrations in traumatized individuals. Effects within urine samples ($k = 3$), within individuals exposed to interpersonal traumatic events ($k = 12$), within individuals exposed to childhood trauma ($k = 15$), within women ($k = 7$), within non-clinical samples ($k = 14$), within studies that recruited participants community-based ($k = 12$) and within studies that did not match the comparison groups for covariates ($k = 14$) remained heterogeneous. Within cerebrospinal fluid samples ($k = 2$), within individuals exposed to war-related traumatic events ($k = 3$), within individuals repeatedly exposed to traumatic events ($k = 2$), within individuals exposed to adulthood trauma ($k = 3$), within men ($k = 3$), within clinical samples ($k = 4$), within studies that recruited participants in hospital ($k = 4$) or military settings ($k = 2$) and within studies that matched the comparison groups for covariates ($k = 3$), no difference between traumatized and non-traumatized individuals was detected.

4.3.4.1.2 Correlations

The correlation (k = 16) between oxytocin concentrations and trauma exposure (n = 1,550, FIGURE 4.4) was non-significant (r = -.08 [-.20; .04], p = .19, Q = 57.11; p < .01; I^2 = 71.98).

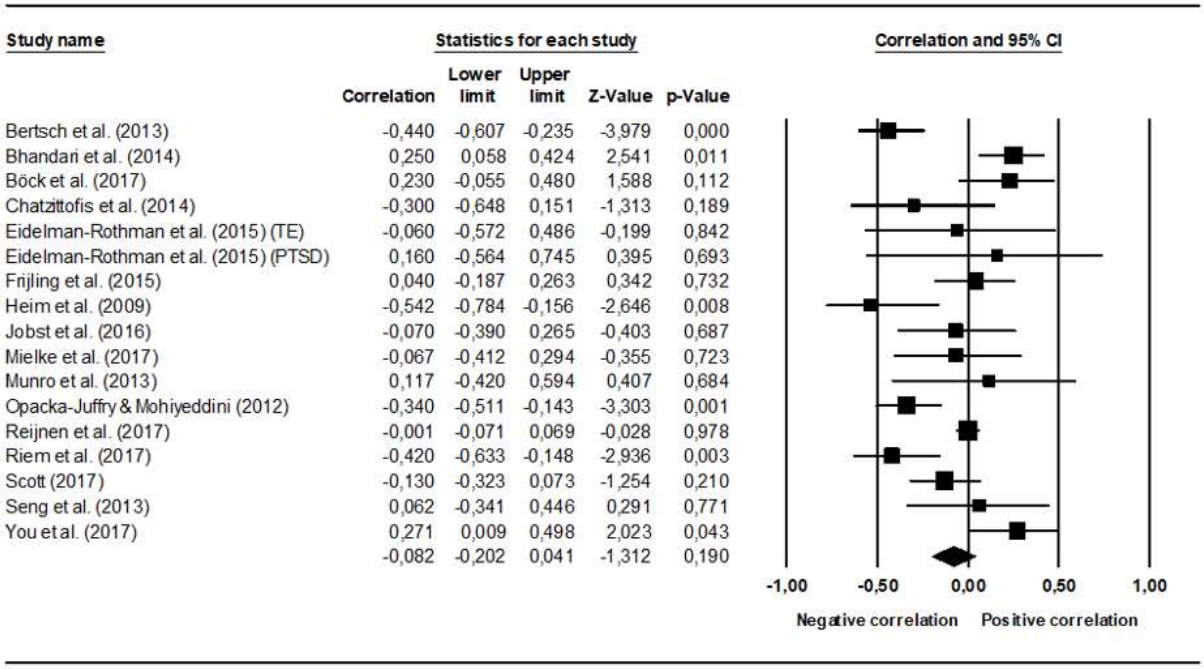

FIGURE 4.4. Correlations of endogenous oxytocin concentrations with trauma exposure. TE = traumatized individuals. PTSD = posttraumatic stress disorder.

Subgroup analyses (see TABLE 4.6) indicated that sex and health status did not significantly reduce heterogeneity, but extraction, assessment scale, restrictive traumatic event criterion, mental disorder, and recruitment setting did.

TABLE 4.6. Results of the subgroup analyses - correlation between endogenous oxytocin concentrations and trauma exposure

(Sub)group	Number of studies	Number of individuals	*r*[a]	*p*	Confidence interval[a]	Q	*p*	I^2
All studies	16	1,550	-.08	.19	-.20; .04	57.11	<.01	71.98
Details of oxytocin assessment								
Specimen								
Blood	11	1,209	-.06	.41	-.21; .08	33.99	<.01	67.64
Saliva	5	344	-.02	.84	-.24; .20	17.07	<.01	70.71
CSF	2		-.22	.60	-.75; .48	5.46	.02	81.67
Extraction								
Yes	9	1,302	-.01	.77	-.06; .05	39.30	<.01	79.64
No	3	148	-.31	.01	-.52; -.07	3.73	.15	46.33
Combinations of specimens and extraction								
Blood, extracted	5	983	.01	.92	-.23; .25	22.56	<.01	82.27
Blood, unextracted	2	126	-.27	<.01	-.43; -.10	1.93	.16	48.17
Saliva, extracted	3	319	-.06	.68	-.31; .21	16.73	<.01	82.07
Details of traumatic event exposure								
Age at traumatic event								
Childhood	15	1,527	-.07	.30	-.19; .06	56.26	<.01	75.11
Adulthood	3	655	.02	.54	-.05; .10	2.25	.52	0.00
Assessment scale								
CTQ	7	424	-.16	.22	-.39; .09	37.67	<.01	84.07
ETISR-SF	2	865	.00	.94	-.06; .07	0.74	.74	0.00
Restrictive traumatic event criterion								
Actual or threatened death, serious injury or sexual violence	3	710	.03	.36	-.04; .11	0.20	.98	0.00
Type								
Interpersonal	11	1,321	-.01	.23	-.24; .06	47.10	<.01	78.76
War-related	2	634	.03	.40	-.04; .11	0.19	.91	0.00
Sample details								
Sex								
Female	8	354	-.06	.61	-.30; .18	32.02	<.01	78.14
Male	4	948	-.19	.14	-.41; .06	16.99	<.01	76.45
Health status								
Clinical	4	144	-.15	.44	-.49; .23	12.75	<.01	76.48
Non-clinical	12	1,286	-.06	.39	-.19; .08	35.71	<.01	66.39
Mental disorder								
Borderline personality disorder	2	110	-.28	.14	-.59; .10	3.63	.06	72.55
Recruitment setting								
Community	7	362	-.11	.43	-.37; .16	35.95	<.01	83.31
Hospital	3	95	.04	.82	-.26; .33	3.83	.15	47.79
Military or police	3	888	.00	.93	-.06; .07	0.31	.96	0.00

Note. [a] negative values indicate negative correlations between oxytocin concentrations and trauma exposure. Significant *Q* coefficients and I^2 scores of 25-49 indicate mild, 50-74 moderate, and ≥75 high heterogeneity. Analyses were conducted if a minimum of two studies were available per group. Accordingly, subgroup analyses for urine samples ($k = 0$), unextracted saliva ($k = 0$), unextracted cerebrospinal fluid samples (CSF, $k = 1$), extracted CSF samples ($k = 0$) or other assessment scales than CTQ (childhood trauma questionnaire) and ETI (early trauma inventory, $k = 1$, respectively), accidental traumatic events ($k = 0$), exposure to a single traumatic event ($k = 1$) or repeated traumatic event exposure (k = 1) were not conducted. All studies measured oxytocin in adulthood ($k = 13$), impeding the differential investigation of age at oxytocin assessment. Samples were considered as clinical if they consisted of individuals with a physical or mental disorder and samples were considered as non-clinical if they consisted of healthy individuals. Clinical samples were either suffering from borderline personality disorder ($k = 2$), various psychiatric disorders ($k = 1$) or migraine ($k = 1$), impeding the differential investigation of specific physical or mental disorders, other than borderline personality disorder. Of note, both studies investigating patients with borderline personality disorder also included healthy controls.

The effect within extracted samples (irrespective of specimen, $k = 9$) remained heterogeneous, but within unextracted samples (irrespective of specimen, $k = 3$), it indicated a moderate negative correlation between trauma exposure and oxytocin. The extraction effect also occurred when taking specific assay and specimen combinations into account: Within extracted blood ($k = 5$), within extracted saliva samples (k = 3), within individuals exposed to interpersonal traumatic events ($k = 11$), and within studies that recruited participants community-based ($k = 7$) the effect remained heterogeneous. Within unextracted blood samples ($k = 2$), the effect indicated a small negative correlation between trauma exposure and oxytocin. Within studies assessing childhood trauma exposure ($k = 15$) and specifically within studies using the Childhood Trauma Questionnaire (Bernstein et al., 2003; Bernstein & Fink, 1998) effects remained heterogeneous. Within studies assessing adulthood trauma ($k = 3$), within studies using the Early Trauma Inventory (Bremner, Bolus, & Mayer, 2007) for childhood trauma exposure ($k = 2$), within studies using a restrictive traumatic event criterion ($k = 3$), within individuals exposed to war-related traumatic events ($k = 2$), within studies investigating borderline personality disorder patients ($k = 2$), and within studies that recruited participants in hospital ($k = 3$) or military or police settings ($k = 3$), no correlation was found between trauma exposure and oxytocin.

4.3.4.2 Endogenous oxytocin concentrations and PTSD

4.3.4.2.1 Group comparisons

Comparisons ($k = 8$) between PTSD patients ($n = 131$) and individuals without PTSD ($n = 835$, FIGURE 4.5) yielded a non-significant effect size ($g = 0.07$ [-0.19; 0.33], $p = .60$, $Q = 10.31$; $p = .24$; $I^2 = 22.41$). Egger's regression test (Egger et al., 1997) indicated no publication bias and the trim-and-fill procedure (Duval & Tweedie, 2000) did not impute any studies.

4.3.4.2.2 Correlations

The correlation ($k = 8$) between oxytocin and PTSD severity ($n = 962$, FIGURE 4.6) was non-significant ($r = -.08$ [-.25; .09], $p = .34$, $Q = 22.54$; $p < .01$; $I^2 = 68.94$).

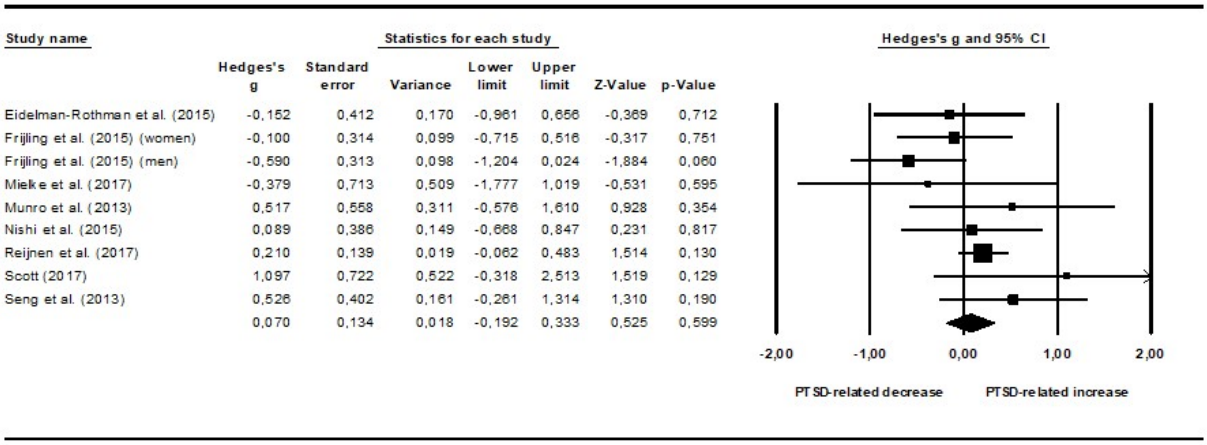

FIGURE 4.5. Comparisons of endogenous oxytocin concentrations between posttraumatic stress disorder (PTSD) patients and individuals without PTSD. Negative values indicate that oxytocin concentrations were lower in PTSD patients than in individuals without PTSD.

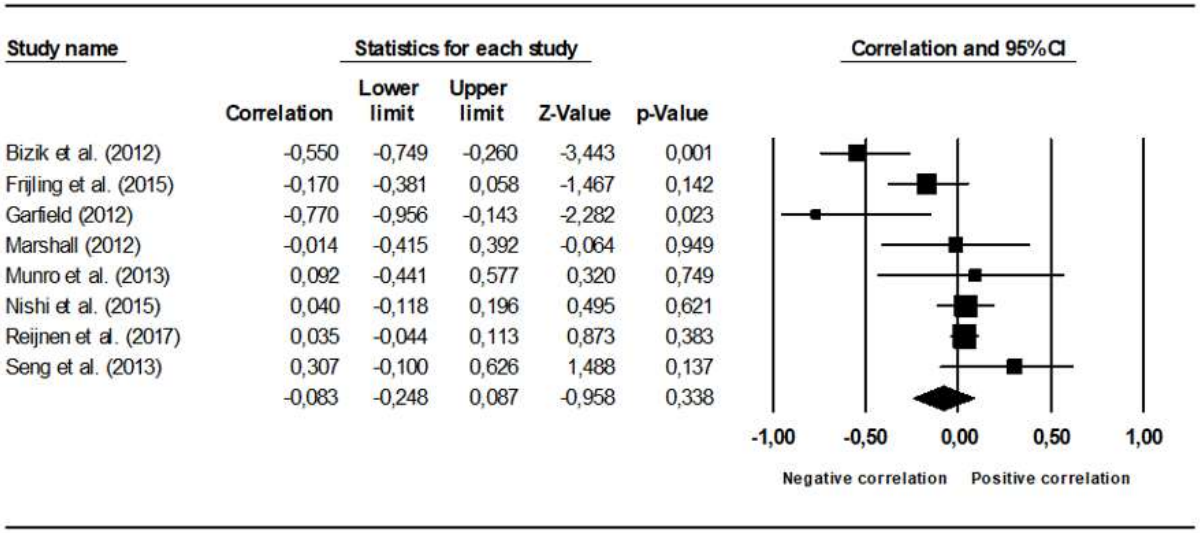

FIGURE 4.6. Correlations of endogenous oxytocin concentrations with posttraumatic stress disorder (PTSD) symptoms.

TABLE 4.7. Results of the subgroup analyses - correlation between endogenous oxytocin concentrations and posttraumatic stress disorder (PTSD) symptoms

(Sub)group	Number of studies	Number of individuals	*r*[a]	*p*	Confidence interval[a]	Q	*p*	I^2
All studies	8	962	-.08	.34	-.25; .09	22.54	<.01	68.94
Details of oxytocin assessment								
Specimen								
Blood	6	862	-.08	.46	-.29; .14	20.22	<.01	75.27
Extraction								
Yes	4	871	.02	.57	-.05; .09	2.94	.40	0.00
Combinations of specimens and extraction								
Blood, extracted	3	795	.04	.30	-.03; .11	0.04	.98	0.00
Details of traumatic event exposure								
Age at traumatic event								
Childhood	2	23	-.39	.46	-.90; .59	4.37	.04	77.11
Adulthood	2	232	-.05	.64	-.25; .15	2.21	.14	54.84
Sample details								
Age at oxytocin assessment								
Adulthood	7	928	-.00	>.99	-.12; .12	10.33	.11	41.93
Sex								
Male	3	647	-.19	.37	-.55; .23	4.82	.09	58.50
Health status								
Non-clinical	6	903	-.02	.72	-.14; .10	8.33	.14	39.99
Recruitment setting								
Hospital	3	189	-.05	.83	-.47; .39	7.31	.03	72.64
Military or police	2	700	-.04	.69	-.23; .15	2.79	.09	64.16

Note. [a] negative values indicate negative correlations between oxytocin concentrations and PTSD symptoms. Significant *Q* coefficients and I^2 scores of 25-49 indicate mild, 50-74 moderate, and ≥75 high heterogeneity. Analyses were conducted if a minimum of two studies were available per group. Accordingly, subgroup analyses for saliva samples ($k = 1$), urine samples ($k = 1$) or cerebrospinal fluid samples (CSF, $k = 0$), unextracted samples across specimens ($k = 0$), assessment scales others than CAPS ($k = 1$, respectively), underaged samples at the timepoint of oxytocin assessment ($k = 0$), female samples ($k = 1$), clinical samples ($k = 1$) or community-based recruitment ($k = 1$) were not conducted. Samples were considered as clinical if they consisted of individuals with a physical or mental disorder and samples were considered as non-clinical if they consisted of healthy individuals.

Subgroup analyses (see TABLE 4.7) showed that effects remained heterogeneous within blood samples (irrespective of extraction, $k = 6$), within individuals exposed to childhood trauma ($k = 2$), and within studies that recruited participants in hospital settings ($k = 3$). Within extracted samples (irrespective of specimen, $k = 4$), within extracted blood samples ($k = 3$), within individuals exposed to trauma in adulthood ($k = 2$), within men ($k = 3$), and within studies that recruited participants in military or police settings ($k = 2$), no correlation between oxytocin and PTSD severity was detected. For the effect within adults at oxytocin assessment ($k = 7$), we also performed Egger's regression test (Egger et al., 1997), which indicated no publication bias and the trim-and-fill procedure (Duval & Tweedie, 2000), which imputed no missing studies. The same applied to the effect within non-clinical samples ($k = 6$). Other moderators of interest could not be tested due to an insufficient number of studies within subgroups.

4.3.4.2 OXTR genotype and PTSD

Assuming that trauma exposure cannot impact genotype and genotype can only indirectly impact trauma exposure, we did not meta-analytically test associations between trauma exposure and OXTR genotype. In line with our assumptions on possible interactions between trauma processing and oxytocin parameters, we meta-analytically investigated associations between OXTR genotypes and PTSD. Two studies provided data to correlate rs53567 genotype variation (GG vs. AG/AA) with PTSD severity ($n = 909$, (Dunn et al., 2014; Lucas-Thompson & Holman, 2013). As one study (Lucas-Thompson & Holman, 2013) provided PTSD scores at two time points, both time points were considered in two separate analyses. The effect based on data from the first available time point, i.e. earlier PTSD symptoms, was small and significant ($r = -.07$ [-.14; -.01], $p = .03$, $Q = 0.11$; $p = .73$; $I^2 = 0.00$), indicating higher PTSD severity in A allele carriers. The effect based on data from the second available time point, i.e. later PTSD symptoms, was no longer significant ($r = -.02$ [-.08; .04], $p = .55$, $Q = 0.29$; $p = .59$; $I^2 = 0.00$). OXTR genotype data were not sufficient for conducting group comparisons between individuals with and without PTSD.

4.4 Discussion

4.4.1 Summary of evidence

We conducted a systematic review on k = 66 studies relating trauma exposure or PTSD to oxytocin. Most primary studies investigated endogenous oxytocin concentrations (k = 41), followed by OXTR genotype (k = 25) and OXTR methylation studies (k = 4). Studies were systematically described with regard to samples, design, quality and timing of traumatic events, of oxytocin and of psychological assessment.

In addition, we meta-analytically compared endogenous oxytocin concentrations between traumatized and non-traumatized individuals (k = 17) and correlated trauma exposure (k = 16) and PTSD symptoms (k = 8) with oxytocin. All main effects were heterogeneous. Specimen, extraction, age at trauma exposure, age at oxytocin assessment, psychometric assessment scale, restrictive trauma criterion, type of traumatic event, frequency of trauma exposure, sex, health status and recruitment setting were identified as moderators, yielding homogeneous effects in subgroup analyses. Effects within subgroups created according to methodological aspects of oxytocin assessment, that is, specimen and extraction, were inconsistent. Effects within subgroups created according to all other trauma-related and sample-related aspects were homogeneous and non-significant. The meta-analytic comparison of endogenous oxytocin concentrations between individuals with and without PTSD (k = 8) yielded a homogeneous, non-significant effect with no indication of publication bias.

The meta-analytic correlation between OXTR polymorphisms and PTSD symptoms (k = 2) yielded inconsistent results. OXTR methylation data were not sufficient to conduct meta-analyses.

4.4.2 Discussion and interpretation of results

Empirical research provides two main approaches to examine the association of oxytocin with trauma exposure and PTSD: first, group comparisons between traumatized and non-traumatized individuals and individuals with and without PTSD; and second, correlations of trauma exposure and PTSD severity with oxytocin. We considered both approaches concomitantly in our study.

Concerning the comparison of traumatized and non-traumatized individuals, as well as correlations with trauma exposure and PTSD, our main analyses on endogenous oxytocin yielded non-significant, heterogeneous effects. With regard to effects of trauma exposure, reduction of heterogeneity was achieved by considering methodological aspects, specifically specimen and extraction. Unfortunately, no clear, conclusive pattern emerged from these subgroup analyses regarding a potential hyper- or hypoactivity of the oxytocin system in traumatized individuals. Under the assumption that oxytocin can be measured comparably in different extracted and unextracted specimens, it seems implausible that we detected a negative correlation between trauma exposure and oxytocin in unextracted samples but found no effect in extracted samples. Moreover, it also seems implausible that within saliva samples, oxytocin concentrations were lower in traumatized than in non-traumatized individuals, whereas extracted blood samples showed a trauma-related increase. Publication bias detected for the latter effect might partly explain this discrepancy. However, this finding might also be related to a common problem in research on endogenous oxytocin: the lack of knowledge about the validity of measurements in different specimens (Hoffman, Brownley, Hamer, & Bulik, 2012; Valstad et al., 2017) and using different assays (Leng & Sabatier, 2016; Szeto et al., 2011). Concentrations measured in peripheral specimens do not reflect central or even brain region-specific availability (Valstad et al., 2017). Thus, behavioral effects correlated with peripheral concentrations do not necessarily correspond with oxytocin's central actions.

A recent study reported that oxytocin concentrations in saliva were more strongly correlated with central oxytocin compared to those in plasma (Martin et al., 2018), but the exact pathways of oxytocin distribution through the human body (Valstad et al., 2017), the dependence of the effects of oxytocin on receptor function (Dadds et al., 2014) as well as the validity of different oxytocin assays (Leng & Sabatier, 2016), especially those applying unextracted measurements (Szeto et al., 2011), are not yet comprehensively understood. Our meta-analysis demonstrates that a lack of understanding of these basic processes limits the informative value of research on endogenous oxytocin in mental disorders.

We identified various relevant demographic and trauma-related moderators, but all homogeneous effects identified in our subgroup analyses were non-significant. Moreover, the homogeneous overall comparison between individuals with and without PTSD revealed no significant difference. Thus, the

present original data indicate that unstimulated oxytocin does not seem to be a biomarker either for trauma exposure or for PTSD. Given the pivotal role of oxytocin in modulating the neuroendocrine stress axis (Ditzen et al., 2009; Heinrichs et al., 2003; Windle et al., 1997), it might be worthwhile to investigate trauma- or PTSD-related differences in the oxytocin response to acute stress. In future studies, we recommend applying traumatic stress reactivity paradigms rather than unstimulated measurements. Furthermore, although no effects were detected with regard to current PTSD symptoms, endogenous oxytocin concentrations might be altered at different stages of PTSD development or recovery (Galatzer-Levy et al., 2018). Therefore, it might be useful to prospectively investigate the impact of endogenous oxytocin concentrations on traumatic event processing and PTSD symptom development. In addition, it would be interesting to assess endogenous oxytocin as a possible correlate of PTSD symptom reduction in psychotherapeutic or pharmacological interventions, but also in natural recovery.

Due to a lack of sufficient comparable data from studies on OXTR methylation, we were unable to meta-analytically summarize epigenetic findings. We meta-analytically correlated OXTR genotype with PTSD severity, but the results were inconsistent. Whereas one analysis yielded a small effect, indicating higher PTSD severity in A allele carriers, this effect was not present when using data from the same study but at a different time point of PTSD assessment. Thus, it might be worthwhile to consider the timing of trauma exposure and PTSD development in research on OXTR function. To date, research on both markers, OXTR genotype and methylation, is still too sparse to draw meaningful conclusions, and further investigation is therefore warranted in this field.

4.4.3 Limitations and future directions

However, it needs to be admitted that the genetic arm of our systematic review and meta-analysis had a very narrow focus, as we only included studies that specifically investigated OXTR genotype or methylation. Expanding this focus by additionally aggregating data from genome-wide association studies would significantly increase the body of available studies to summarize. Therefore, we highly recommend this approach for future meta-analyses in the field.

Furthermore, it is worth discussing that we defined broad inclusion criteria with regard to traumatic events and posttraumatic stress symptoms. Consequently, our systematic review described studies that substantially differed with regard to their underlying conceptualizations of traumatic events and posttraumatic stress symptoms. Many traumatic events that were investigated in the included primary studies indeed did not fulfill the criteria of common classification systems, and in a stricter sense might rather be described as adverse experiences. Therefore, our broad inclusion criteria might have contributed to the heterogeneity observed in all main analyses on endogenous oxytocin. However, as this is the first systematic review and meta-analysis on this topic, we prioritized drawing a complete picture of the current state of research over summarizing a smaller, strictly defined pool of studies. This allowed for a comprehensive overview of this field of research and can serve as a basis for future, more specific investigations.

Another limitation of the present study lies in our conceptualization of non-traumatized comparison groups. We classified individuals who did not report exposure to specific traumatic events of interest in the primary studies as non-traumatized. However, this does not imply that these individuals had not experienced any traumatic event at all. This problem is particularly relevant in terms of the large number of primary studies that based their assessments on questionnaires which were designed to investigate exposure to specific traumatic events but did not comprehensively measure lifetime trauma. With regard to our meta-analyses, this might have caused heterogeneity within non-traumatized comparison groups, which we were unable to control for. Future empirical studies might address this problem by administering comprehensive, standardized clinical interviews on lifetime traumatic experiences in their control groups.

4.4.4 Conclusions

The current state of research does not allow for conclusions about the associations between OXTR genotype or methylation and trauma exposure or PTSD. Concerning endogenous oxytocin, we showed that unstimulated measurements are not an adequate biomarker for trauma exposure or PTSD. The inconsistent impact of specimen and extraction shows that more fundamental, methodological knowledge is needed in order to interpret the differential findings on trauma exposure in a meaningful

way. Future research might investigate oxytocin stress reactivity rather than unstimulated measurements and focus on PTSD symptom development and recovery rather than cross-sectional comparisons of manifest symptom statuses. Therefore, longitudinal studies, particularly prospective ones or therapeutic intervention studies, are warranted. Moreover, we summarized primary studies using a broad range of conceptualizations of traumatic events and posttraumatic stress. To increase consistency within this field of research, we recommend that future studies should precisely define these concepts according to established diagnostic systems and comprehensively assess lifetime trauma exposure. Finally, in order to comprehensively evaluate oxytocin system functioning in traumatic event processing and PTSD, future studies might combine assessments of all currently established parameters, that is, endogenous concentrations, OXTR genotype and methylation, as they are interdependent (Dadds et al., 2014; Gregory et al., 2009).

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Conflicts of interest

Mrs. Engel, Mrs. Klusmann, Mr. Laufer, Dr. Pfeifer, Prof. Dr. Ditzen, Dr. van Zuiden, Prof. Dr. Knaevelsrud and Dr. Schumacher have no conflicts of interest to declare.

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*reference was included in systematic review.

Contributions

Sinha Engel, Ann-Christin Pfeifer and Sarah Schumacher designed the study.

Sinha Engel developed the search strategy, supervised the literature search and study screening. Bettina Westerhoff performed the literature search. Bettina Westerhoff and Sophie Ibert performed the study screening.

Sinha Engel and Anna-Lena Sznur performed the data extraction, under Sarah Schumacher's supervision.

Hannah Klusmann and Sebastian Laufer performed the quality rating, under Sinha Engel's supervision.

Sinha Engel contacted the authors of all primary studies for which data for the meta-analysis were not reported in the publication, performed the statistical analyses and drafted the manuscript. All authors critically revised the manuscript for important intellectual content.

Sarah Schumacher, Christine Knaevelsrud, Beate Ditzen and Mirjam van Zuiden supervised the study.

Online supplementary material

The following supplementary material is available online:

ONLINE SUPPLEMENTARY MATERIAL 4.1. Full electronic search strategy

ONLINE SUPPLEMENTARY MATERIAL 4.2. Full list of studies included in systematic review

ONLINE SUPPLEMENTARY MATERIAL 4.3. Abbreviations and references of assessment scales reported in the manuscript and tables

ONLINE SUPPLEMENTARY MATERIAL 4.4. Genomic coordinates of sites investigated with regard to traumatic events or posttraumatic stress disorder

ONLINE SUPPLEMENTARY MATERIAL 4.5 Detailed results of the Newcastle-Ottawa Quality Assessment Scale ratings

Available online at:

https://www.sciencedirect.com/science/article/abs/pii/S0149763419300466?via%3Dihub

CHAPTER 5

Oxytocin and vasopressin in internet-based cognitive-behavioral treatment for posttraumatic stress disorder

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Oxytocin and vasopressin in internet-based cognitive-behavioral treatment for posttraumatic stress disorder

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Abstract

Background. Posttraumatic stress disorder (PTSD) is characterized by impairments in extinction learning and social behavior, which are targeted by trauma-focused cognitive behavioral treatment (TF-CBT). The biological underpinnings of TF-CBT can be understood better by adding biomarkers to the clinical evaluation of interventions. Due to their involvement in social functioning and fear processing, oxytocin and arginine vasopressin might be informative biomarkers for TF-CBT, but to date, this has never been tested.

Objective. To differentiate the impact of traumatic event exposure and PTSD symptoms on endogenous oxytocin and vasopressin concentrations. Further, to describe courses of PTSD symptoms, oxytocin and vasopressin during an internet-based TF-CBT and explore interactions between these parameters.

Method. We compared oxytocin and vasopressin between three groups of active and former male service members of the German Armed Forces (n = 100): PTSD patients (n = 39), deployed healthy controls who experienced a deployment-related traumatic event (n = 33) and non-deployed healthy controls who never experienced a traumatic event (n = 28). PTSD patients underwent a 5-week internet-based TF-CBT. We correlated PTSD symptoms with oxytocin and vasopressin before treatment onset. Further, we analyzed courses of PTSD symptoms, oxytocin and vasopressin from pre- to post-treatment and 3 months follow-up, as well as interactions between the three parameters.

Results. Oxytocin and vasopressin did not differ between the groups and were unrelated to PTSD symptoms. PTSD symptoms were highly stable over time, whereas the endocrine parameters were not, and they also did not change in mean. Oxytocin and vasopressin were not associated with PTSD symptoms longitudinally.

Conclusions. Mainly due to their insufficient intraindividual stability, single measurements of endogenous oxytocin and vasopressin concentrations are not informative biomarkers for TF-CBT. We discuss how the stability of these biomarkers might be increased and how they could be better related to the specific impairments targeted by TF-CBT.

Keywords: Psychotherapy, Cognitive behavioral therapy, Neuropeptide, Online intervention, Soldiers, Military

Highlights (Summary of the article in lay terms)

To understand better why trauma-focused psychotherapy is effective, biomarkers can be added to intervention studies.

We tested whether the hormones oxytocin and vasopressin are informative biomarkers.

We found out that they are not, as they change too quickly within individuals.

5.1 Introduction

Posttraumatic stress disorder (PTSD) is a burdensome disease that causes severe impairments in individuals' lives. PTSD can develop in response to a traumatic event, that is, exposure to actual or threatened death, serious injury or sexual violence, and includes symptoms such as intrusions, avoidance, negative alterations in cognition and mood, as well as marked alterations in arousal and reactivity (American Psychiatric Association, 2013). Globally, PTSD lifetime prevalence rates range from 1.3 to 8.9%, depending on country-specific, but also individual risk factors (Atwoli, Stein, Koenen, & McLaughlin, 2015). Among the latter, belonging to professional groups characterized by frequent and severe traumatic event exposure, such as rescue workers (Berger et al., 2012), war reporters (Feinstein, Owen, & Blair, 2002) or soldiers (Hoge et al., 2004; Wittchen et al., 2012) increases PTSD risk. Furthermore, social factors, such as exposure to an interpersonal traumatic event (Shalev et al., 2019), particularly sexual (Schumm, Briggs-Phillips, & Hobfoll, 2006; Tolin & Foa, 2006) or intimate partner violence (Forbes et al., 2014), as well as low perceived social support after traumatic event exposure (Andrews, Brewin, & Rose, 2003; Brewin, Andrews, & Valentine, 2000; Ozer, Best, Lipsey, & Weiss, 2003; Schumm et al., 2006) increase individuals' risk to develop PTSD.

From a neurocognitive perspective, PTSD development and maintenance can be explained by deficient extinction learning (for overviews, see Parsons & Ressler, 2013; Zuj, Palmer, Lommen, & Felmingham, 2016). Fear learning represents a normal process after traumatic event exposure that can be regarded as adaptive. Learning that certain cues or contexts that co-occurred with a traumatic event might indicate danger helps individuals to avoid dangerous situations in the future. However, subsequent impaired extinction learning can be regarded as a maladaptive process after traumatic event exposure, as fear responses to trauma reminders are maintained even after repeatedly experiencing that the reminders no longer co-occur with the anticipated event (Zuj & Norrholm, 2019). Several studies reported deficient extinction learning in PTSD, as reflected in increased general and differential fear responses during extinction acquisition (Orr et al., 2000; Peri, Ben-Shakhar, Orr, & Shalev, 2000; Steiger, Nees, Wicking, Lang, & Flor, 2015; Wessa & Flor, 2007), as well as impaired extinction recall (Garfinkel et al., 2014; Milad et al., 2008; Milad et al., 2009).

Extinction learning is promoted in trauma-focused cognitive behavioral therapy (TF-CBT), which is one of the gold standard PTSD treatments (National Institute of Clinical Excellence, 2018). TF-CBT encompasses programs such as cognitive processing therapy (Monson et al., 2006; Resick & Schnicke, 1993), (prolonged) exposure therapy (Foa et al., 2005; Foa, Rothbaum, Riggs, & Murdock, 1991) or narrative exposure therapy (Hermenau, Hecker, Schaal, Maedl, & Elbert, 2013; Neuner, Schauer, Klaschik, Karunakara, & Elbert, 2004). They aim at promoting the adaptive processing of traumatic memories which is, among other techniques, achieved by psychotherapist-guided exposure. During exposure, PTSD patients repeatedly confront themselves with their traumatic memories by describing their sensory perceptions, behaviors, emotions, physiological responses and cognitions during the traumatic event, in a safe environment and supported by their psychotherapist. Over several exposure sessions, analogous to extinction learning, their initially strong, negative emotional response to the traumatic memories decreases. In recent years, technological advances made it possible to deliver TF-CBT via the internet. This helps to overcome emotional, social and practical barriers to care (Hoge et al., 2004; Hoge et al., 2014) and thereby, to reach an increasing number of individuals who would otherwise not commit to psychotherapy. Meta-analytic research has proven that internet-based and face-to-face TF-CBTs are equally effective (Kuester, Niemeyer, & Knaevelsrud, 2016).

Given that effective treatments for PTSD exist and innovative methods to disseminate them have been developed, it is a current challenge to better understand the exact mechanisms of action underlying TF-CBT. By identifying its active ingredients, its efficacy and effectiveness can be further improved, accelerating remission and increasing response rates (Stojek, McSweeney, & Rauch, 2018). Therefore, it has been suggested to add measurements of biological correlates of behaviors, cognitions or emotions of interest to clinical evaluations of TF-CBT (Fischer & Ehlert, 2019; Yehuda, Flory, Southwick, & Charney, 2006). In this regard, two neuroanatomically closely related neuropeptides and hormones, that is, oxytocin and arginine vasopressin, seem particularly interesting. Oxytocin has previously been related to a range of positive social behaviors in healthy humans, such as caring parenting behaviors (Feldman et al., 2012; Feldman, Gordon, & Zagoory-Sharon, 2011), trust (Kosfeld, Heinrichs, Zak, Fischbacher, & Fehr, 2005; Mikolajczak, Pinon, Lane, Timary, & Luminet, 2010), cooperative communication (Ditzen et al., 2009), or interpersonal closeness (Riem et al., 2019). In contrast,

vasopressin has been associated with aggression (Coccaro, Kavoussi, Hauger, Cooper, & Ferris, 1998), decreased differentiation between neutral and threatening social stimuli (Thompson, Gupta, Miller, Mills, & Orr, 2004) and increased physiological responses to threatening social stimuli (Thompson, George, Walton, Orr, & Benson, 2006). Oxytocin's and vasopressin's involvement in social functioning already suggest their investigation as biomarkers of TF-CBT, as social factors contribute to PTSD development (Andrews et al., 2003; Brewin et al., 2000; Ozer et al., 2003; Schumm et al., 2006; Shalev et al., 2019) and a positive therapeutic relationship is a crucial basis for successful TF-CBT (Cloitre, Stovall-McClough, Miranda, & Chemtob, 2004). Further, as both neuropeptides influence fear and extinction learning, the neurocognitive process underlying PTSD development and exposure therapy. In this regard, oxytocin has often been associated with anxiolytic effects, whereas vasopressin's effects appear to be fear-promoting: In rats, central administration of synthetic oxytocin during fear conditioning decreased, whereas the same dose of vasopressin increased fear responses (Roozendaal et al., 1992). Further, post-conditioning central administration of oxytocin hexapeptide fragments decreased, while administration of vasopressin hexapeptide fragments increased fear recall (Stoehr, Cramer, & North, 1992). In healthy humans, intranasal administration of oxytocin decreased fear responses during extinction acquisition and recall (Acheson et al., 2013; Eckstein et al., 2015), whereas animal studies indicated that least high doses of centrally administered vasopressin delayed this process (Hayes & Chambers, 2005). However, it is worth noting that, oxytocin's and vasopressin's effects on social functioning and fear processing they are not uniform, but instead strongly modulated by contextual and individual factors (Olff et al., 2013; Roozendaal et al., 1992; Toth, Neumann, & Slattery, 2012). Regarding the investigation of these neuropeptides in research on PTSD treatments, evidence is still scarce. Some studies evaluated the effects of a single intranasal oxytocin administration on selected functions that are impaired in PTSD (e.g. Koch et al., 2019; Nawijn et al., 2016, 2017; Sack et al., 2017), and so far, one study evaluated the effects of intranasal oxytocin as potential TF-CBT enhancer (Flanagan, Sippel, Wahlquist, Moran-Santa Maria, & Back, 2018). The present study is the first one that included measurements of both, oxytocin and vasopressin into the clinical evaluation of TF-CBT.

Specifically, this study aimed at answering three main scientific questions:

First, we aimed at differentiating the impact of traumatic event exposure and PTSD on endogenous oxytocin and vasopressin concentrations. Therefore, we cross-sectionally compared oxytocin and vasopressin between PTSD patients, healthy controls who were exposed to a deployment-related traumatic event and healthy controls who were never deployed.

Second, we aimed at exploring the associations of oxytocin and vasopressin with PTSD symptoms. Therefore, we tested whether these parameters were correlated in PTSD patients before onset of a 5-week, internet-based TF-CBT.

Third, we aimed at describing courses and interactions between oxytocin, vasopressin and PTSD during an online psychotherapeutic intervention. Therefore, we examined courses of these three parameters from pre- to post-treatment and 3 months follow-up. We were particularly interested to explore whether oxytocin and vasopressin interacted with PTSD symptoms over time.

5.2 Methods and materials

5.2.1 Participants

Participants were active and former service members of the German Armed Forces (n = 100). We initially intended to recruit female and male service members. However, given the significantly lower proportion of women in the German Armed Forces (approximately 12%), which is even lower among deployed service members, it became evident that it was not possible to recruit a number of women that was sufficiently high for the purpose of this investigation, especially as additional factors such as menstrual cycle phase need to be considered in endocrine studies in women. Therefore, all participants in this study were male. Three groups were investigated: PTSD patients (n = 39; M = 37.74 years; SD = 9.62 years), deployed healthy controls (n = 33; M = 38.36 years; SD = 7.98 years) and non-deployed healthy controls (n = 28; M = 26.36 years; SD = 4.30 years). The group of PTSD patients consisted of service members who were treatment seeking for PTSD. Both healthy control groups did not fulfill criteria for PTSD or any other mental disorder, as confirmed by the German version of the Mini-

International Neuropsychiatric Interview (Ackenheil et al., 1999; Sheehan et al., 1998). The first healthy control group consisted of service members who were deployed abroad and reported a deployment-related traumatic event according to the DSM-5 criterion (American Psychiatric Association, 2013), as confirmed in a telephone screening interview and by the List of the Mental Health Advisory Team (Zimmermann et al., 2014). The second healthy control group consisted of service members who were never deployed abroad and reported no traumatic event according to the DSM-5 criterion (American Psychiatric Association, 2013). General exclusion criteria that applied to all groups were acute psychosis, acute manic episode, current substance abuse or dependence, current suicidal ideation, neurological disorder, acute somatic disease, concurrent psychotherapeutic treatment, or unstable psychotropic medication.

Participants were recruited via advertisements in military journals, on websites and in online chat rooms for service members. Printed flyers and posters were distributed in a number of health service centers and German Armed Forces Military Hospitals. Moreover, unit commanders distributed flyers in after-deployment seminars and the study was introduced to military psychologists and psychiatrists at German Armed Forces mental health conferences.

5.2.2 Study design

This study is part of a randomized waitlist-controlled trial investigating the feasibility, acceptability and efficacy of a 5-week internet-based TF-CBT in German Armed Forces service members (Niemeyer et al., 2020). The study design is explained and illustrated in TABLE 5.1. The intervention was based on the treatment protocols of Interapy (Lange et al., 2003) and Integrative Testimonial Therapy (Knaevelsrud, Böttche, Pietrzak, Freyberger, & Kuwert, 2017) and adapted to the military context. It encompassed 10 modules of writing assignments completed by the PTSD patients for which they received written feedback by the therapists. It was structured in three treatment phases: biographical reconstruction, exposure and cognitive restructuring. Before assignment to the trial, PTSD patients were - based on a computer-generated randomization list - assigned to the waitlist or non-waitlist condition. Patients in the waitlist condition waited for six weeks before starting treatment, patients in the non-waitlist condition started treatment immediately. Patients in the waitlist condition completed four

assessments at the German Armed Forces Military Hospital Berlin, in which psychological and biological data was collected: a pre-waiting, pre-treatment, post-treatment and follow-up assessment. Patients in the non-waitlist condition completed three assessments: pre-treatment, post-treatment and follow-up. Healthy controls completed one assessment.

In order to address our first scientific question, that is, cross-sectional group comparisons, data of each group's respective first assessment was used. For the purpose of the second and third scientific question, that is, the investigation of interactions between PTSD symptoms and endocrine parameters in relation to the internet-based TF-CBT, PTSD patients' pre-treatment, post-treatment and follow-up data was used. Data was collected between July 2016 and July 2018.

The study was pre-registered in the Australian Clinical Trials Registry (ACTRN 12616000956404). After internal approval by the German Armed Forces, the study was approved by the Ethics committee of Freie Universität Berlin (reference number: 85/2014; addendum: 116 /2016).

5.2.3 Psychological assessments

The German translation of the Clinician Administered PTSD Scale for DSM-5 (CAPS; Weathers et al., 2018) was used to assess PTSD symptoms and diagnosis. The CAPS measures PTSD symptoms in the four domains: re-experiencing symptoms, avoidance symptoms, negative alterations in cognition and mood and alterations in arousal and reactivity. By means of a standardized interview with the patient, a clinician (a trained master's level or a PhD student) rated the severity of symptoms experienced during the last month on a 5-point scale ranging from 0 = *not present* to 4 = *extreme*.

TABLE 5.1 Overview of assessments, flow of participants and available data

	Pre-waiting	Pre-treatment	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	Post-treatment	Follow-up
Assessments														
Deployed healthy controls	-	✓											-	-
Non-deployed healthy controls	-	✓											-	-
PTSD patients, waitlist condition	✓	✓											✓	✓
PTSD patients, non-waitlist condition	-	✓											✓	✓
Flow of participants														
n (deployed healthy controls)	0	33	0	0	0	0	0	0	0	0	0	0	0	0
n (non-deployed healthy controls)	0	28	0	0	0	0	0	0	0	0	0	0	0	0
n (PTSD patients, waitlist condition)	19	17	17	16	16	13	13	13	13	13	13	13	13	12
n (PTSD patients, non-waitlist condition)	0	20	14	13	12	9	9	9	8	8	8	8	8	7
Available data														
CAPS														
n (deployed healthy controls)	0	33											0	0
n (non-deployed healthy controls)	0	0											0	0
n (PTSD patients, waitlist condition)	19	17											13	12
n (PTSD patients, non-waitlist condition)	0	20											8	7
Oxytocin														
n (deployed healthy controls)	0	33											0	0
n (non-deployed healthy controls)	0	26[1]											0	0
n (PTSD patients, waitlist condition)	19	17											12[2]	11[2]
n (PTSD patients, non-waitlist condition)	0	18[2,3]											8	7
Vasopressin														
n (deployed healthy controls)	0	33											0	0
n (non-deployed healthy controls)	0	28											0	0
n (PTSD patients, waitlist condition)	19	17											13	12
n (PTSD patients, non-waitlist condition)	0	19[3]											8	7

Note. [1] Data from two participants were defined as outliers and therefore removed. [2] Data from one participant was defined as outlier and therefore removed. [3] Data from one participant is missing as assessment was cancelled before blood sampling. M = module; CAPS = Clinician-Administered PTSD Scale; ü = Assessment was conducted in the respective group. - = Assessment was not conducted in the respective group. The rows representing assessments show when and how often posttraumatic stress disorder (PTSD) symptoms, oxytocin and vasopressin were assessed in the respective groups. Deployed and non-deployed healthy controls were assessed at one timepoint and did not receive the internet-based trauma-focused cognitive behavioral treatment. PTSD patients who were randomly assigned to the waitlist condition were assessed at four timepoints: once before a 6-week waiting period (TX), as well as pre-treatment (T1), post-treatment (T2) and at follow-up (T3). PTSD patients who were randomly assigned to the non-waitlist condition were assessed at three timepoints: T1, T2 and T3. The rows representing flow of participants show the number of participants assessed per group and timepoint. They also depict study and therapy dropout. The rows representing available data show the number of available data for our outcomes of interest per group and timepoint. PTSD symptoms were not assessed in non-deployed healthy controls. For cross-sectional baseline comparisons, variables of participants' respective first assessments were compared. TX was the first assessment for PTSD patients assigned to waitlist condition and T1 was the first assessment for PTSD patients assigned to non-waitlist condition and both healthy control groups. For longitudinal analyses, PTSD patients' T1, T2 and T3 data were used. This implies that data from PTSD patients assigned to waitlist- and non-waitlist condition were combined.

5.2.4 Biological assessments

5.2.4.1 Sampling and biochemical analyses

Endogenous oxytocin and vasopressin concentrations were measured in blood. At 8 a.m. on the first day of assessment in the case of a single-day assessment or on the second day in the case of a two-days assessment, participants were invited to the laboratory. Time of blood sampling was recorded and we observed high compliance to the protocol: In 83.33%, blood was drawn exactly at 8 a.m.. Deviations were $M = 3.00$ $SD = 10.00$ [0.00; 75.00] minutes. Patients were instructed not to eat, drink (except for water), consume caffeine or nicotine before sampling. Compliance to these instructions was reported by the patients in 91.67% of the samples (143 samples, eat), 96.79% (151 samples, drink), 89.10% (139 samples, caffeine) and 79.49% (124 samples, nicotine), respectively. Furthermore, age, body weight, body height, and leucocytes were assessed. In 96.15% of the samples (150 samples), leucocyte values were within the normal range, 1.92% (3 samples) were considered as too low, 0.61% (1 sample) as too high and for 1.21% (2 samples), leucocyte values were unavailable.

Blood was collected in 9.00 ml serum tubes (Sarstedt, Germany). After sampling, tubes were softly shaken, then put aside for 30 minutes, protected from light, in order to allow blood to plot. Then, tubes were centrifuged at 1000xg for 10 minutes and serum was pipetted into smaller, 1.50 ml tubes (Eppendorf, Germany). Samples were stored in a freezer at -80°C. After completion of data collection, all samples were sent to the cooperating laboratory. They were extracted and analyzed using a highly sensitive and selective RIA (RIAgnosis, Regensburg, Germany), as described in Landgraf, Neumann, Holsboer, and Pittman (1995) and in Landgraf and Neumann (2004). All samples were measured within the same assay, in order to avoid inter-assay variability. Intra-assay variability was < 10%. The detection limit was 0.1 - 0.5 pg/ml for both oxytocin and vasopressin, depending on the age of the tracers. No sample was below the detection limit. There was no significant cross-reactivity with structurally related peptides including the ring hexapeptides and the terminal tripeptides of oxytocin and vasopressin.

5.2.4.2 Preparation of biological data

Visual inspection and descriptive statistics revealed that oxytocin data was not normally distributed (*M* = 4.50; *SD* = 1.88 [1.77; 13.90]; skewness = 2.76; curtosis: 9.93). Five outliers were identified, deviating more than 3 *SD* from *M*. Outlier removal and log-transformation (LG10(1+oxytocin)) resulted in normally distributed data that was used for analyses (*M* = 0.71; *SD* = 0.09 [0.44; 1.04]; skewness: 0.19; curtosis: 0.55). With regard to vasopressin, visual inspection and descriptive statistics revealed normally distributed data and no outliers (*M* = 3.50; *SD* = 0.53 [2.22; 5.01]; skewness = 0.02; curtosis: -0.72). Accordingly, complete and non-transformed data was used for analyses.

5.2.5 Statistical analyses

5.2.5.1 Group differences in endocrine parameters

For cross-sectional group comparisons, we conducted two one-way ANOVAs, using group (PTSD patients vs. deployed healthy controls vs. non-deployed healthy controls) as factor and oxytocin and vasopressin as outcomes.

5.2.5.2 Correlations before internet-based trauma-focused cognitive behavioral treatment (TF-CBT) onset

We used Pearson's coefficient *r* to correlate pre-treatment oxytocin, vasopressin and PTSD symptoms (total CAPS scores).

5.2.5.3 Courses of endocrine parameters and PTSD symptoms

In order to describe courses of oxytocin, vasopressin and PTSD symptoms, we performed three repeated-measures ANOVAs from pre-treatment over post-treatment to follow-up assessments. Time was used as within-factor, while oxytocin, vasopressin and PTSD symptoms were used as the respective outcomes. As both PTSD patient groups (waitlist and non-waitlist condition) were collapsed for longitudinal analyses, there was no between-factor.

5.2.5.4 Interactions of endocrine parameters with PTSD symptoms over time

In order to explore interactions between oxytocin and vasopressin with PTSD symptoms over time, we tested whether pre-treatment values of one parameter were correlated with post-treatment values of another parameter. In addition, we tested whether post-treatment values of one parameter were correlated with follow-up values of another parameter. Moreover, we created change scores, indicating an increase (positive value) or a decrease (negative value) in oxytocin, vasopressin or PTSD symptoms from one assessment to the next. We tested whether pre- and post-treatment values of one parameter were correlated with change scores from pre- to post-treatment or from post-treatment to follow-up in another parameter. Vice versa, we explored whether change scores from pre-to post-treatment and from post-treatment to follow up in one parameter were correlated with post- or follow-up values of another parameter.

Concerning the analyses in the context of the internet-based TF-CBT, all results that are presented in the manuscript are based on data of those PTSD patients with available PTSD symptoms, oxytocin and vasopressin and data at pre-treatment, post-treatment and follow-up (complete cases, n = 16). This comparatively low number of cases is mainly due to the high dropout rate (as explained in Niemeyer et al., 2020). In order to test the robustness of our results, descriptive and correlational analyses were also conducted in all cases with available data in the respective parameter per assessment (n = 17 - 37). Results were compared and no significant differences were found. An overview of longitudinal PTSD symptoms, oxytocin and vasopressin data, as well as their longitudinal correlations and correlations with change scores is given in this dissertation's SUPPLEMENTARY MATERIAL 5, 6, and 7 for all and complete cases, respectively,

Non-adjusted α was defined as .05. All analyses were performed with SPSS Statistics, version 25 (IBM).

5.3 Results

5.3.1 Flow of participants

The number of participants and available data for our outcomes of interest are illustrated in TABLE 5.1. Baseline demographic, trauma-related and psychological variables are presented in TABLE 5.2. It reveals group differences in age: Non-deployed healthy controls were younger than PTSD patients and deployed healthy controls. As deployment is a common event in most military careers, service members are non- (or not yet-)deployed only at early career stages and thus, at younger age. Except for age, no demographic group differences were detected.

Concerning deployment-related traumatic event exposure, in PTSD patients, having seen destroyed houses or villages (n = 33, 84.62%), knowing someone seriously injured or killed (n = 29, 74.36%) and having seen (parts of) dead bodies (n = 28, 71.79%) were the most frequently reported potentially traumatic events, according to the List of the Mental Health Advisory Team (Zimmermann et al., 2014). In deployed healthy controls, having seen destroyed houses or villages (n = 26, 78.79%), having experienced hostility by civilians (n = 20, 60.61%) and having seen (parts of) dead bodies (n = 19, 57.58%) were the most frequently reported potentially traumatic events. As table 2 shows, PTSD patients and deployed healthy controls did not differ with regard to deployment-related variables, but as expected, they differed with regard to PTSD symptoms.

5.3.2 Group differences in endocrine parameters

The cross-sectional group comparison did not reveal any differences in endogenous oxytocin or vasopressin concentrations between PTSD patients, deployed healthy controls or non-deployed healthy controls (see TABLE 5.2 and FIGURE 5.1).

TABLE 5.2. Baseline comparisons of demographic, posttraumatic stress disorder (PTSD) symptom-related and endocrine variables

	PTSD patients (*n* = 39)	**Deployed healthy controls (*n* = 33)**	**Non-deployed healthy controls (*n* = 28)**	**Statistics**
Demographic information				
Age	37.74 (9.62)[a]	38.36 (7.98)	26.36 (4.30)	$F_{(2, 96)} = 22.09$, $p < .01$
BMI	26.84 (3.06)[a]	26.55 (3.06)[a]	25.54 (2.42)	$F_{(2, 91)} = 1.77$, $p = .18$
Number of cigarettes per day	0.94 (1.12)[a]	0.42 (0.75)	0.55 (0.96)[a]	$F_{(2, 88)} = 2.72$, $p = .07$
Number of deployments	2.84 (3.05)[a]	3.21 (2.76)	-	$F_{(1, 69)} = 0.28$, $p = .60$
Total number of days deployed	357.03 (369.94)[a]	415.31 (574.40)[a]	-	$F_{(1, 66)} = 0.27$, $p = .60$
PTSD symptoms				
PTSD diagnosis (*n*, %)	25, 64.10	0, 0.00	-	
Overall symptoms	35.05 (14.71)	1.88 (4.08)	-	$F_{(1, 70)} = 157.19$, $p < .01$
Re-experiencing symptoms	9.87 (4.32)	0.36 (1.11)	-	$F_{(1, 70)} = 150.83$, $p < .01$
Avoidance symptoms	3.92 (2.07)	0.18 (0.72)	-	$F_{(1, 70)} = 97.43$, $p < .01$
Negative alterations in cognition and mood	11.13 (6.04)	0.18 (0.53)	-	$F_{(1, 70)} = 107.61$, $p < .01$
Alterations in arousal and reactivity	10.13 (4.34)	1.15 (2.42)	-	$F_{(1, 70)} = 111.71$, $p < .01$
Endocrine parameters				
Oxytocin log (pg/ml)	0.72 (0.10)[a]	0.74 (0.09)	0.68 (0.12)[a]	$F_{(2, 93)} = 2.32$, $p = .10$
Vasopressin pg/ml	3.55 (0.60)[a]	3.52 (0.48)	3.54 (0.63)	$F_{(2, 96)} = 0.05$, $p = .95$

Note. [a] Due to missing values, information was not available for all participants (see also TABLE 5.1 for main outcomes). If not indicated differently, descriptive information is presented as *M* (*SD*) and comparisons were conducted based on one-way ANOVAs (in order to compare PTSD patients, deployed healthy controls and non-deployed healthy controls) or t-tests (in order to compare PTSD patients and deployed healthy controls). All PTSD symptom-related variables are based on the Clinician-Administered PTSD Scale (CAPS)

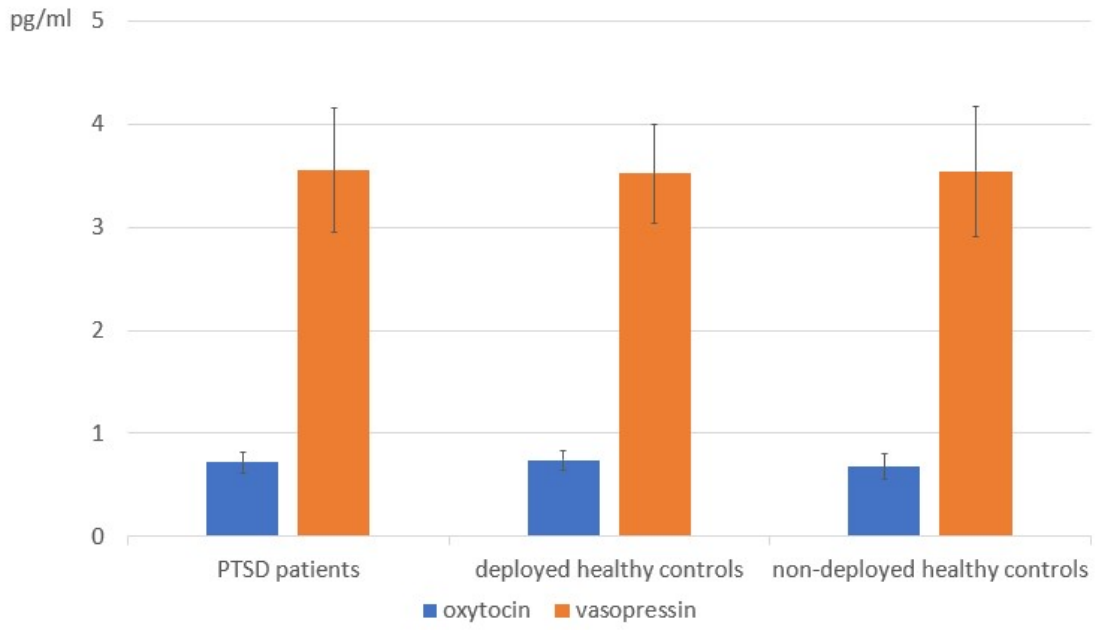

FIGURE 5.1. Endogenous oxytocin and vasopressin concentrations in posttraumatic stress disorder (PTSD) patients, deployed healthy controls and non-deployed healthy controls, as assessed at the participants' first assessments. No significant group differences were detected. *M* and *SD* of log-transformed oxytocin and non-transformed vasopressin concentrations are shown.

5.3.3 Correlations before internet-based TF-CBT onset

PTSD symptoms were neither correlated with oxytocin (r = -.19, p = .47), nor with vasopressin (r = .11, p = .68) before onset of the internet-based TF-CBT. Likewise, no correlations of oxytocin and vasopressin with PTSD symptoms were detected at post-treatment or follow-up assessment (see SUPPLEMENTARY MATERIAL 6).

5.3.4 Courses of endocrine parameters and PTSD symptoms

Courses of PTSD symptoms, oxytocin and vasopressin are illustrated in FIGURE 5.2. There was no change in mean PTSD symptoms from pre- to post-treatment and follow-up ($F_{2, 30}$ = 1.00, p = .38). PTSD symptoms remained stable within individuals (pre- to post-treatment: r = .83, p < .01, post-treatment to follow-up: r = .93, p < .01). Mean oxytocin did not change from pre- to post-treatment and follow-up

($F_{2, 30}$ = 1.41, p = .26). Oxytocin was not stable within individuals over time (pre- to post-treatment: r = .04, p = .89, post-treatment to follow-up: r = .19, p <=.47). Likewise, there was no change in mean vasopressin from pre- to post-treatment and follow-up up ($F_{2, 30}$ = 1.24, p = .28) and it was not stable within individuals over time (pre- to post-treatment: r = .44, p = .08, post-treatment to follow-up: r = .01, p = .98).

5.3.4 Courses of endocrine parameters and PTSD symptoms

Courses of PTSD symptoms, oxytocin and vasopressin are illustrated in FIGURE 5.2. There was no change in mean PTSD symptoms from pre- to post-treatment and follow-up ($F_{2, 30}$ = 1.00, p = .38). PTSD symptoms remained stable within individuals (pre- to post-treatment: r = .83, p < .01, post-treatment to follow-up: r = .93, p < .01). Mean oxytocin did not change from pre- to post-treatment and follow-up ($F_{2, 30}$ = 1.41, p = .26). Oxytocin was not stable within individuals over time (pre- to post-treatment: r = .04, p = .89, post-treatment to follow-up: r = .19, p <=.47). Likewise, there was no change in mean vasopressin from pre- to post-treatment and follow-up up ($F_{2, 30}$ = 1.24, p = .28) and it was not stable within individuals over time (pre- to post-treatment: r = .44, p = .08, post-treatment to follow-up: r = .01, p = .98).

5.3.5 Interactions of endocrine parameters with PTSD symptoms over time

Oxytocin and vasopressin, as assessed pre-treatment, were not correlated with PTSD symptoms post-treatment (oxytocin: r = -.25, p = .35, vasopressin: r = .27, p = .32). Likewise, pre-treatment oxytocin and vasopressin did not predict changes in PTSD symptoms from pre- to post-treatment (oxytocin: r = -.07, p = .79, vasopressin: r = .25, p = .36). Oxytocin and vasopressin post-treatment were not correlated with PTSD symptoms at follow-up (oxytocin: r = .24, p = .36, vasopressin: r = .13, p = .62), nor were they correlated with the change score of PTSD symptoms from post-treatment to follow-up (oxytocin: r = .44, p = .08, vasopressin: r = .04, p = .89).

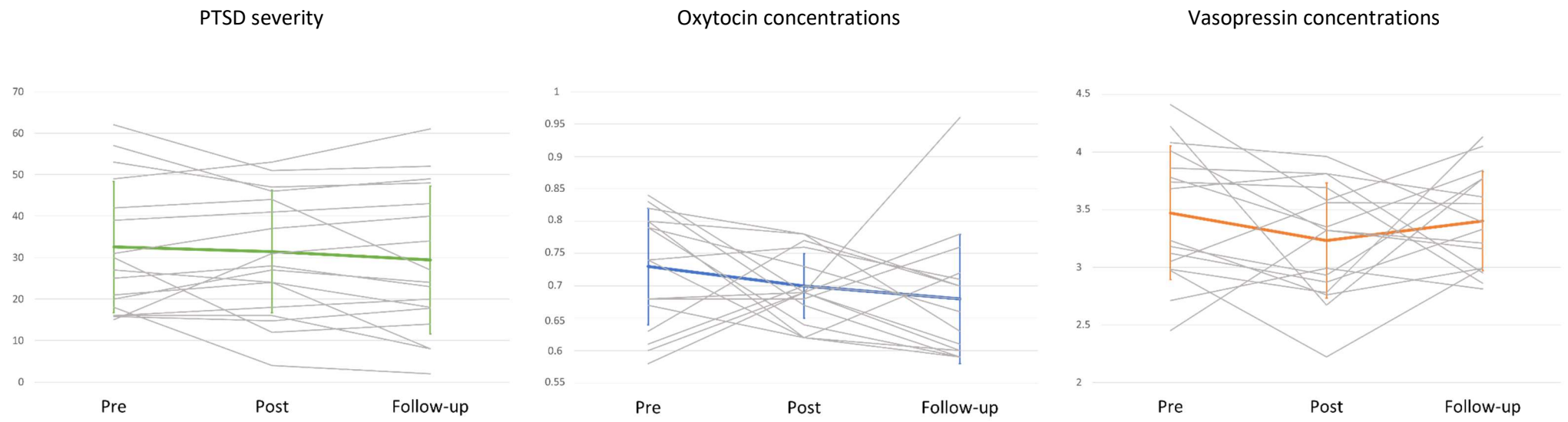

FIGURE 5.2. Courses of posttraumatic stress disorder (PTSD) severity (total Clinician-Administered PTSD Scale scores), oxytocin (log-transformed pg/ml) and vasopressin (pg/ml) from pre-treatment to post-treatment and follow up. There was no significant mean change in any of the outcomes. PTSD severity remained stable within individuals, whereas oxytocin and vasopressin were not correlated within individuals over time.

Vice versa, PTSD symptoms pre-treatment were neither correlated with oxytocin and vasopressin, as assessed post-treatment (oxytocin: $r = .12$, $p = .64$, vasopressin: $r = .22$, $p = .41$), nor were they correlated with changes in oxytocin and vasopressin from pre- to post-treatment (oxytocin: $r = .23$, $p = .38$, vasopressin: $r = .08$, $p = .77$). Likewise, PTSD symptoms, as assessed post-treatment was not correlated with oxytocin and vasopressin, as measured at follow up (oxytocin: $r = -.09$, $p = .73$, vasopressin: $r = -.18$, $p = .63$) and it was not correlated with their change scores from post-treatment to follow-up (oxytocin: $r = -.13$, $p = .62$, vasopressin: $r = -.23$, $p = .39$).

5.4 Discussion

5.4.1 Summary of evidence

Our findings suggest that endogenous oxytocin and vasopressin concentrations are unrelated to PTSD psychopathology during internet-based TF-CBT. We did not detect any difference in these endocrine parameters between PTSD patients, deployed and non-deployed healthy controls. Furthermore, in patients, oxytocin and vasopressin were not correlated with PTSD symptoms when measured simultaneously. Besides evaluating clinical treatment effects, we investigated oxytocin and vasopressin as possible biomarkers of internet-based TF-CBT, in order to explore treatment effects on a biological level. Our results showed that neither PTSD symptoms, nor oxytocin or vasopressin changed during internet-based TF-CBT or to follow-up. Notably, while PTSD symptoms were stable within individuals, the endocrine parameters were not. Our exploratory investigations revealed that oxytocin and vasopressin did not predict PTSD symptoms longitudinally and likewise, PTSD symptoms were not associated with the endocrine parameters longitudinally.

5.4.2 Interpretation of results

In this study, we used single measurements of oxytocin and vasopressin and yielded unsatisfactory intraindividual stability. A prerequisite to use biological measurements as biomarkers for mental disorders during psychotherapeutic or pharmacological interventions is the possibility to measure these parameters repeatedly. Only then can potential changes in biological measurements clearly be related to the intervention effect. Our study showed that this prerequisite is not given when measuring oxytocin

and vasopressin only once, even though this is the most frequently applied approach for these neuropeptides in PTSD research (Engel, Klusmann et al., 2019). With regard to future intervention studies, it can clearly be recommended to use endocrine parameters with higher intraindividual stability, for instance by increasing the number of measurements and merging them into more conclusive parameters such as the area under the curve (Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003). Furthermore, confounders of oxytocin and vasopressin need to be controlled for (Engel, Laufer et al., 2019). In the present study, blood sampling was highly standardized, by scheduling it at a precise time and providing precise instructions with regard to behaviors before measurements. Further, our sample was comparatively homogeneous, as it included only men with the same profession. Especially amidst the low intraindividual stability of both endocrine parameters, it remains unclear which factors actually determined the observed values. Our study's results indicate that the values were not determined by traumatic event exposure, PTSD symptoms, sex, profession, time of day and behaviors before measurement, but it remains unresolved which factors actually influenced them.

Despite a strong theoretical rationale to investigate oxytocin and vasopressin as biomarkers of TF-CBT, which has been derived from animal studies and experimental studies in humans, the transfer to the clinical setting did not succeed. Besides their involvement in social functioning and fear processing, oxytocin and vasopressin are involved in a variety of other physiological, behavioral and psychological functions (for overviews, see Boll, Almeida de Minas, Raftogianni, Herpertz, & Grinevich, 2018; Donaldson & Young, 2008; Gimpl & Fahrenholz, 2001; Lawson, 2017; MacDonald & Feifel, 2014; MacDonald & MacDonald, 2010; Winter & Jurek, 2019; Yang, Wang, Han, & Wang, 2013). It appears that experimental paradigms that specifically target either social functioning or fear processing might be more appropriate to investigate oxytocin or vasopressin as potential underpinnings of psychopathological functions in a more hypothesis-driven manner. Alternatively, with regard to psychotherapy, oxytocin and vasopressin might be measured repeatedly within single sessions, such as exposure sessions. Moving from the macro- to the micro-level of psychotherapy evaluation research might be more helpful to identify the active ingredients of TF-CBT.

5.4.3 Limitations

Contrasting previous findings (Kuester et al., 2016), the internet-based TF-CBT evaluated in the present study was not effective in reducing PTSD symptoms (Niemeyer et al., 2020). The limited number of PTSD patients who started and completed therapy impeded a differential investigation of therapy effectiveness compared with a waiting period. As merging all patients into one group in order to quantify changes during the internet-based TF-CBT and follow-up period did not result in any effects, it was not necessary to compare these null-effects with a passive condition, anyway. Still, in general, an effective treatment is necessary to identify its active ingredients, and this prerequisite was not given here. The small number of patients observed limits the informative value of the observed null-findings on both, clinical and endocrine parameters. However, the results of the present study remained stable when using all values instead of those of completers only, which indicates that the findings are still plausible.

5.4.4 Conclusion

In the present investigation, oxytocin and vasopressin were not informative biomarkers of TF-CBT. Previous evidence from animal studies and non-clinical samples indicated that they were involved in social functioning and fear processing, two functions that are important in PTSD development and treatment. However, the informative value of single measurements of oxytocin and vasopressin was restricted, mainly due to the low intraindividual stability of these parameters. More basic research is needed in order to identify parameters of the endogenous oxytocin and vasopressin system that are more stable and less sensitive to physiological confounders. Only then can oxytocin and vasopressin successfully be implemented into the clinical evaluation of TF-CBT.

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Data availability statement

Upon request, the data that were used in the present study can be made available by the corresponding author.

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Disclosure statement

OFA Heinrich Rau and OTA Dr. Gerd-Dieter Willmund are employed by the German Armed Forces. Their employment influenced neither the study design nor the collection, analysis, and interpretation of the data. There are no conflicts of interest among the other authors.

Ethical Standard

The study was approved by the Ethics committee of Freie Universität Berlin (reference number: 85/2014; addendum: 116 /2016), after internal approval by the German Armed Forces.

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Contributions

Christine Knaevelsrud and Sarah Schumacher designed the study.

Christine Knaevelsrud obtained the funding.

Sebastian Burchert, Sinha Engel, Hannah Klusmann, Christine Kersjes, Annika Küster, Beate Muschalla, Heinrich Rau, Jan-Peter Spies and Deborah Weiss collected the data.

Sarah Schumacher and Helen Niemeyer performed the internet-based trauma-focused cognitive behavioral treatment.

The staff of the German Armed Forces Centre of Military Mental Health, Berlin provided administrative, technical and material support.

Rainer Landgraf analyzed the oxytocin and vasopressin samples and provided assistance in analysis and interpretation of the data.

Sinha Engel performed the statistical analyses and drafted the manuscript. All authors critically revised the manuscript for important intellectual content.

Sarah Schumacher, Christine Knaevelsrud and Gerd-Dieter Willmund supervised the study.

Supplementary material

The following supplementary material is included in in this dissertation:

SUPPLEMENTARY MATERIAL 5. Means and standard deviations of posttraumatic stress disorder symptoms, oxytocin and vasopressin concentrations over time

SUPPLEMENTARY MATERIAL 6. Correlations between posttraumatic stress disorder symptoms, oxytocin and vasopressin concentrations over time: All cases

SUPPLEMENTARY MATERIAL 7. Correlations between posttraumatic stress disorder symptoms, oxytocin and vasopressin concentrations over time: Complete cases

CHAPTER 6

Does oxytocin impact the psychotherapeutic process? An explorative investigation of internet-based cognitive-behavioral treatment for posttraumatic stress disorder

Engel, S., Schumacher, S., Niemeyer, H., Küster, A., Burchert, S., Rau, H., Willmund, G.-D., Knaevelsrud, C. (2020). Does oxytocin impact the psychotherapeutic process? An explorative investigation of internet-based cognitive-behavioral treatment for posttraumatic stress disorder. *Verhaltenstherapie*, 1–13.

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Does oxytocin impact the psychotherapeutic process? An explorative investigation of internet-based cognitive-behavioral treatment for posttraumatic stress disorder

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Abstract

Background. Oxytocin might promote favorable psychotherapy outcomes by strengthening the therapeutic alliance. Its involvement in psychotherapeutic processes, especially regarding the therapeutic alliance, needs further investigation.

Patients and Methods. Blood oxytocin of 35 male German Armed Forces service members who were seeking treatment for posttraumatic stress disorder (PTSD) was analyzed before the onset of a 5-week internet-based, trauma-focused psychotherapy. We investigated whether oxytocin influenced patients' ratings of the therapeutic alliance components "agreement on collaboration" and "emotional bond," assessed during and after treatment. We further explored oxytocin's impact on general change mechanisms of psychotherapy and on psychotherapy expectation and evaluation.

Results. Oxytocin had no significant impact on early agreement on collaboration, which significantly predicted psychotherapy outcome. Early emotional bond was not predicted by oxytocin and was not predictive for psychotherapy outcome. Descriptive analyses showed that patients with higher pretreatment oxytocin concentrations provided higher ratings of general change mechanisms of psychotherapy. On a descriptive level, the associations between psychotherapy expectation and evaluation and oxytocin were mixed.

Discussion and Conclusion. We found positive effects of higher pretreatment oxytocin concentrations in PTSD patients. This descriptive study is limited by its small sample size and needs replication in larger, independent samples. However, results indicate possible benefits of oxytocin on trauma-focused psychotherapy.

Keywords: Oxytocin, Therapeutic alliance, Cognitive behavioral therapy

6.1 Theoretical background

Research into the neurotransmitter and hormone oxytocin originated in biology. Recently, however, social neuroscience and clinical psychology have taken up this branch of research and identified oxytocin as a "social hormone." Pioneering studies have shown that the central nervous injection of synthetic oxytocin, which under natural conditions is released during pregnancy, childbirth, and lactation (Neumann, Russell, & Landgraf, 1993), triggered nurturing behavior in rats (Pedersen & Prange, 1979), while the injection of an antagonist reduced it (Neumann, Douglas, Pittman, Russell, & Landgraf, 1996). Similarly, endogenous oxytocin concentrations in human fathers and mothers have been associated with parental behaviors such as nurturing, touching (Feldman et al., 2012), social engagement, affect synchrony, and positive communication with children (Feldman, Gordon, & Zagoory-Sharon, 2011). Oxytocin also increases interest in social interactions beyond the context of parent-child relationships: central nervous injection of an oxytocin antagonist reduced social exploration behavior in rats, whereas stress-induced social avoidance behavior was reduced by central nervous injection of oxytocin (Lukas et al., 2011). Experimental studies in humans have shown that intranasal oxytocin administrations promoted trust (Kosfeld, Heinrichs, Zak, Fischbacher, & Fehr, 2005; Mikolajczak, Pinon, Lane, Timary, & Luminet, 2010) and positive communication during a partnership conflict (Ditzen et al., 2009), to mention just two of many prominent examples that have been summarized in numerous reviews (Donaldson & Young, 2008; Feldman, 2012; Heinrichs, Dawans, & Domes, 2009; MacDonald & MacDonald, 2010).

However, it would be insufficient to call the effects of oxytocin exclusively "prosocial." For example, it was observed that patients with borderline personality disorder showed a lower level of trust after administration of intranasal oxytocin, compared to a placebo condition (Bartz, Simeon et al., 2011; Ebert et al., 2013). This shows that findings from experimental studies of healthy people are not readily transferable to clinical populations.

The social salience hypothesis is an attempt to explain these opposite observations (Shamay-Tsoory & Abu-Akel, 2016). It postulates that oxytocin increases the salience of social stimuli independently of their valence. Depending on individual traits and context, oxytocin fosters attention in relation to

positive and negative social stimuli. Some relevant moderators have already been identified, including traumatic experiences in childhood and symptoms of mental disorders (Olff et al., 2013; Shamay-Tsoory & Abu-Akel, 2016). But it is clear that more research is needed to precisely predict the social effects of intranasal oxytocin administration in specific clinical populations.

If we transfer this branch of research into clinical psychology, it seems particularly interesting to study psychotherapy as a specific form of social interaction. The psychotherapeutic success is promoted by general change mechanisms (Grawe, 2004). Previous studies have suggested that oxytocin in particular could promote the therapeutic alliance, one of the general mechanisms of change of psychotherapy. Specifically, it was postulated that intranasal oxytocin administration could contribute to an improvement of the therapeutic alliance and thus indirectly to an improvement of symptoms (Koch et al., 2014). However, since psychopathology is known to be a moderator according to the social salience hypothesis, concerns were also raised about potentially harmful effects of such administrations (Hurlemann, 2017). So far, a comprehensive review of the question of whether oxytocin actually has helpful or harmful effects in psychotherapeutic treatment remains to be performed. In this regard, two studies that evaluated the effects of intranasal oxytocin administration in patients with posttraumatic stress disorder (PTSD) are particularly noteworthy: in one study, patients who received intranasal oxytocin instead of a placebo before undergoing a total of 10 weekly exposure therapy sessions reported lower PTSD symptoms and a more positive therapeutic alliance during therapy, although the effects were not statistically significant (Flanagan, Sippel, Wahlquist, Moran-Santa Maria, & Back, 2018). Another study examined the underlying biological mechanisms: compared to placebo, intranasal oxytocin administration in PTSD patients normalized the reduced reactivity to reward in the left anterior insula, part of the neural “salience network” that processes personally relevant information (Downar, Crawley, Mikulis, & Davis, 2002). It was concluded that oxytocin could help PTSD patients to better perceive social support, which could bolster the therapeutic alliance (Nawijn et al., 2017).

The present study is intended to expand these preliminary findings by casting more light on the still open question of whether endogenous measurements that reflect the activity of the endogenous oxytocin system can also influence the psychotherapeutic process. Specifically, we examined the effect of

endogenous oxytocin concentrations, measured before the start of psychotherapy, on psychotherapy process variables. We assumed a positive influence on the therapeutic alliance and explored the influence on general mechanisms of change of psychotherapy as well as on psychotherapy expectation and evaluation, which the subjects were asked about before, during, and after an internet-based cognitive-behavioral treatment (CBT) for PTSD.

6.2 Methods

6.2.1 Study design

The present work is based on data from a randomized, waiting-list-controlled study, which evaluated the feasibility, acceptance, and effectiveness of a 5-week internet-based program of CBT for PTSD in German Armed Forces service members (Niemeyer et al., 2020).The study was preregistered in the Australian Clinical Trials Registry (ACTRN 12616000956404). After internal approval by the German Armed Forces, the study was authorized by the Ethics Committee of the Freie Universität Berlin (reference number: 85/2014; addendum: 116/2016). All study participants were thoroughly informed about the study goals, the study procedure, and the voluntary nature of participation, and were asked for written consent.

The patients were randomly assigned, by means of a computer-generated randomization list, to either the waiting list condition or the immediate treatment condition. Then the PTSD patients, either after an initial diagnostic examination and the subsequent 6-week passive waiting period (waiting list condition) or immediately after inclusion in the study (immediate treatment condition), underwent a diagnostic examination at the German Armed Forces Military Hospital Berlin (E1). Sociodemographic, psychological, behavioral, and biological data were collected. The blood test for measurement of oxytocin and the determination of the severity of the PTSD symptoms are particularly relevant to the aim of our study.

In the 5 weeks after E1, the patients completed the internet-based CBT, which consisted of 10 sessions (2 per week). The treatment was evaluated using internet-based psychological questionnaires that patients filled out before session 1 (E2), session 4 (E3), session 8 (E4), and after session 10 (E5). The

psychotherapy process variables that were compiled before, during, and after treatment are particularly relevant for this study. Immediately after the end of the internet-based CBT (E6) and at a follow-up point 3 months later, the patients were invited to the German Armed Forces Military Hospital Berlin for further diagnostic examinations, which included assessing the severity of the PTSD symptoms.

Since the present study relates to the psychotherapeutic process, the examinations relevant to the overall study before the start of the waiting period and the follow-up examination were excluded from the analyses. All data records were used to investigate the effect of endogenous oxytocin concentrations before the beginning of treatment (E1) on psychotherapy process variables before, during, and after the internet-based CBT (from E2 to E5), regardless of whether the patients were assigned to the waiting list or immediate treatment condition.

6.2.2 Participants

Active and former German Armed Forces service members who wanted psychotherapeutic treatment for PTSD were eligible for the study. Exclusion criteria were acute psychosis, an acute manic episode, current substance abuse or substance dependency, current suicidal thoughts, a neurological disorder, an acute physical illness, simultaneous psychotherapeutic treatment, or irregular use of psychotropic drugs. The patients were recruited by advertisements in military magazines and on websites and forums for German Armed Forces service members. Leaflets and posters were also distributed in German Armed Forces health centers and German Armed Forces Military Hospitals. Finally, study staff and commanding officers distributed leaflets at follow-up seminars after deployments abroad, and the study was presented at conferences to German Armed Forces psychologists and psychiatrists.

6.2.3 Internet-based cognitive-behavioral treatment

The internet-based CBT consisted of 10 twice-weekly sessions. It is based on the protocols of *Interapy* (Lange et al., 2003) and *Integrative Testimonial Therapy* (Knaevelsrud, Böttche, Pietrzak, Freyberger, & Kuwert, 2017) and was adapted to the military context. Each session consisted of a writing task that was performed by the patients. The text was then sent on a password-protected platform to one of two randomly assigned, licensed cognitive-behavioral therapists (H.N., S.Sch.). Both therapists had

completed specific training for the internet-based CBT. The patients received written therapeutic feedback on their texts within one workday; it was based on a standardized treatment manual and was tailored to the specific situation of the patient. In the feedback, the patient's participation was recognized and positively reinforced, which was intended to promote patients' motivation. If the patients had difficulty with the content of a writing task, the feedback also contained further assistance. Patients received no separate feedback on sessions 2 and 5. For those, feedback was given after the following session for the previous two writing tasks.

Treatment was divided into three phases: biographical reconstruction (sessions 1 to 3), exposure (sessions 4 to 7), and cognitive restructuring (sessions 8 to 10). In the phase of the biographical reconstruction, the patients reflected on their previous life experiences from childhood to the time of the traumatic event. They described both positive and difficult experiences that they had successfully handled. Psychoeducational texts and support from the therapists then prepared the patients for the exposure sessions. In the four exposure texts, patients were asked to repeatedly describe the worst traumatic event they had experienced. They were instructed to write in the first person and the present tense, to put into writing the most painful aspects, emotions, and sensory impressions. The phase of cognitive restructuring, on the other hand, aimed to give the patient a new perspective on the traumatic event. To achieve this, they were instructed to reflect on feelings such as guilt and shame, to question dysfunctional patterns in their thoughts and behaviors, to correct unrealistic assumptions, to consider possible positive consequences of the traumatic event, and to plan how they wanted to deal with such things in the future. A more detailed description of the treatment manual and a safety protocol in the event of crises can be found in Niemeyer et al. (2020).

6.2.3 Oxytocin

6.2.3.1 Measurement and data preparation

Oxytocin was measured in the blood of n = 36 patients (n = 1 patient completed the diagnostic examination, but did not show up for blood sampling), which was collected in 9.00-mL serum tubes (Sarstedt, Germany). After the blood was drawn, the tubes were gently swirled, then rested in the dark

for 30 min to allow the blood to clot. The tubes were then centrifuged at 1,000 *g* for 10 min and the serum was pipetted into smaller 1.50-mL tubes (Eppendorf, Germany). These samples were stored in a freezer at –80°C. At the end of the data collection, all samples were sent to the laboratory (RIAgnosis, Sinzing, Germany). They were extracted and analyzed using a highly sensitive and selective radioimmunoassay, as described by Landgraf, Neumann, Holsboer, and Pittman (1995) and Landgraf and Neumann (2004). The intra-assay variability was <10%. All samples were analyzed using the same assay, eliminating inter-assay variability. The detection limit was between 0.1 and 0.5 pg/mL, depending on the age of the tracer. None of the samples were below the detection limit. There was no significant cross-reactivity with structurally related peptides, including the ring hexapeptides and the terminal tripeptides of oxytocin and vasopressin.

The distribution of the oxytocin concentrations was visually examined and statistically described (n = 36, M = 4.53, SD = 1.82, skewness = 3.05, kurtosis = 13.78). An outlier that was more than 3 SD above the M had to be removed. The remaining values were normally distributed (n = 35, M = 4.29, SD = 1.08, skewness = 0.12, kurtosis = –1.22) and were included in the statistical analyses.

6.2.3.2 Confounding variables

Blood sampling was scheduled for 8 a.m. on the day of the examination. The exact time of the sampling was recorded and we were able to establish a high level of agreement with the protocol: in 88.57% of patients (31 samples), the blood was drawn exactly at 8 a.m. The deviations were 5, 10, and 15 min (2.86%, one sample each) and the time was not recorded for one sample and was therefore unknown. Patients were instructed not to drink anything except water, not to eat anything, not to consume caffeine, and not to smoke before the blood test. Compliance with these instructions was reported for 97.14% of the patients (34 samples) for drinking, 88.57% (31 samples) for food and caffeine consumption, and 71.42% (25 samples) for smoking. Age, body weight and height (Table 2), and leukocyte values were also recorded. The latter were within the normal range for 91.43% of the patients (32 samples) and for one sample each (2.86% each) they were too low, too high, or not available. Age (r = 0.20, p = 0.24), body mass index (r = –0.01, p = 0.96), and leukocyte values (r = –0.06, p = 0.75) were not significantly correlated with oxytocin.

6.2.4 Psychological variables

The severity of PTSD symptoms was determined using the German translation of the Clinician-Administered PTSD Scale for DSM-5 (Weathers et al., 2018), a standardized interview.

The Scales for the Multiperspective Assessment of General Change Mechanisms in Psychotherapy (SACiP; Mander et al., 2013) which comprise 21 items, were used to measure the therapeutic alliance. The subscale *Emotional Bond* (3 items) is based on the scale of the same name in the Working Alliance Inventory-Short Revised (WAI-SR; Munder, Wilmers, Leonhart, Linster, & Barth, 2010) and the subscale *Agreement on Collaboration* (6 items) combines the WAI-SR scales *Agreement on Goals* and *Agreement on Tasks*. Furthermore, the SACiP scales *Resource Activation* (3 items), *Clarification of Meaning* (3 items), *Problem Actuation* (3 items), and *Mastery* (3 items) were used. These were adapted from the Bern Post-Session Report for Patients and for Therapists (Flückinger, Regli, Zwahlen, Hostettler, & Caspar, 2010) and pertain to the general change mechanisms of psychotherapy according to Grawe (2004). The items of the SACiP can have values between 1 and 5, whereby higher values indicate a higher Emotional Bond, Agreement on Collaboration, Resource Activation, Clarification of Meaning, Problem Actuation, and Mastery. The SACiP was filled out at E3, E4, and E5, and thus during and after treatment.

The German version of the Patient Questionnaire on Therapy Expectation and Evaluation (Schulte, 2005) comprised a total of 11 items across the three subscales *Hope for Improvement* (4 items), *Fear of Change* (4 items), and *Suitability* (3 items). The items can have values between 1 and 5, whereby higher values indicate a higher Hope for Improvement, Fear of Change, and Suitability. This questionnaire was completed at E2, E3, E4, and E5, and thus before, during, and after treatment.

6.2.5 Statistical analyses

The directed hypotheses that could be derived from the previous research related to the possible positive influence of oxytocin on the therapeutic alliance. Thus, inferential statistical tests were only performed for this variable, while the influence of oxytocin on the other psychotherapy process variables was only examined descriptively.

Since the therapeutic alliance in early phases of conventional face-to-face psychotherapy has proven to be particularly important for preventing termination of therapy and improving the symptoms of disorders (Horvath, Del Re, Fluckiger, & Symonds, 2011), and its predictive value in internet-based CBT is still disputed (Berger, 2017), we tested this relationship. Two linear regression analyses were performed for that purpose. Emotional Bond and Agreement on Collaboration, each assessed at E3, served as predictors, and the severity of the PTSD symptoms after the end of therapy served as a dependent variable. The severity of PTSD symptoms before the start of therapy was considered a control variable.

Since it has been explicitly stated that oxytocin may influence the therapeutic alliance (Koch et al., 2014), we conducted two additional linear regression analyses. In these, the oxytocin concentrations measured before the start of treatment served as predictors. Emotional Bond and Agreement on Collaboration, measured at E3, were examined as dependent variables. The patients' age was added as a control variable, because it differed between the two groups that were formed later on the basis of oxytocin concentrations.

The relationship between the independent and dependent variables examined in the regression analyses was further clarified by bivariate correlations. Partial correlations were also calculated, whereby the relationship between the independent and dependent variables was adjusted for the influence of the respective control variable of the regression. The α error level was set at 5% in each case.

Given the exploratory nature of the additional analyses, we only performed descriptive analyses of the influence of the oxytocin concentrations measured before the start of treatment on the later evaluations of the therapeutic alliance and on the other psychotherapy process variables. We compared the progression of the psychotherapy process variables of those patients with high and those with low oxytocin concentrations. The patients were assigned to the two groups based on a median split.

All analyses were performed with the program SPSS, version 25 (IBM).

6.3 Results

6.3.1 Patients

The number of patients and data at the examination time points are shown in TABLE 6.1. All study participants were male. An overview of the total number of patients included in the internet-based CBT and a list of the reasons why patients stopped participating in the study may be found in Niemeyer et al. (2020) and **study 4** of this dissertation. A sample description of all patients and the two groups that were differentiated based on their oxytocin concentrations before the start of treatment is presented in TABLE 6.2.

As TABLE 6.2 shows, the patients with low oxytocin concentrations before the start of treatment were significantly younger than those with high oxytocin concentrations before the start of treatment. There were no group differences with regard to assignment to the group that was treated immediately, the waiting list condition, additional demographic variables, the severity of the PTSD symptoms, or termination of therapy.

TABLE 6.1. Number of patients and existing data.

	E1	E2	S1	S2	S3	E3	S4	S5	S6	S7	E4	S8	S9	S10	E5	E6
			Biographical reconstruction				Exposure					Cognitive restructuring				
Participants	35	30	30	28	27	21	21	21	21	20	20	20	20	20	20	20
Low oxytocin	17	14	14	12	12	8	8	8	8	8	8	8	8	8	8	8
High oxytocin	18	16	16	16	15	13	13	13	13	12	12	12	12	12	12	12
Oxytocin	35															
Low oxytocin	17															
High oxytocin	18															
CAPS	35															20
Low oxytocin	17															8
High oxytocin	18															12
SACiP																
Emotional Bond						20					20			19		
Low oxytocin						7					8			7		
High oxytocin						13					12			12		
Agreement on Collaboration						20					20			19		
Low oxytocin						7					8			8		
High oxytocin						13					12			11		
Resource Activation						19					20			19		
Low oxytocin						7					8			8		
High oxytocin						12					12			11		
Motivational Clarification						20					20			20		
Low oxytocin						7					8			8		
High oxytocin						13					12			12		
Problem Actuation						18					20			20		
Low oxytocin						6					8			8		
High oxytocin						12					12			12		
Mastery						20					20			20		
Low oxytocin						7					8			8		
High oxytocin						13					12			12		
PATHEV																
Hope for Improvement		28				20					20			20		
Low oxytocin		12				7					8			8		
High oxytocin		16				13					12			12		
Fear of Change		28				20					20			20		
Low oxytocin		12				7					8			8		
High oxytocin		16				13					12			12		
Suitability		26				20					20			19		
Low oxytocin		10				7					8			8		
High oxytocin		16				13					12			11		

Note. The table shows the number of patients. Oxytocin was measured in the diagnostic examination (E)1. The German translation of the Clinician-Administered PTSD Scale for DSM-5 (CAPS) was used for E1 and E6. The German translation of the Patient Questionnaire on Therapy Expectation and Evaluation (PATHEV) was filled out for E2, E3, E4, and E5. The Scales for the Multiperspective Assessment of General Change Mechanisms in Psychotherapy (SACiP) were filled out for E3, E4, and E5. n = 37 patients completed the examination before treatment started, but the present study only applies to those n = 35 patients with valid oxytocin measurements. One patient's oxytocin measurement was lacking because the patient completed the test but did not provide blood, and one patient's score was excluded as an outlier. The patients were assigned to the groups with high or low oxytocin based on a median split. S = session.

TABLE 6.2. Demographic, clinical, and endocrine profiles before treatment begin

	PTSD patients (*n* = 35)	**Low oxytocin (*n* = 17)**	**High oxytocin (*n* = 18)**	**Comparison**
Randomized allocated condition				
Waiting list (immediate treatment)	17 (18)	9 (8)	8 (10)	$\chi^2 = 0.25$ $p = 0.43$
Demographic information before the start of treatment				
Age[a]	37.91 (10.04)	34.00 (7.33)	41.39 (11.00)	$F_{(1,32)} = 5.17$ $p = 0.03$
BMI[a]	27.01 (3.16)	27.19 (3.85)	26.86 (2.62)	$F_{(1,30)} = 0.08$ $p = 0.77$
Number of cigarettes per day[a]	0.97 (1.14)	1.25 (1.18)	0.72 (1.07)	$F_{(1,32)} = 1.86$ $p = 0.18$
Number of deployments abroad[a]	2.85 (3.19)	3.12 (4.38)	2.61 (1.65)	$F_{(1,32)} = 0.21$ $p = 0.65$
Total number of days deployed abroad[a]	379.77 (386.90)	428.27 (521.41)	331.27 (182.10)	$F_{(1,28)} = 0.46$ $p = 0.52$
Dropout				
Dropouts, *n* (%)	15 (42.86)	9 (52.94)	6 (33.33)	$\chi^2 = 1.37$ $p = 0.24$
Therapy duration in days[a, b]	80.60 (31.21)	92.25 (38.13)	72.83 (24.34)	$F_{(1,18)} = 1.95$ $p = 0.18$
PTSD severity before beginning of treatment				
CAPS total value	33.60 (15.27)	36.06 (14.91)	31.28 (15.67)	$F_{(1,33)} = 0.85$ $p = 0.36$
Endocrine profile before beginning of treatment				
Oxytocin, pg/mL	4.29 (1.08)	3.34 (0.48)	5.18 (0.63)	$F_{(1,33)} = 92.88$ $p < 0.01$

Note. The patients were assigned to the groups with high or low oxytocin based on a median split. [a] Due to missing values, this information was not available for all patients. [b] Refers only to the patients who completed the treatment. Unless otherwise noted, M (SD) are given. The comparisons were conducted with univariate ANOVAs or $\chi 2$ tests. BMI = body mass index; PTSD = posttraumatic stress disorder; CAPS = German translation of the Clinician-Administered PTSD Scale for DSM-5.

6.3.2 The therapeutic alliance as a predictor of symptom improvement

On average, the patients evaluated the therapeutic alliance as positive and their ratings continued to increase in the course of the internet-based CBT (Emotional Bond: M = 3.82, SD = 0.83 at E3; 4.02 ± 0.79 at E4; 4.23 ± 0.59 at E5; Agreement on Collaboration: 2.90 ± 0.99 at E3; 3.04 ± 1.02 at E4; 3.30 ± 1.01 at E5). Statistically controlling for the severity of the PTSD symptoms before the start of treatment did not reveal Emotional Bond as a significant predictor (β = –0.14, t = –1.05, p = 0.31). However, Agreement on Collaboration was a significant predictor of lower severity of PTSD symptoms after treatment (β = –0.27, t = –2.19, p = 0.04). FIGURE 6.1 illustrates the bivariate correlations between Emotional Bond and the severity of PTSD symptoms after treatment (r = –0.16, p = 0.52) and between Agreement on Collaboration and the PTSD symptoms after treatment (r = –0.44, p = 0.06). Controlling for the severity of symptoms before the start of treatment, the partial correlations were even more negative (Emotional Bond: r = –0.25, p = 0.31; Agreement on Collaboration: r = –0.48. p = 0.04).

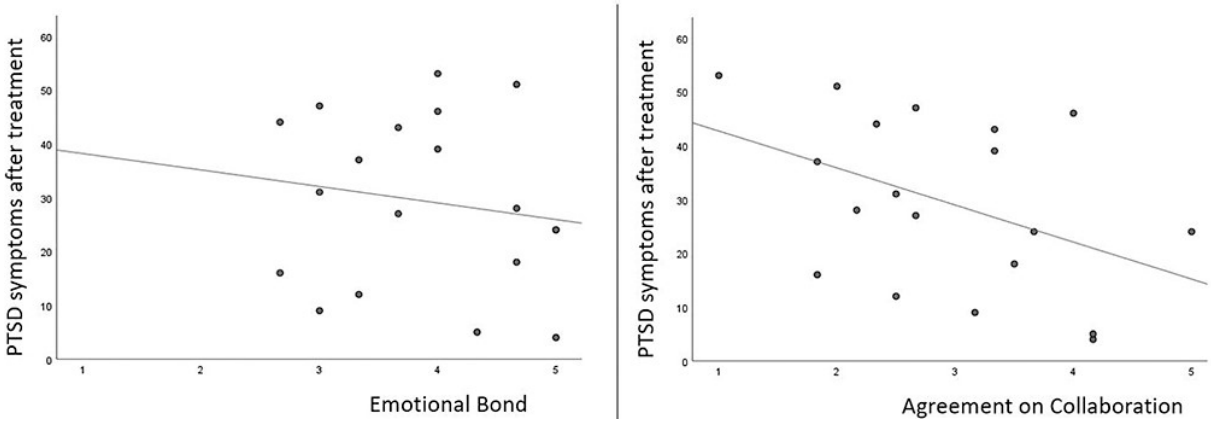

FIGURE 6.1 The bivariate relationship between the two components of the therapeutic alliance (Emotional Bond: r = -0.16, p = 0.52 and Agreement on Collaboration: r = -0.44, p = 0.06), measured during the examination (E)3, and the symptoms of posttraumatic stress disorder (PTSD) after treatment (E6).

6.3.3 Oxytocin as a predictor of the therapeutic alliance

The oxytocin concentrations before the start of treatment did not significantly predict Emotional Bond ($\beta = 0.14$, $t = 0.61$, $p = 0.55$). The effect of oxytocin on Agreement on Collaboration was also not statistically significant ($\beta = 0.23$, $t = 0.99$, $p = 0.34$). Also controlling for the age of the patients, neither predictor was significant (Emotional Bond: $\beta = 0.12$, $t = 0.51$, $p = 0.62$; Agreement on Collaboration: $\beta = 0.09$, $t = 0.37$. $p = 0.72$). The bivariate correlations between oxytocin and Emotional Bond ($r = 0.14$, $p = 0.55$) and between oxytocin and Agreement on Collaboration ($r = 0.23$, $p = 0.34$) are shown in FIGURE 6.2. Controlling for age, the partial correlations turned out to be less positive (Emotional Bond: $r = 0.13$, $p = 0.62$; Agreement on Collaboration: $r = 0.09$, $p = 0.72$).

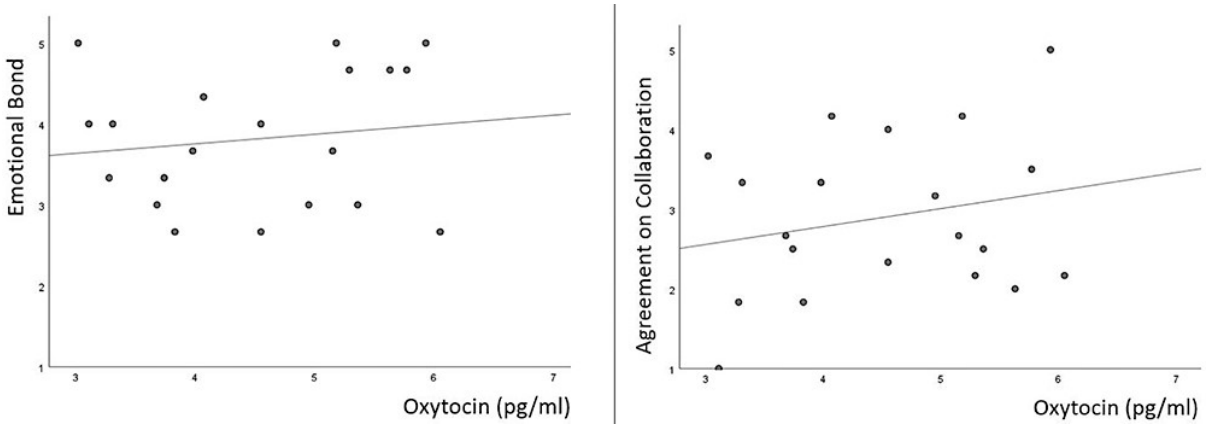

FIGURE 6.2 The bivariate relationship between oxytocin concentrations, measured before the start of treatment in the diagnostic examination (E)1, and the two components of the therapeutic alliance (Emotional Bond: $r = 0.14$, $p = 0.55$ and Agreement on Collaboration: $r = 0.23$, $p = 0.34$), measured at E3.

The effect of oxytocin on evaluation of the therapeutic relationship, which was queried at later time points, is shown in FIGURE 6.3. In accordance with the results of the regression analyses, the descriptive analyses showed that patients with high oxytocin concentrations before the start of treatment reported higher Emotional Bond during treatment (high oxytocin: M = 3.92, SD = 0.84 at E3; 4.11 ± 0.81 at E4;

4.33 ± 0.62 at E5; low oxytocin: 3.62 ± 0.72 at E3; 3.87 ± 0.69 at E4; 4.05 ± 0.42 at E5) and Agreement on Collaboration (high oxytocin: 3.17 ± 0.92 at E3; 3.24 ± 0.96 at E4; 3.53 ± 1.00 at E5; low oxytocin: 2.40 ± 0.86 at E3; 2.75 ± 0.98 at E4; 2.98 ± 0.87 at E5).

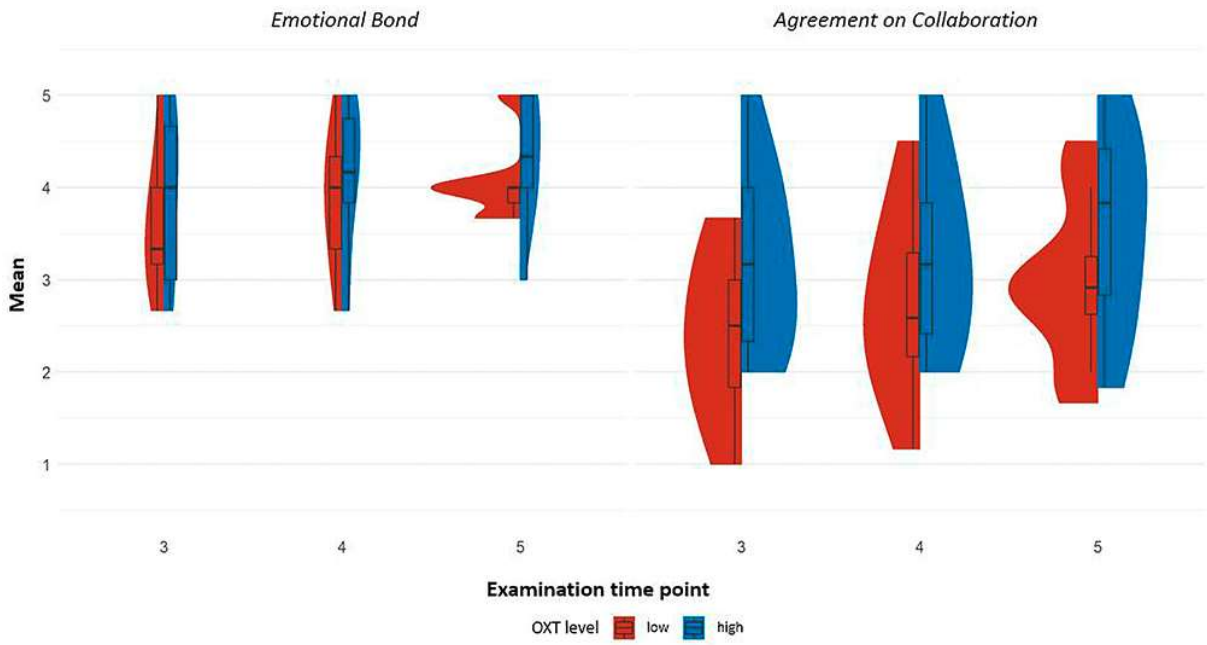

FIGURE 6.3. Evaluations of the therapeutic alliance (Emotional Bond and Agreement on Collaboration), measured during and after treatment at the examination time points (E)3, E4, and E5, as a function of the oxytocin (OXT) level before the start of treatment (E1). The patients were assigned to the groups with high or low OXT based on a median split.

6.3.4 The influence of oxytocin on general change mechanisms of psychotherapy

FIGURE 6.4 illustrates the influence of oxytocin concentrations before the start of treatment on the general change mechanisms of psychotherapy, namely Resource Activation, Clarification of Meaning, Problem Actuation, and Mastery. The figure shows that the levels of the general change mechanisms increased in the course of the internet-based CBT. It also shows that patients with high oxytocin

concentrations reported higher Resource Activation prior to treatment (high oxytocin: M = 3.19, SD = 0.96 at E3; 3.14 ± 1.04 at E4; 3.52 ± 0.82 E5; low oxytocin: 2.19 ± 0.96 at E3; 2.25 ± 0.72 at E4; 2.83 ± 0.93 at E5), as well as higher Clarification of Meaning (high oxytocin: 2.77 ± 0.95 at E3; 2.78 ± 1.20 at E4; 3.36 ± 0.92 at E5; low oxytocin: 1.86 ± 0.94 at E3; 2.42 ± 1.00 at E4; 2.92 ± 0.85 at E5), higher Problem Actuation (high oxytocin: 3.92 ± 0.82 at E3; 4.42 ± 0.47 at E4; 4.31 ± 0.55 at E5; low oxytocin: 3.33 ± 0.86 at E3; 3.71 ± 0.65 at E4; 4.08 ± 0.60 at E5), and a higher level of Mastery (high oxytocin: 2.56 ± 0.93 at E3; 2.97 ± 1.12 at E4; 3.11 ± 1.10 at E5; low oxytocin: 1.62 ± 0.65 at E3; 2.33 ± 0.97 at E4; 2.58 ± 0.97 at E5).

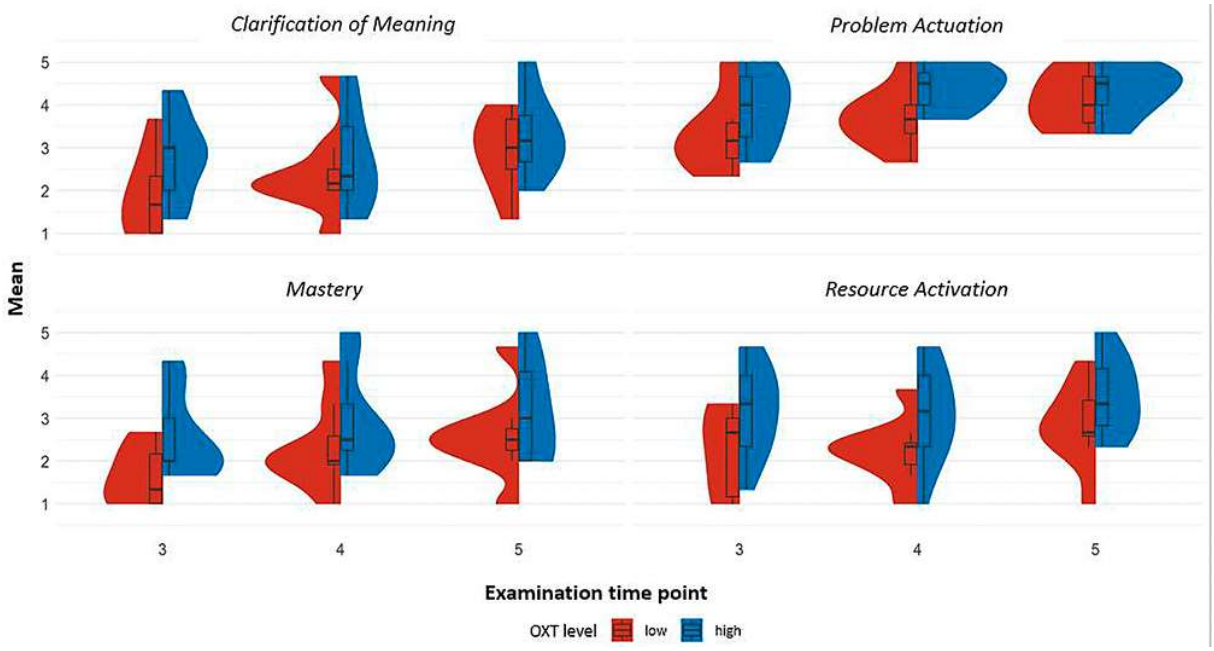

FIGURE 6.4. General change mechanisms (Resource Activation, Clarification of Meaning, Problem Actuation, and Mastery), measured during and after treatment at the examination time points (E)3, E4, and E5, as a function of the oxytocin (OXT) level before the start of treatment (E1). The patients were assigned to the groups with high or low oxytocin based on a median split.

6.3.5. The influence of oxytocin on psychotherapy expectation and evaluation

FIGURE 6.5 shows the influence of the oxytocin concentrations before the start of treatment on psychotherapy expectation and evaluation, especially on the variables Hope for Improvement, Fear of Change, and Suitability. The visualizations show that patients with high oxytocin concentrations before the start of treatment evaluated the Hope for Improvement (high oxytocin: M = 3.58, SD = 0.85 at E2; 3.65 ± 0.88 at E3; 3.71 ± 0.93 at E4; 3.67 ± 0.89 at E5; low oxytocin: 3.48 ± 0.93 at E2; 3.14 ± 1.07 at E3; 3.16 ± 1.02 at E4; 3.28 ± 1.09 at E5) and Suitability (high oxytocin: 3.55 ± 0.69 at E2; 3.60 ± 0.75 at E3; 3.54 ± 0.95 at E4; 3.66 ± 0.88 at E5; low oxytocin: 3.45 ± 0.61 at E2; 3.21 ± 0.75 at E3; 3.12 ± 0.84 at E4; 3.31 ± 1.03 at E5) during treatment as higher and Fear of Change at E2 as lower (high oxytocin: 1.94 ± 0.82; low oxytocin: 2.00 ± 0.76). However, Fear of Change at the later measurement points was reported as higher by this group (high oxytocin: 2.08 ± 0.75 at E3; 1.92 ± 0.87 at E4; 1.83 ± 0.75 at E5; low oxytocin: 1.81 ± 0.56 at E3; 1.83 ± 0.50 at E4; 1.71 ± 0.68 at E5).

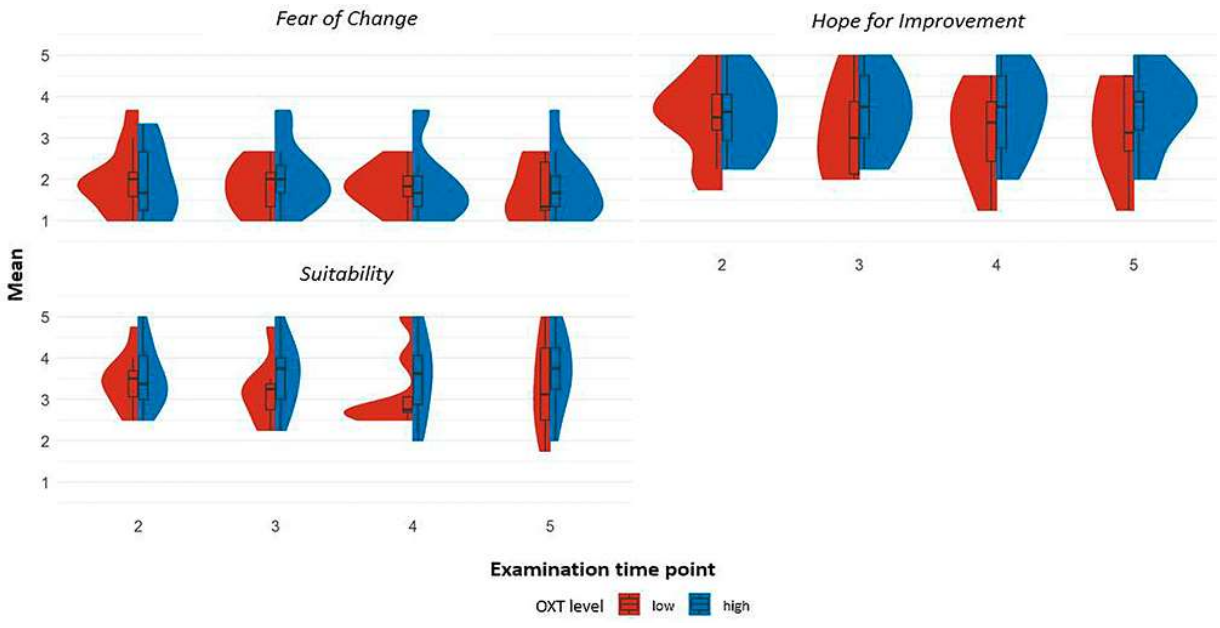

FIGURE 6.5. Psychotherapy expectation and evaluation (Hope for Improvement, Fear of Change, and Suitability), measured before, during, and after treatment at the examination time points (E)2, E3, E4, and E5, as a function of the oxytocin (OXT) level before the start of treatment (E1). The patients were assigned to the groups with high or low oxytocin based on a median split.

6.4 Discussion

6.4.1 Summary of results

In the present study, the influence of endogenous oxytocin concentrations that were measured in the blood of *n* = 35 German Armed Forces service members before the start of a trauma-focused, internet-based CBT was explored for variables that reflect the psychotherapeutic process. Oxytocin was positively but not significantly correlated with Agreement on Collaboration, a component of the therapeutic alliance, in the early stages of therapy. Agreement on Collaboration, in turn, predicted significant improvements in symptoms. The second component of the therapeutic alliance, Emotional Bond, was positively but not significantly influenced by oxytocin and, in turn, did not significantly predict symptom improvements. Descriptive analyses showed that oxytocin had a positive influence on the other general effects of psychotherapy, namely Resource Activation, Clarification of Meaning, Problem Actuation, and Mastery. Furthermore, patients with higher concentrations of oxytocin before treatment reported higher Hope for Improvement, Fear of Change, and Suitability.

6.4.2 Interpretation of the results

The positive evaluations of the therapeutic alliance in the internet-based CBT examined here are in line with previous findings on the therapeutic alliance for internet-based treatments (Berger, 2017; Sucala et al., 2012). Previous research on internet-based CBT has provided mixed findings regarding the relationship between the therapeutic alliance and symptom improvements (Berger, 2017). Our study could help explain this heterogeneity, since we analyzed two components of the therapeutic alliance differentially. The Emotional Bond in the early treatment phase could not predict any symptom changes, but Agreement on Collaboration in the early treatment phase predicted reduced severity of the PTSD symptoms after the treatment was completed. It was previously conjectured that a ceiling effect, based on uniformly high ratings of Emotional Bond and, associated with it, a lack of variance in the predictor variable, was responsible for the fact that no relationship between the therapeutic alliance and symptom improvements was discovered in internet-based treatments (Berger, 2017). Our results, however, contradict this assumption. Although the Emotional Bond was rated as high on average, the variance

indicators nevertheless showed sufficient inter-individual variability. Our results indicate that the process-oriented component of the therapeutic alliance, that is, the Agreement on Collaboration, is more relevant for Internet-based treatments than the more emotion-oriented component, the Emotional Bond.

The influence of oxytocin on evaluations of the therapeutic alliance in the early treatment phase was statistically verified. The inferential and descriptive statistics consistently showed that oxytocin did not significantly influence the Emotional Bond. The correlation between oxytocin and Agreement on Collaboration was also not significant, but was higher at first. However, the strength of this relationship was reduced by the additional consideration of age.

We also explored the influence of oxytocin on the later evaluations of the therapeutic alliance, on other general change mechanisms of psychotherapy, and on psychotherapy expectation and evaluation. The general change mechanisms of psychotherapy were rated as more positive by patients with high oxytocin concentrations before the start of treatment than by patients with low oxytocin concentrations before the start of treatment. Regarding psychotherapy expectation and evaluation, patients with high concentrations of oxytocin before treatment reported higher Hope for Improvement and Suitability across all treatment phases and lower Fear of Change in the early treatment phase. However, this group reported higher Fear of Change in the later treatment phases. It also cannot be ruled out that the group differences are at least partially due to response tendency biases.

According to the social salience hypothesis, it is assumed that oxytocin increases the salience of social stimuli and thus, depending on individual characteristics, achieves either pro- or antisocial effects (Shamay-Tsoory & Abu-Akel, 2016). Based on studies of borderline personality disorder (Bartz, Simeon et al., 2011; Ebert et al., 2013) psychopathology was previously discussed as a moderator that explained the harmful effects of oxytocin in clinical populations (Bartz, Zaki, Bolger, & Ochsner, 2011; Olff et al., 2013; Shamay-Tsoory & Abu-Akel, 2016). In the present study with PTSD patients, however, prosocial effects of oxytocin were described at the descriptive level. This suggests that specific symptoms, such as chronic interpersonal insecurities (Bartz, Simeon et al., 2011), regulate the effects of oxytocin rather than generally increased vulnerability due to psychological problems.

6.4.3 Limitations

The most important limitation of the present study was the limited possibility of reaching statistical conclusions. This was due to the small sample size and our decision to avoid a large number of statistical tests by not using hypothesis-validating statistical procedures for most psychotherapy process variables. An a priori power analysis we performed to estimate the number of patients we needed in order to evaluate the effectiveness of internet-based CBT, resulted in a sample size of $n = 100$ (Niemeyer et al., 2020). Any additional analysis, such as the role of biological markers in treatment, would have required an even larger number of patients to reach statistically valid conclusions. Problems with recruitment and dropouts, which had been previously reported in military samples (Hoge et al., 2014), resulted in a significantly reduced sample size compared to our goal. That was the reason for our decision to reduce the number of inferential statistical tests to a minimum and instead focus on descriptive evaluations. Our evaluations should therefore be regarded as exploratory and our results, which can be viewed as preparatory work for further studies, must be confirmed in larger, independent samples.

Another limitation concerns the meaningfulness of endogenous oxytocin concentrations. On the one hand, their measurement is presented as an important noninvasive tool to study interactions between the oxytocin system and psychological processes (Crockford, Deschner, Ziegler, & Wittig, 2014). On the other hand, psychological processes are controlled by the central nervous system, whereby peripheral oxytocin concentrations only reflect the central nervous availability of oxytocin under specific conditions, such as after acute stress or intranasal administration (Valstad et al., 2017). Furthermore, endogenous oxytocin concentrations are not stable over time and are susceptible to the influence of confounding variables. In the present sample, the oxytocin concentrations before the start of treatment and immediately after treatment were not correlated ($r = 0.04$, $p = 0.89$, see Engel et al., in preparation) This makes it clear that the group differences in the later therapy phases, which have a longer time interval to the oxytocin measurements, should especially be interpreted with caution. The use of psychotropic drugs should be mentioned as a possible relevant confounding variable. Although this was intraindividually stable in the present study, it could vary interindividually.

It should also be noted that the patients with lower oxytocin concentrations were younger than those with higher oxytocin concentrations. The reduction in the relationship between oxytocin and the variables of the therapeutic alliance at the beginning of treatment, after taking age into account, suggests that age differences could also be relevant for the group differences.

The psychotherapeutic treatment that we studied was delivered through a specific medium: the internet. It has been well demonstrated that internet-based CBT effectively reduces PTSD symptoms, with effect sizes that are comparable to those of conventional face-to-face psychotherapy (Kuester, Niemeyer, & Knaevelsrud, 2016). However, internet-based CBT has some specific characteristics, particularly with regard to the social interaction between patient and therapist. So far, little research has been done on the mechanisms underlying symptom improvements in internet-based CBT; however, there are indications that these differ in part from the mechanisms of action in face-to-face psychotherapy (Andersson, Cuijpers, Carlbring, Riper, & Hedman, 2014). The predictive value of the therapeutic alliance for symptom improvement in internet-based CBT is still disputed (Berger, 2017), whereas the therapeutic alliance of face-to-face psychotherapy has been established as a general change mechanism (Horvath et al., 2011). Although internet-based CBT represents an interesting context to investigate the influence of oxytocin on psychotherapy process variables, it remains unclear to what extent the results are also applicable to face-to-face psychotherapy.

6.4.4 Conclusion

The present study provides evidence that endogenous oxytocin concentrations have prosocial effects in PTSD patients. This was shown in positive evaluations of the therapeutic alliance and other general psychotherapeutic mechanisms of change, which were compiled in the context of an internet-based CBT program. It is necessary to validate these results in a larger, independent sample using inferential statistics and to confirm them outside the specific context of internet-based CBT.

It has been debated whether stimulation of the endogenous oxytocin system by intranasal administration of synthetic oxytocin (van IJzendoorn, Bhandari, van der Veen, Grewen, & Bakermans-Kranenburg, 2012) might be contraindicated in clinical populations (Hurlemann, 2017). In our study, however, at

least with regard to endogenous oxytocin concentrations, evidence of possible positive effects was found. Future studies could examine a possible therapeutic benefit of oxytocin nasal sprays for the effectiveness of trauma-focused psychotherapy. The therapeutic alliance is particularly important in the early treatment phase, in order to keep patients in treatment and to stimulate symptom improvement (Horvath et al., 2011). Therefore, future studies could specifically investigate whether trauma-focused psychotherapy could be promoted by stimulating the oxytocin system in the early treatment phase.

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Contributions

Christine Knaevelsrud and Sarah Schumacher designed the study.

Christine Knaevelsrud obtained the funding.

Sebastian Burchert, Sinha Engel, Hannah Klusmann, Christine Kersjes, Annika Küster, Beate Muschalla, Heinrich Rau, Jan-Peter Spies and Deborah Weiss collected the data.

Sarah Schumacher and Helen Niemeyer performed the internet-based trauma-focused cognitive behavioral treatment.

The staff of the German Armed Forces Centre of Military Mental Health, Berlin provided administrative, technical and material support.

Rainer Landgraf analyzed the oxytocin samples and provided assistance in analysis and interpretation of the data.

Sinha Engel performed the statistical analyses and drafted the manuscript. Susan Welsh translated the original German version of the manuscript to English. All authors critically revised the manuscript for important intellectual content.

Lars Schulze created FIGURES 6.3, 6.4 and 6.5.

Sarah Schumacher, Christine Knaevelsrud and Gerd-Dieter Willmund supervised the study.

CHAPTER 7

Demographic, sampling- and assay-related confounders of endogenous oxytocin concentrations: A systematic review and meta-analysis

Engel, S., Laufer, S., Miller, R., Niemeyer, H., Knaevelsrud, C., & Schumacher, S. (2019). Demographic, sampling- and assay-related confounders of endogenous oxytocin concentrations: A systematic review and meta-analysis. *Frontiers in Neuroendocrinology*, *54*, 100775.

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Demographic, sampling- and assay-related confounders of endogenous oxytocin concentrations: A systematic review and meta-analysis

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Abstract

Studies on endogenous oxytocin concentrations are often criticized for the debatable comparability between specimens and the variation in reported values. We performed meta-regressions on k = 229 studies (n = 12 741 participants), testing whether specimen, extraction, sex, age, time of day, or fasting instructions influenced oxytocin measurements. Predicted oxytocin concentrations differed depending on specimen and extraction: Measurements were extremely high in unextracted blood, compared to extracted blood and other specimens. Measurements were higher in samples with more female participants and higher age. Instructions not to smoke before sampling were correlated with higher oxytocin in unextracted samples. There was no impact of instructions to refrain from eating, drinking, consume caffeine, alcohol or exercising. Oxytocin concentrations increased from morning to afternoon. Our results showed that oxytocin is differentially reflected in blood, saliva, urine and cerebrospinal fluid. Extraction impacts oxytocin measurements, particularly in blood. Considering relevant confounders might increase comparability between studies.

Keywords: Covariate; Extraction; Specimen; Sex; Age; Sampling; Nicotine, Smoking; Diurnal rhythm; Time of day

7.1. Introduction

The neuropeptide oxytocin is an important agent in clinical psychoneuroendocrinological research. There is a large body of research investigating endogenous oxytocin concentrations as a possible indicator of neuroendocrine dysregulations in patients with mental disorders (Cochran, Fallon, Hill, & Frazier, 2013). At the same time, the reliability of this approach has been strongly disputed (McCullough, Churchland, & Mendez, 2013).

Oxytocin is synthesized in magnocellular neurons of the paraventricular and supraoptic nuclei of the hypothalamus. From there, it is transmitted to the posterior lobe of the pituitary and released into the bloodstream (Brownstein, Russell, & Gainer, 1980). Peripheral oxytocin receptors have been identified in the reproductive organs, mammary tissue, kidney, heart, thymus, fat cells, pancreas and adrenal gland (Gimpl & Fahrenholz, 2001; Jurek & Neumann, 2018; Song & Albers, 2018). In addition to its function as a hormone, oxytocin acts centrally as a neurotransmitter and neuromodulator. From both, magnocellular neurons of the hypothalamic paraventricular and supraoptic nuclei and parvocellular neurons of the paraventricular nucleus, it is directly transmitted to various brain sites (Knobloch & Grinevich, 2014).

A variety of complementary methodological approaches is available to determine oxytocin's functions. In animal models, brain site-specific effects of endogenous oxytocin can be determined by means of microdialysis, as well as oxytocin receptor agonist or antagonist injections (Lee, Park, & Park, 2008; Neumann, 2008). In addition, behavioral consequences of synthetic oxytocin injection to different brain regions can be observed (Neumann, 2008). The methodological approaches available in human research measure oxytocin's effects in a less specific manner. Intranasally administered synthetic oxytocin passes the blood-brain barrier (Born et al., 2002), but targets a variety of brain sites through different pathways (Quintana, Alvares, Hickie, & Guastella, 2015). Therefore, behavioral consequences can only be interpreted brain site-unspecifically. Endogenous oxytocin concentrations can be measured in different body fluids, such as blood, saliva, urine, and cerebrospinal fluid (McCullough et al., 2013), but, again, these effects cannot be attributed to specific central mechanisms of action.

7.1.1 Oxytocin's behavioral and psychological functions

Besides oxytocin's role in physiological processes such as birth and lactation (Kendrick, Keverne, Chapman, & Baldwin, 1988), it influences key behavioral and psychological processes related to mental well-being.

7.1.1.1 Evidence from animal studies

Fear conditioning and safety learning paradigms have been used to study oxytocin's anxiolytic effects and related them to oxytocin receptor expressing structures of the neural fear network (Eckstein et al., 2019; Maroun & Wagner, 2016). For instance, central administration of synthetic oxytocin to rats prior to fear acquisition enhanced, whereas injection of an oxytocin receptor antagonist impaired extinction. Oxytocin administration after fear acquisition, on the other hand, impaired extinction, indicating a time-dependency of the effects (Toth, Neumann, & Slattery, 2012). Additionally, a region-dependency was detected when studying the effects of synthetic oxytocin and oxytocin receptor agonist injection to rats' infralimbic prefrontal cortex, basolateral and central amygdala pre- and post-fear acquisition on extinction (Lahoud & Maroun, 2013). In summary, these results show that, depending on temporal dynamics, oxytocin has anxiolytic effects that are mediated via its receptors at different brain sites.

In addition, oxytocin modulates the endocrine stress response (Engelmann, Landgraf, & Wotjak, 2004; Hostinar, Sullivan, & Gunnar; Winter & Jurek, 2019). Increased oxytocin release in the paraventricular nucleus of the hypothalamus in response to stress was observed in male rats (Babygirija, Bülbül, Yoshimoto, Ludwig, & Takahashi, 2012). This process is assumed to downregulate neuroendocrine stress responses, as in virgin rats, intracerebroventricular infusion of an oxytocin antagonist increased secretion of corticotropin and corticosterone both under basal conditions and in response to stress (Neumann, Torner, & Wigger, 2000). Moreover, originating from research that linked centrally injected synthetic oxytocin to maternal behavior of rats (Pedersen, Caldwell, Peterson, Walker, & Mason, 1992) or to partner preferences of female monogamous prairie voles (Williams, Insel, Harbaugh, & Carter,

1994), oxytocin became known for its prosocial effects (Baribeau & Anagnostou, 2015; Heinrichs, Dawans, & Domes, 2009; Macdonald & MacDonald, 2010).

7.1.1.2 Transfer to human studies

Oxytocin's anxiolytic, stress-reducing and prosocial effects were also transferred to human subjects: Intranasal oxytocin administration after fear acquisition enhanced extinction recall (Acheson et al., 2013) and increased prefrontal cortex and electrodermal activity during the early extinction phase, followed by a stronger decline of electrodermal activity (Eckstein et al., 2015).

Oxytocin's modulation of the human endocrine stress response seems to be particularly relevant in social contexts: Intranasal oxytocin administration led to lower cortisol concentrations, along with decreased subjective stress, in response to a social stress paradigm in healthy men (Heinrichs, Baumgartner, Kirschbaum, & Ehlert, 2003). In addition, it decreased the cortisol response of heterosexual partners during a couple conflict paradigm and promoted positive communication (Ditzen et al., 2009). Concerning its prosocial effects, oxytocin has been associated with more complex social behaviors in humans. Examples include a promotion of trust, as investigated by means of intranasal administration in relation to trust game paradigms (Baumgartner, Heinrichs, Vonlanthen, Fischbacher, & Fehr, 2008; Kosfeld, Heinrichs, Zak, Fischbacher, & Fehr, 2005), or an involvement in romantic attachment as investigated by means of elevated endogenous oxytocin concentrations measured in newly attached romantic partners compared with singles (Schneiderman, Zagoory-Sharon, Leckman, & Feldman, 2012). However, it is worth noting that oxytocin's functions vary between persons and contexts rather than being beneficial for social behavior in general (Olff et al., 2013).

7.1.2 How reliable is current research on endogenous oxytocin?

Impairments in fear processing, stress coping and social behavior are characteristics of several mental disorders. Therefore, oxytocin appears to be an interesting target for clinical researchers. The most frequently applied approach to investigate the endogenous oxytocin system in humans is to measure endogenous oxytocin concentrations in blood, saliva, urine, or CSF. To date, a number of studies have used this approach to investigate dysregulations of the oxytocin system in clinical populations (Cochran

et al., 2013). However, the reliability of measuring endogenous oxytocin concentrations has been strongly questioned (McCullough et al., 2013; Szeto et al., 2011).

For instance, it remains unclear whether peripheral measures reflect the central availability of oxytocin. This is of particular relevance for biomarker research, as oxytocin's psychological functions are assumed to be regulated by its central actions (Meyer-Lindenberg, Domes, Kirsch, & Heinrichs, 2011). A recent meta-analysis underlined this concern, as it found no correlation between oxytocin concentrations from central and peripheral specimens under basal conditions (Valstad et al., 2017). Thus, basal peripheral concentrations do not seem to reflect central ones. However, correlations were found under challenged conditions. Such paradigms might be used to estimate central oxytocin release by means of peripheral measurements (Crockford, Deschner, Ziegler, & Wittig, 2014; Valstad et al., 2017). Evidence from animal studies further emphasizes that the consistency between peripheral and central measurements crucially depends on the specific brain site investigated. For instance, early studies applying push-pull perfusion and microdialysis in rats showed that the increased peripheral availability of oxytocin during birth (Higuchi, Tadokoro, Honda, & Negoro, 1986) was independent of oxytocin release in the septum and hippocampus (Landgraf, Neumann, & Pittman, 1991), whereas it was successfully associated with oxytocin release in the supraoptic and paraventricular hypothalamic nuclei (Neumann, Russell, & Landgraf, 1993). In line with this, central injection of an oxytocin antagonist led to decreased suckling-induced oxytocin release in both the supraoptic nucleus and blood (Neumann, Ludwig, Engelmann, Pittman, & Landgraf, 1993). Furthermore, increased oxytocin concentrations were found both in blood and in the hypothalamic supraoptic and paraventricular nuclei of rats in response to stress (Wotjak et al., 1998).

In addition, it is problematic that there is an extremely high variability of reported oxytocin values between studies, even within similar populations and contexts (Szeto et al., 2011). These inconsistencies can partly be explained by different measurement methods. Not only the correlation between central and peripheral specimens (Valstad et al., 2017), but also that between different peripheral specimens has been called into question (Hoffman, Brownley, Hamer, & Bulik, 2012). Furthermore, the comparability between studies that differ with regard to analysis (e.g. enzyme immunoassay (EIA) or

radioimmunoassay (RIA) and sample preparation (e.g. extraction) methods is doubtable (Leng & Sabatier, 2016; Szeto et al., 2011). Sample extraction precedes the actual peptide measurement and prevents matrix components and molecules other than oxytocin from being detected by the assay (Szeto et al., 2011). Various methods to extract samples exist (Cool & DeBrosse, 2003; Szeto et al., 2011). Comparisons showed that concentrations derived from unextracted samples were (several) hundred-fold higher than from extracted ones and that both measurements were uncorrelated (Robinson, Hazon, Lonergan, & Pomeroy, 2014; Szeto et al., 2011). There are indications that measurements in unextracted samples are more influenceable by sampling-related factors (Robinson et al., 2014) and it was suggested that, next to oxytocin, assays detect multiple other immunoreactive products from unextracted samples (Szeto et al., 2011).

In addition to these methodological issues, potentially confounding variables, such as sex, age, time of day or fasting might influence measurements of endogenous oxytocin concentrations and therefore explain variability between studies. To date, a number of empirical studies has investigated these potential confounders of endogenous oxytocin concentrations, but this primary work has not been summarized in a systematic overview, yet.

Oxytocin's physiological and psychological functions relating to reproductive and sexual behavior (Veening, Jong, Waldinger, Korte, & Olivier, 2015) and its interactions with sex hormones (Patisaul, Scordalakes, Young, & Rissman, 2003) suggest sex differences in endogenous oxytocin concentrations. A review on interindividual differences in the response to intranasal oxytocin administration indeed mentioned that there are some indications that plasma oxytocin levels differ between the sexes (Macdonald, 2013). The idea that endogenous oxytocin concentrations might vary with age has been discussed due to the role of oxytocin in attachment (Huffmeijer, van IJzendoorn, & Bakermans-Kranenburg, 2013). Throughout the lifespan, persons are committed to various kinds of relationships and experience changes in their social roles. Interactions between these events and the oxytocin system seem evident. However, to date, it remains unclear whether there are age-related differences in endogenous concentrations (Huffmeijer et al., 2013). Concerning the possible role of the time of day of sampling as a confounder, there is only a small amount of data from humans, which does not allow

definitive conclusions. Nevertheless, these data, as well as data from animal studies, indicate diurnal fluctuations, as suggested in a review on oxytocin's therapeutic potential (Macdonald & Feifel, 2013). Evidence from animal studies also indicate that food (Burlet, Jhanwar-Uniyal, Chapleur-Chateau, Burlet, & Leibowitz, 1992), nicotine (Russell & Chaudhury, 1972), or caffeine intake (Wu et al., 2017) as well as physical exercise (Bakos et al., 2007) might impact oxytocin measures. Oxytocin's regulatory impact on metabolism and food intake has been extensively studied (Ding, Leow, & Magkos, 2018). Concerning the reverse impact of dietary behaviors on endogenous oxytocin concentrations, a recent review systematically described 13 primary studies on this topic, but, lacking sufficient data, could not provide an effect size (Skinner, Garg, Dayas, Fenton, & Burrows, 2018).

In conclusion, different kinds of specimen and assay-related aspects, such as sample preparation and analysis method, cause variation in reported values of endogenous oxytocin concentrations. Additionally, confounders of endogenous oxytocin might constitute a source of variance. As yet, there is no systematic overview of such confounders, and accordingly, guidelines in this field are lacking. Therefore, researchers presumably apply heterogeneous sampling protocols and statistically control for different variables in their analyses. This might explain the significant variation in concentrations reported by studies, decreasing the trust in the reliability of current clinical research on oxytocin (McCullough et al., 2013; Szeto et al., 2011).

7.1.3 Objectives

This is the first systematic review and meta-analysis to address these issues related to measurements of endogenous oxytocin. We performed meta-regressions to test whether possible heterogeneity in basal endogenous oxytocin concentrations of healthy humans might be explained by specimen, considering the impact of extraction. Moreover, we determined the impact of participants' sex and age, time of day of sampling, and fasting instructions. All analyses were controlled for assay-related variance.

According to the recommended reporting system by the PRISMA group (Moher, Liberati, Tetzlaff, Altman, & PRISMA Group, 2009), our main systematic research question can be described as follows: As population, we included healthy, adult and non-pregnant humans. We included studies without

special consideration of intervention or comparison group. In terms of outcome, we extracted basal measures of endogenous oxytocin concentrations. We included between-, within-, and single-group designs.

7.2. Methods

7.2.1 Review protocol, data, analysis and results accessibility

We published a protocol explaining the rationale and methods of this systematic review and meta-analysis on PROSPERO (Registration number: CRD42017072306) on the 17th July 2017. It is available online at: http://www.crd.york.ac.uk/PROSPERO/display_record.php?ID=CRD42017072306

Moreover, we published our data, analysis methods and results on an Open Science Framework repository on the 14th November 2018. It is available online at:

https://osf.io/4jsah/

7.2.2 Eligibility criteria

Studies were eligible if they included a sample or subsample of healthy, adult (mean age ≥ 18 years) and non-pregnant humans. Samples or subsamples consisting of animals, children (< 18 years), persons with mental or physical illness or injuries, and pregnant or postpartum women (≤ 1 year after birth) were excluded. Studies applying reactivity designs (e.g. stress paradigms or pharmacological challenges) or evaluating an intervention (e.g. psychotherapy or pharmacotherapy) were included if they reported pre-challenge or pre-intervention basal oxytocin concentrations. We did not define any restrictions with regard to comparison groups. A single sample or subsample of participants meeting our criteria was sufficient for our study aims. In terms of outcome, studies were required to measure basal measures of endogenous oxytocin concentrations in blood, saliva, urine, or CSF. Despite the problems associated with unextracted measurements (Leng & Sabatier, 2016; Robinson et al., 2014; Szeto et al., 2011), we included studies measuring oxytocin in extracted and unextracted samples in order to meet the standard of a comprehensive systematic review. We included studies applying between-, within- and single-group

designs but excluded case studies and reviews. Published and unpublished studies in the English and German language were considered. There were no restrictions concerning publication year.

7.2.3 Identification and selection of studies

7.2.3.1 Information sources and electronic search strategy

The literature search was conducted following the search strategies recommended by Lipsey and Wilson (2001). We searched the following electronic bibliographic databases and registers up to 28th March 2017: *PsycINFO, PubPsych, PsycARTICLES, PubMed, Web of Science, BIOSIS, ProQuest Dissertations and Theses Global* and *Clinicaltrials.gov*. We searched titles, abstracts and keywords for the terms ((oxytocin) AND (blood OR plasma OR serum OR CSF OR cerebrospinal fluid OR urine OR urinary OR saliva OR salivary)). We searched the grey literature by examining the abstracts of conference contributions, posters and commentaries, and contacted experts in the field. Moreover, we screened reference lists of reviews and articles selected for inclusion in this review.

7.2.3.2 Study selection

In a first step, titles and abstracts were screened, excluding duplicates and studies meeting our exclusion criteria (i.e., studies published in languages other than English or German, animal studies, studies with children, studies with exclusively pregnant or post-partum women, studies with exclusively mentally or physically ill participants, reviews, qualitative studies, studies investigating biochemical micro-processes, e.g. in-vitro studies, studies that did not assess endogenous oxytocin, studies that did not assess endogenous oxytocin under basal conditions, study registers without reported data, and studies without available abstracts). Regarding study registers, we contacted the corresponding author to ask whether data were accessible and, if possible, included them in the screening process. If abstracts or full texts were not available, we contacted the corresponding author and requested access to the paper.

In a second step, full-text articles were screened to decide whether studies were eligible for inclusion.

In a third step, we descriptively summarized all eligible studies and included those with all necessary data obtainable in the meta-analytic procedure.

Steps one and two were performed by one researcher (SE). Step three was conducted as follows: Studies considered as eligible were randomized based on a computerized tool and assigned to a team of two researchers (SE and AW or SL and HK). Researchers within one team independently extracted data and, based on this, decided whether all information necessary for the meta-analytic procedure was obtainable. In the case of disagreements, a third independent person (SL or SE, respectively) was consulted for the final decision.

7.2.4 Data extraction and preparation

7.2.4.1 Data collection process

Data from individual studies were extracted according to a predefined coding manual which the researchers had been trained to use. Extraction was performed by the same teams and using the same strategies as decisions regarding inclusion. If data were not accessible or values could only be estimated, the corresponding authors of the primary studies were contacted in order to gather as many data as possible and to obtain data that were as precise as possible. The authors were contacted by e-mail and a reminder was sent after 10 days if no reply was received. If authors were unable to provide us with the data or did not respond to the emails, we estimated the data if possible, or otherwise considered them as unavailable. Studies without available data or studies containing data that overlapped with other samples were descriptively summarized but excluded from the meta-analysis. With regard to overlaps, we first extracted data from all studies, and then included those studies which provided a larger sample or more useable data in the meta-analytic procedure.

7.2.4.2 Data items

In terms of our outcome measure, we extracted *M* and *SEM* of basal endogenous oxytocin concentrations. If studies reported more than one basal value, we extracted the chronologically first one. From crossover designs in which participants underwent different challenges in varying orders, we extracted values measured before their first challenge whenever possible. Otherwise, we picked one of the challenges at random and extracted the corresponding value that was gathered beforehand. If values were not reported in texts or tables but were shown in figures, we used an online plot digitizer (Rohatgi,

2015).This tool has been previously applied in a meta-analysis on endogenous oxytocin and yielded good results (Valstad et al., 2017). If *M* and *SEM* were not directly extractable, we estimated them from other descriptive parameters (i.e. *Md*, *SD*, *Var*, *Min,* and *Max*, (Hozo, Djulbegovic, & Hozo, 2005) and transformed all units into pg/ml. For the respective formulas, see ONLINE SUPPLEMENTARY MATERIAL 7.1.

We extracted sample sizes and considering assay-related variables, the type of specimen (blood, saliva, urine, or CSF), extraction (0 = no, 1 = yes), assay (e.g. EIA or RIA), location of the biochemical laboratory and assay manufacturer. If the assay manufacturer was not reported, the information was imputed from the laboratory location to enable the estimation of assay-related heterogeneity in oxytocin concentrations.

Regarding potential confounders, we extracted *M* and *SEM* of age and sex distribution of each sample. Furthermore, we extracted the time of sampling and whether participants were asked to refrain from eating, drinking, consuming caffeine, smoking, consuming alcohol, or exercising before sample collection. In addition, we extracted study type (e.g. journal article, dissertation), information about overlaps, and location of data collection.

7.2.5 Assessment of risk of bias and appropriateness to the aim of our study

Our inclusion criteria were defined with the aim of gathering a large and representative pool of studies. Therefore, we included studies with heterogeneous designs. As most validated tools for the assessment of risk of bias refer to specific study designs, there was no available tool which was appropriate to all studies in our pool. We did not wish to refrain from estimating study quality, as we consider this to be an important aspect of systematic reviews. Therefore, in line with the notion of the Weight of Evidence framework (Gough, 2007), we developed an instrument that included items on general study quality and the appropriateness to our study. To estimate general study quality, we used one item referring to the handling of missing data. To estimate the appropriateness to our study, we used two items referring to the assessment of physical and mental health, as health constituted a central inclusion criterion.

The rating process was carried out by the same teams and with the same strategies as decisions about inclusion and extraction. We used the final summary score of these three items, indicating quality and appropriateness to our review aims, as a regressor in our meta-regression in order to control for risk of bias and appropriateness to the study aim when estimating the influence of the respective confounders.

7.2.6 Meta-analytic procedure

We performed meta-regressions using our predefined potential confounders as sample-level regressors to explain between (sub-)samples heterogeneity in basal endogenous oxytocin concentrations in log-scaled pg/ml (Higgins, White, & Anzures-Cabrera, 2008).

In a first step, we defined a baseline regression model that served as the basis for all following analyses. It included two variance components to account for study-and assay-related heterogeneity. Study-related heterogeneity was considered as variance component as in some cases, we included several independent samples from the same study. Assay-related heterogeneity was considered as variance component to control for the impact of different laboratories, assay manufacturers and assay types (RIA or EIA). In this way, we ensured that the influence of the regressors added to the model was estimated irrespective of similarities within studies and differences between laboratories, assay manufacturers or assay types.

The baseline regression model included sample-level moderators representing specimen (reference: blood, comparisons: saliva, urine, CSF) and extraction (reference: unextracted samples). A highly accurate imputation model was fitted to handle missing values in the extraction variable. Main, as well as interactive effects of specimen and extraction were tested. In addition, the baseline regression model comprised main effects of the regressors year of publication (reference: 2 000 years AD, scale: 10 years), risk of bias and appropriateness score, and the standard error of the reported oxytocin concentration (in order to adjust for bias using the PET-PEESE method (Stanley & Doucouliagos, 2014)) as sample-level moderators.

In the following steps, we entered the possible confounders into the model as sample-level moderators. Sex was defined as a metric variable indicating the percentage of women per sample. The metric variable age was referenced at 30 years and scaled with 10 years. The metric variable time of day was referenced

at 12 a.m./midnight and scaled with units of three hours. Instructions to fast were divided into seven variables: instructions to refrain from eating, drinking, consuming caffeine, smoking, consuming alcohol, or exercising, as well as one combined variable indicating whether any of these instructions was given. Fasting variables were coded dichotomously, indicating whether participants received the respective instructions (1) or not (0). Missing values in the fasting variables were imputed to 0, as we assumed that if no such instructions were reported in the paper, they were not given.

Unextracted blood was set as reference for all analyses of possible confounders. In order to test whether extraction moderated their possible impact, we performed subgroup analyses. This implies that the main analysis which was initially conducted across all primary studies was performed separately within those studies that used unextracted samples and within those that used extracted samples. All analyses were performed using the *metafor* package (Viechtbauer, 2017) and *R* statistical software (R Core Team, 2017).

7.3. Results

7.3.1 Study selection

The number of studies that were screened, assessed for eligibility, excluded for predefined reasons or included in the qualitative or quantitative part of this review are shown in a flow diagram (FIGURE 7.1). ONLINE SUPPLEMENTARY MATERIAL 7.2 contains the full reference list. We included 326 studies in the qualitative analysis and 229 studies (comprising 12 741 participants from 339 subsamples) in the meta-analytic procedure.

7.3.2 Description of primary studies and consideration of potential confounders

TABLE 7.1 shows the data of the 229 studies upon which the meta-analytic procedure was based. It reports size, age and sex distribution of each sample, as well as time of sampling, fasting instructions, specimen and oxytocin concentrations.

As comprehensive information was extracted from all 326 primary studies, the remaining results of the qualitative analyses are presented as supplementary information. The ONLINE SUPPLEMENTARY MATERIAL 7.3 reports study type, sample overlaps, location of data collection, as well as number of participants, sex distribution, and age. It also shows the time of sampling, fasting instructions and specimen. The results of our risk of bias and appropriateness ratings for all 326 studies are shown in the ONLINE SUPPLEMENTARY MATERIAL 7.4.

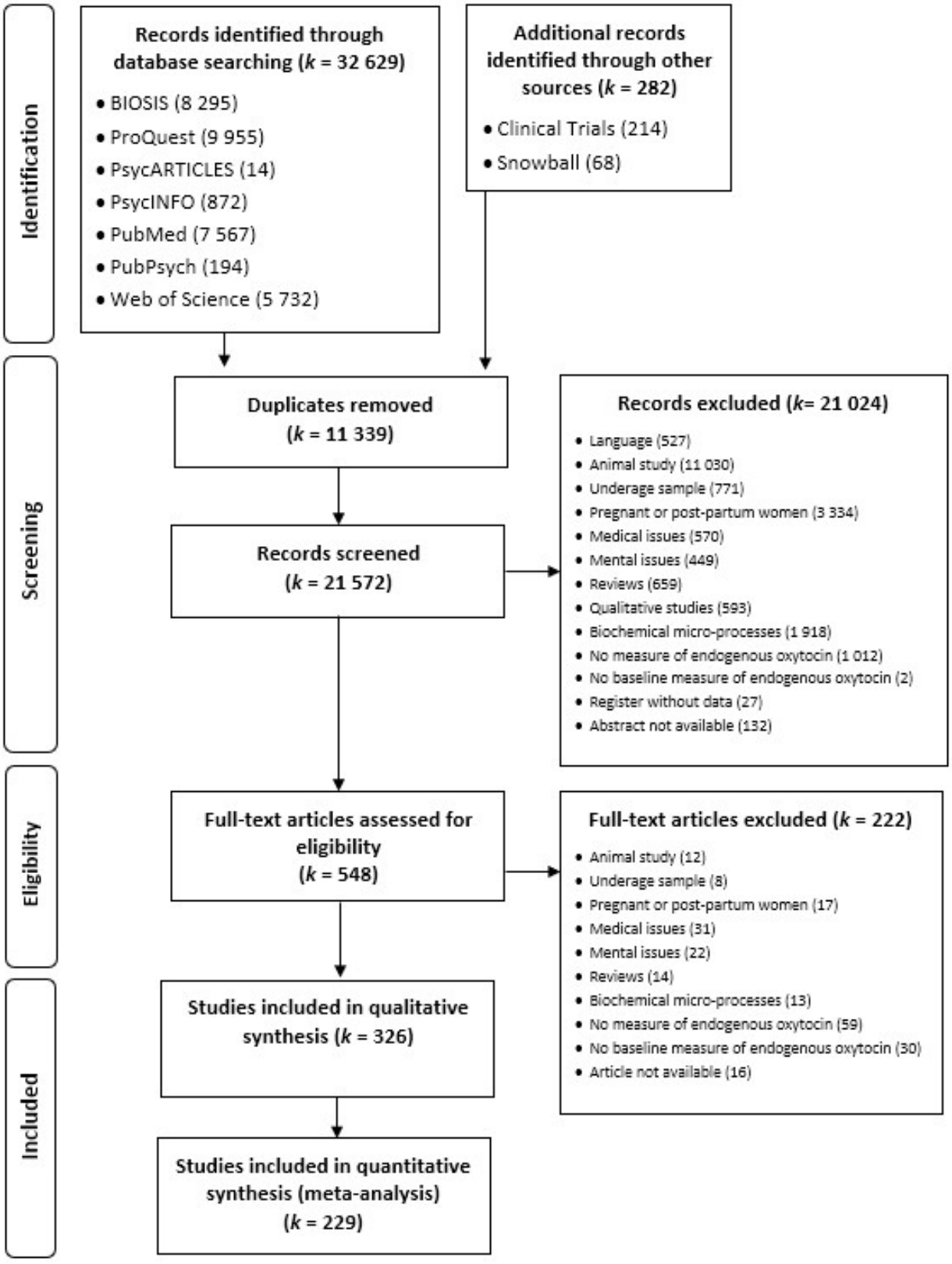

FIGURE 7.1. Flow diagram of identification and selection of primary studies.

TABLE 7.1. Description of studies included in meta-analytic procedure.

Authors	Year	*n*[a]	% female[b]	Age *M*	Time of day[c]	Fast[d]	Fast[e]	Eat[e]	Drink[e]	Caffeine[e]	Smoking[e]	Alcohol[e]	Exercise[e]	Specimen	Oxy *M*[f]	Oxy*SD*[f]	Oxy *M*log[g]	Oxy *SD*log[g]
Afinogenova	2015	34	100.00	23.90	N/A	yes	1	0	0	0	0	0	0	blood	1501.00	664.73	7.22	0.42
Ahmed et al.	2015	23	52.17	72.00	N/A	yes	1	0	0	0	0	0	0	blood	179.00	95.00	5.06	0.50
Altemus et al.	1999	26	46.15	35.00	9:00am - 10:00am	yes	1	1	1	1	0	1	1	CSF	3.70	1.02	1.27	0.27
Altemus et al.	2001	8	100.00	34.20	7:50am - 9:00am	yes	1	1	0	1	1	1	1	blood	1.67	0.28	0.50	1.68
Althaus et al.	2016	30	0.00	22.60	9:10am or 2:10pm	yes	0	0	0	1	1	1	0	blood	0.68	0.78	-0.81	0.92
Amico, Seif & Robinson	1981a	17	0.00	N/A	morning and afternoon	yes	1	1	0	1	1	1	1	blood	3.00	0.49	1.09	0.16
		8	100.00												2.85	0.34	1.04	0.12
		5	100.00												7.65	1.90	2.00	0.24
		5	40.00												35.32	5.70	3.55	0.16
Amico, Seif & Robinson	1981b	5	100.00	26.00	9:00am	yes	0	0	0	1	1	1	0	blood	1.90	0.94	0.53	0.47
Amico et al.	1983	6	0.00	N/A	6:00am	yes	0	0	0	1	1	1	0	blood	1.33	0.42	0.24	0.31
		6	100.00												2.50	1.22	0.81	0.46
Amico et al.	1985	18	100.00	N/A	N/A	N/A	0	0	0	0	0	0	0	blood	1.33	0.63	0.18	0.45
		18	100.00												2.83	0.51	1.02	0.18
		18	0.00												1.22	0.42	0.14	0.34
		18	0.00												2.83	0.64	1.02	0.22
		5	100.00												1.33	0.16	0.28	0.12
		5	100.00												7.67	1.90	2.01	0.24
		4	100.00												0.50	0.20	-0.75	0.39
		4	100.00												2.67	0.54	0.96	0.20
		3	0.00												3.17	1.00	1.11	0.31
Amico & Johnston	1985	8	50.00	N/A	9:00am - 10:00am	yes	1	0	1	1	1	1	0	blood	2.17	0.48	0.75	0.22
		6	0.00												1.67	0.73	0.42	0.42

Authors	Year	*n*[a]	% female[b]	Age *M*	Time of day[c]	Fast[d]	Fast[e]	Eat[e]	Drink[e]	Caffeine[e]	Smoking[e]	Alcohol[e]	Exercise[e]	Specimen	Oxy *M*[f]	Oxy*SD*[f]	Oxy *M*log[g]	Oxy *SD*log[g]
Amico, Ulbrecht, Robinson	1987	4	0.00	N/A	8:00am	yes	1	0	0	1	1	1	0	urine	7.82	3.60	1.96	0.44
		9	N/A		9:00am - 12:00pm										17.00	6.99	2.76	0.40
		8												blood	0.83	0.48	-0.33	0.54
Andari	2014	15	43.30	23.67	10:00am	yes	0	0	0	0	1	0	0	blood	3.67	0.32	1.30	0.02
		20	N/A	N/A	N/A	N/A	0	0	0	0	0	0	0		1.50	0.18	0.41	0.03
Anderberg & Uvnäs-Moberg	2000	30	100.00	N/A	8:00am - 9:00am	yes	1	0	0	0	0	0	0	blood	18.14	4.70	2.87	0.25
Apter-Levi, Zagoory-Sharon & Feldman	2014	48	0.00	29.30	1:00pm - 4:00pm	yes	1	1	0	0	0	0	0	blood	391.18	159.72	5.89	0.39
Bagdy & Arato	1998	7	0.00	31.70	7:30am	yes	1	0	0	0	0	0	1	blood	8.28	3.81	2.02	0.44
		5	100.00	33.00											6.49	1.77	1.83	0.27
Barraza	2010	144	51.00	20.80	1:00pm - 4:00pm	yes	0	0	0	1	0	1	0	blood	456.45	300.24	5.94	0.60
Beavin	2014	30	0.00	49.17	N/A	N/A	0	0	0	0	0	0	0	blood	341.96	175.72	5.72	0.48
		56	0.00	21.66											1.97	2.15	0.29	0.89
Bello	2007	13	0.00	33.29	1:30pm - 9:00pm	yes	1	1	1	1	0	1	0	blood	281.60	95.55	5.58	0.33
Bello et al.	2008	14	0.00	N/A	1:30pm - 9:00pm	yes	1	1	1	1	1	1	0	blood	219.56	106.23	5.29	0.46
Bershad et al.	2015	11	54.55	22.50	9:00am	yes	0	0	0	1	1	1	0	blood	37.80	45.77	3.18	0.95
		7	0.00	25.50											6.36	2.54	1.78	0.38
Bhandari et al.	2014	93	100.00	19.86	9:00am or 12:00pm or 3:00pm	yes	0	0	0	0	1	0	0	saliva	2.65	1.32	0.86	0.47
Bialik	2007	30	53.30	28.70	N/A	N/A	0	0	0	0	0	0	0	blood	129.70	71.70	4.73	0.52
Bick	2011	43	100.00	42.10	6:00am - 8:00pm	yes	0	0	0	1	1	1	0	urine	0.87	0.07	-0.14	0.08
Blagrove et al.	2012	17	50.00	20.45	10:00pm	yes	1	1	1	1	1	1	1	saliva	12.63	3.35	2.50	0.26
Blaicher et al.	1999	12	100.00	N/A	N/A	N/A	0	0	0	0	0	0	0	blood	11.53	6.08	2.32	0.50

Authors	Year	*n*[a]	% female[b]	Age *M*	Time of day[c]	Fast[d]	Fast[e]	Eat[e]	Drink[e]	Caffeine[e]	Smoking[e]	Alcohol[e]	Exercise[e]	Specimen	Oxy *M*[f]	Oxy*SD*[f]	Oxy *M*log[g]	Oxy *SD*log[g]
Bosch et al.	2015	16	0.00	23.90	8:30am	yes	0	0	0	1	1	1	0	blood	17.49	8.46	2.76	0.46
Bossmar, Forsling & Åkerlund	1995	10	100.00	55.20	7:00am - 8:30am	yes	0	0	0	0	1	0	0	blood	2.52	1.01	0.92	0.13
Breuil et al.	2014	1097	100.00	71.80	12:00pm - 3:00pm	no	0	0	0	0	0	0	0	blood	1.00	1.90	-0.76	1.24
Breuil et al.	2015	552	0.00	65.00	8:00am	yes	1	1	1	1	0	1	0	blood	7.81	9.40	1.61	0.95
Brondino, Fusar-Poli & Politi	2017	22	100.00	20.82	4:00pm - 5:00pm	yes	1	1	1	1	1	1	1	saliva	6.52	2.86	1.79	0.42
Cao et al.	2014	106	0.00	N/A	N/A	N/A	0	0	0	0	0	0	0	blood	101.59	55.89	4.49	0.52
Carmichael et al.	1987	9 9	0.00 100.00	28.00 27.50	N/A	N/A	0	0	0	0	0	0	0	blood	1.86 2.58	0.12 0.20	0.62 0.94	0.06 0.08
Carson et al.	2012	17	35.30	35.47	10:00am - 2:00pm	N/A	0	0	0	0	0	0	0	blood	263.10	103.40	5.50	0.38
Carter et al.	2007	8 8	0.00 0.00	N/A	N/A	N/A	0	0	0	0	0	0	0	blood saliva	175.00 1.52	23.08 0.80	5.16 0.30	0.13 0.49
Chang et al.	2014	82	54.88	34.69	N/A	N/A	0	0	0	0	0	0	0	blood	26.26	5.87	3.24	0.22
Chicharro et al.	2001	10 12	0.00 0.00	23.00 26.00	8:00am - 11:00am	yes	0	0	1	1	0	1	1	blood	0.18 0.11	0.03 0.02	-1.73 -2.22	0.17 0.18
Chiodera, Louis & Legros	1984	3 6	100.00 0.00	N/A N/A	N/A	yes	0	0	0	0	1	0	0	blood	5.40 5.10	0.52 0.73	1.68 1.62	0.10 0.14
Chiodera & Coiro	1991	7 7	0.00 0.00	N/A N/A	9:00am	yes	1	0	0	0	0	0	0	blood	2.57 2.40	0.20 0.20	0.94 0.87	0.08 0.08
Chiodera & Coiro	1992	7	0.00	N/A	8:00am	yes	1	0	0	0	1	0	1	blood	2.55	0.10	0.94	0.04
Chiodera et al.	1992	8 8	0.00 0.00	N/A N/A	8:00am	yes	1	0	0	0	0	0	0	blood	2.59 2.90	0.68 0.37	0.92 1.06	0.26 0.13
Chiodera et al.	1995	7	0.00	N/A	8:00am	yes	1	0	0	0	0	0	0	blood	2.52	0.26	0.92	0.10
Chiodera et al.	1996a	14	0.00	N/A	9:00am	yes	1	0	0	0	1	0	0	blood	2.61	0.45	0.94	0.17
Chiodera et al.	1996b	6	0.00	N/A	8:30am	yes	1	0	0	0	1	0	1	blood	2.43	0.86	0.83	0.34

Authors	Year	*n*[a]	% female[b]	Age *M*	Time of day[c]	Fast[d]	Fast[e]	Eat[e]	Drink[e]	Caffeine[e]	Smoking[e]	Alcohol[e]	Exercise[e]	Specimen	Oxy *M*[f]	Oxy*SD*[f]	Oxy *M*log[g]	Oxy *SD*log[g]
Chiodera et al.	1998a	6	0.00	N/A	8:00am	yes	1	0	0	0	1	0	0	blood	2.49	0.17	0.91	0.07
Chiodera et al.	1998b	7	0.00	N/A	8:30am	yes	1	0	0	0	0	0	1	blood	2.32	0.53	0.82	0.23
Christensen et al.	2014	13	38.46	31.40	morning	yes	0	0	0	1	1	1	0	blood	14.51	5.23	2.61	0.35
Coiro & Chiodera	1991	7	0.00	N/A	8:30am	yes	1	0	0	0	0	0	1	blood	2.19	0.45	0.76	0.20
Coiro et al.	1992	8	100.00	N/A	9:00am	yes	1	0	0	0	0	0	1	blood	2.44	0.25	0.89	0.10
		8	100.00	N/A											2.37	0.28	0.86	0.12
Cong et al.	2015	19	0.00	35.60	1:09pm	yes	1	1	1	1	1	1	1	saliva	41.25	25.74	3.56	0.57
Daubenbüchel et al.	2016	73	56.16	37.23	N/A	yes	1	1	0	0	0	0	0	saliva	5.55	3.82	1.52	0.62
Daughters et al.	2015	40	0.00	20.98	N/A	yes	0	0	1	1	1	1	0	saliva	77.93	74.46	4.03	0.80
		40	0.00	20.98											45.96	33.27	3.62	0.65
de Groot et al.	1995	6	0.00	33.17	N/A	no	0	0	0	0	0	0	0	blood	1.23	0.25	0.19	0.20
de Jong et al.	2015	17	41.18	36.44	6:00pm - 12:00am	yes	1	1	1	1	1	1	0	saliva	1.55	0.29	0.42	0.18
		30	50.00	N/A	12:58pm		1	1	1	1	0	1	1		1.42	0.19	0.34	0.13
Demitrack et al.	1990	11	100.00	25.50	9:00am - 09:30am	yes	1	1	0	0	0	0	1	CSF	8.01	3.43	2.00	0.41
Ditzen et al.	2007	22	100.00	26.80	4:00pm - 7:00pm	yes	1	1	0	1	1	1	1	blood	10.05	7.04	2.30	0.15
		22	100.00	26.60											11.74	7.74	2.45	0.14
		17	100.00	25.70											9.22	7.34	2.20	0.19
Dolder et al.	2017	60	50.00	25.00	N/A	yes	1	1	0	1	1	0	0	blood	6.15	2.63	1.73	0.41
Domes et al.	2010	16	100.00	24.20	9:00am - 6:00pm	yes	1	1	1	1	1	1	1	blood	5.70	8.10	1.19	1.05
Dumont et al.	2009	15	20.00	21.10	10:25am	no	0	0	0	0	0	0	0	blood	0.81	1.16	-0.77	1.06
Eisenach, Tong & Curry	2015	20	60.00	37.00	morning	yes	1	1	1	1	0	1	0	CSF	9.50	3.10	2.20	0.32
Emeny et al.	2015	952	47.69	75.59	morning	no	0	0	0	0	0	0	0	blood	310.96	460.07	5.16	1.08
Engert et al.	2016	65	100	40.18	12:0pm - 6:00pm	no	0	0	0	0	0	0	0	blood	0.47	0.08	-0.77	0.17
		49	0.00	40.18											0.37	0.07	-1.01	0.19

Authors	Year	*n*[a]	% female[b]	Age *M*	Time of day[c]	Fast[d]	Fast[e]	Eat[e]	Drink[e]	Caffeine[e]	Smoking[e]	Alcohol[e]	Exercise[e]	Specimen	Oxy *M*[f]	Oxy*SD*[f]	Oxy *M*log[g]	Oxy *SD*log[g]
Fancourt et al.	2016	72	80.60	56.86	7:00pm	N/A	0	0	0	0	0	0	0	saliva	5.14	0.32	1.64	0.06
		66	81.60	59.69											5.15	0.35	1.64	0.07
Feldman, Samson & O'Dorisio	1988	7	25.00	31.00	N/A	yes	1	1	0	0	0	0	0	blood	171.00	47.62	5.10	0.27
Feldman et al.	2010	41	0.00	29.10	1:00pm - 4:00pm	N/A	0	0	0	0	0	0	0	saliva	7.09	3.95	1.82	0.52
Feldman, Gordon & Zagoory-Sharon	2011	41	0.00	29.10	1:00pm - 4:00pm	N/A	0	0	0	0	0	0	0	blood	405.10	151.88	5.94	0.36
		41	0.00	29.10										saliva	7.09	2.24	1.91	0.33
		41	0.00	29.10										urine	9.81	13.00	1.78	1.01
Feldman et al.	2012	121	0.00	N/A	4:00pm - 7:00pm	N/A	0	0	0	0	0	0	0	blood	378.19	220.07	5.79	0.54
		80	50.00	N/A											318.82	171.04	5.63	0.50
Ferreira et al.	1998	17	100.00	34.40	9:00am	yes	1	1	0	1	1	1	0	blood	10.88	4.43	2.31	0.39
		16	100.00	32.90											12.19	3.86	2.45	0.31
Fisher, Baylis & Frier	1987	6	0.00	24.00	N/A	yes	1	1	1	1	1	1	1	blood	0.71	0.24	-0.40	0.34
Fisher et al.	1989	6	0.00	N/A	N/A	yes	1	0	0	0	0	0	0	blood	0.71	0.24	-0.40	0.34
Floyd, Pauley & Hesse	2010	50	100.00	26.83	N/A	N/A	0	0	0	0	0	0	0	blood	268.48	293.94	5.20	0.89
		50	0.00	26.83											319.20	293.94	2.46	0.78
Forsling et al.	1998	15	0.00	25.00	5:00pm	yes	0	0	0	0	1	1	1	blood	1.89	1.20	0.47	0.58
		9	0.00	70.00											3.69	1.65	1.21	0.43
Forsling, Wheeler & Williams	1999	8	0.00	21.00	2:30pm	yes	1	1	1	1	1	1	1	blood	1.71	1.13	0.35	0.60
Forsling & Williams	2002	15	0.00	N/A	2:00pm	yes	0	0	0	0	1	1	1	blood	2.36	1.28	0.73	0.51
Francis et al.	2016	10	20.00	25.80	9:00am - 9:20am	yes	0	0	0	0	0	1	0	urine	3.50	2.00	1.11	0.53
Frank et al.	2000	17	100	23.40	N/A	N/A	0	0	0	0	0	0	0	CSF	6.31	1.96	1.80	0.30
Frijling et al.	2015	20	0.00	41.40	9:30am - 7:00pm	yes	1	1	1	1	1	1	1	saliva	3.00	3.54	0.66	0.93
		19	100.00	38.40											2.42	3.77	0.27	1.11
Fruhstorfer et al.	1988	7	0.00	N/A	8:00am	yes	0	0	0	1	1	1	0	blood	0.82	2.20	-1.25	1.45

Authors	Year	*n*[a]	% female[b]	Age *M*	Time of day[c]	Fast[d]	Fast[e]	Eat[e]	Drink[e]	Caffeine[e]	Smoking[e]	Alcohol[e]	Exercise[e]	Specimen	Oxy *M*[f]	Oxy*SD*[f]	Oxy *M*log[g]	Oxy *SD*log[g]
Fujiwara et al.	2012	50	100.00	35.90	11:00am - 2:00pm	N/A	0	0	0	0	0	0	0	urine	181.50	63.67	5.14	0.34
		31	0.00	36.90											188.00	70.17	5.17	0.36
Gerra et al.	2017	18	0.00	33.20	8:00am	yes	0	0	0	1	1	0	0	blood	252.70	89.70	5.47	0.34
Glovinsky et al.	1994	15	33.33	30.00	9:00am	N/A	0	0	0	0	0	0	0	CSF	8.92	3.01	2.13	0.33
Goldman et al.	2008	5	42.86	34.70	7:30pm	no	0	0	0	0	0	0	0	blood	240.00	224.00	5.17	0.79
Gordon et al.	2008	45	53.30	24.63	4:30pm - 6:30pm	yes	1	1	0	1	0	1	0	blood	258.76	257.66	5.21	0.83
Gordon et al.	2010a	76	0.00	29.45	4:00pm - 8:00pm	yes	1	1	0	0	0	0	0	blood	401.98	360.28	5.70	0.77
Gordon et al.	2010b	43	0.00	28.08	4:00pm - 8:00pm	yes	1	1	0	0	0	0	0	blood	370.59	255.54	5.72	0.62
Gordon et al.	2010c	37	0.00	28.81	4:00pm - 8:00pm	yes	1	1	0	0	0	0	0	blood	306.01	181.14	5.57	0.55
Gossen et al.	2012	8	0.00	26.40	1:00pm - 7:00pm	yes	1	1	1	1	1	1	0	blood	1.70	2.49	-0.04	1.07
Gouin et al.	2010	74	50.00	38.47	7:45am	yes	0	0	0	0	1	0	0	blood	308.26	226.28	5.15	0.66
Gouin, Pournajafi-Nazarloo & Carter	2015	59	47.50	23.81	9:30am - 12:30pm	yes	1	1	0	1	0	0	0	blood	234.24	60.21	5.42	0.25
Graugaard-Jensen et al.	2014	22	0.00	25.60	8:00a.m.	yes	0	0	1	1	1	1	1	blood	2.25	0.66	0.77	0.29
		15	100.00	25.50											1.98	0.97	0.58	0.64
Grenbäck, Hulting & Petersson	2007	4	0.00	47.74	morning	yes	1	0	0	0	0	0	0	blood	13.85	6.38	2.53	0.44
		9	100.00	57.11											13.09	5.19	2.50	0.38
Grewen et al.	2005	38	0.00	29.26	N/A	N/A	0	0	0	0	0	0	0	blood	1.53	1.17	0.19	0.68
		38	100.00	27.66											1.65	1.23	0.28	0.67
Grewen et al.	2008	25	100.00	27.40	12:00pm - 2:00pm	yes	0	0	0	0	1	0	0	blood	3.90	2.75	1.16	0.64
		23	100.00	27.40											7.05	4.08	1.81	0.54
Handlin et al.	2011	10	100.00	53.00	evening (most)	N/A	0	0	0	0	0	0	0	blood	169.71	110.21	4.96	0.59
		10	100.00	42.00											210.10	197.48	5.03	0.80
Handlin et al.	2012	10	100.00	53.00	N/A	N/A	0	0	0	0	0	0	0	blood	169.71	110.29	4.96	0.59

Authors	Year	*n*[a]	% female[b]	Age *M*	Time of day[c]	Fast[d]	Fast[e]	Eat[e]	Drink[e]	Caffeine[e]	Smoking[e]	Alcohol[e]	Exercise[e]	Specimen	Oxy *M*[f]	Oxy*SD*[f]	Oxy *M*log[g]	Oxy *SD*log[g]
Heim et al.	2009	8	100.00	31.30	4:00pm	yes	1	1	1	1	1	1	1	CSF	12.32	3.71	2.51	0.11
		14	100.00	31.30											17.15	3.55	2.84	0.06
Hermann et al.	1993	21	56.00	29.00	N/A	N/A	0	0	0	0	0	0	0	blood	26.24	8.25	3.22	0.31
Hew-Butler et al.	2008	82	29.00	43.00	6:30am - 8:30am	N/A	0	0	0	0	0	0	0	blood	1.87	0.97	0.51	0.49
Higashida et al.	2012	101	N/A	N/A	2:00pm - 5:00pm	yes	1	1	1	1	0	1	0	blood	198.20	24.70	4.82	0.97
Hoge et al.	2008	20	45.00	35.50	8:15am	N/A	0	0	0	0	0	0	0	blood	145.00	52.90	4.91	0.35
Hoge et al.	2012	27	37.04	40.00	1:00pm - 4:00pm	N/A	0	0	0	0	0	0	0	blood	354.00	181.00	5.75	0.48
Hogenelst et al.	2016	40	50.00	22.10	9:00am - 2:30pm	yes	1	1	0	0	0	1	0	blood	2.07	0.66	0.68	0.31
Holbrook, Hahn-Holbrook & Holt-Lunstad	2015	34	55.90	21.82	9:30am - 5:30pm	no	0	0	0	0	0	0	0	saliva	6.07	2.56	1.72	0.41
Honer et al.	1986	6	0.00	28.00	9:00am - 9:45am	yes	1	0	0	0	0	0	0	blood	1.44	0.71	0.26	0.47
Imamura et al.	2016	198	100.00	74.80	9:00am - 3:00pm	no	0	0	0	0	0	0	0	blood	140.00	133.00	4.62	0.80
		119	0.00	73.90											50.00	38.00	3.68	0.68
Jaeggi et al.	2015	31	0.00	37.80	5:50am - 11:30am	no	0	0	0	0	0	0	0	saliva	30.24	18.11	3.26	0.55
Jansen et al.	2006	14	7.14	21.00	10:00am - 4:00pm	yes	1	1	1	1	1	1	1	blood	7.51	8.87	1.97	0.31
Javor et al.	2014	30	0.00	26.67	9:00am - 13:00pm	yes	1	1	1	1	1	1	0	saliva	18.77	9.62	2.82	0.48
		30	0.00	26.67										blood	32.45	19.50	3.33	0.56
Jobst et al.	2014a	21	100.00	N/A	8:00am - 11:00am	N/A	0	0	0	0	0	0	0	blood	447.00	214.38	6.10	0.10
Jobst et al.	2014b	45	0.00	24.60	8:00am - 9:00am	N/A	0	0	0	0	0	0	0	blood	376.00	229.91	5.77	0.56
Jobst et al.	2015	19	31.58	46.58	8:00am - 11:00am	yes	1	1	1	1	1	1	0	blood	518.58	236.64	6.25	0.10

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John	2014	72	69.40	19.34	N/A	N/A	0	0	0	0	0	0	0	saliva	2.39	0.89	0.81	0.36
		116	60.30	19.34											2.73	1.11	0.93	0.39
		95	65.30	19.82											2.31	0.95	0.76	0.40
Johnson et al.	1990a	6	100.00	N/A	N/A	yes	0	0	0	1	1	1	0	blood	1.11	0.24	0.08	0.22
Johnson et al.	1990b	6	100.00	N/A	N/A	yes	0	0	0	1	1	1	1	blood	0.99	0.64	-0.18	0.59
		6	100.00	N/A											1.04	0.71	-0.15	0.62
Jokinen et al.	2012	19	36.84	30.00	7:45am - 8:45am	yes	1	0	0	0	0	0	0	blood	4.90	3.80	1.35	0.69
		18	36.98	30.00	8:00am - 9:00am									CSF	19.20	15.10	2.71	0.69
Kacheva et al.	2014	4	50.00	40.30	N/A	N/A	0	0	0	0	0	0	0	blood	13.80	1.70	2.62	0.12
Katsarou	2016	7	28.57	50.14	morning	yes	0	0	0	1	1	0	0	saliva	8.14	6.62	1.84	0.71
Keeler et al.	2015	4	50.00	N/A	4:00pm - 5:00pm	yes	1	1	1	1	1	1	1	blood	201.80	104.20	5.19	0.49
Kirkpatrick et al.	2014	12	14.29	25.40	9:20am	yes	1	1	0	0	1	1	0	urine	18.84	15.85	2.67	0.73
Kling et al.	1994	6	33.33	35.30	30 hours	N/A	0	0	0	0	0	0	0	CSF	3.80	0.98	1.30	0.25
Koch et al.	1990	6	66.67	N/A	12:00pm - 2:00pm	yes	1	0	0	0	0	0	0	blood	24.30	11.20	3.09	0.43
		6	66.67	N/A											17.00	5.70	2.78	0.33
Kostoglou-Athanassiou et al.	1996	8	100.00	N/A	5:00pm	yes	0	0	0	0	1	1	1	blood	2.50	0.93	0.85	0.36
Kostoglou-Athanassiou et al.	1998a	10	0.00	N/A	5:00pm	yes	0	0	0	0	0	1	1	blood	6.31	4.59	1.63	0.65
		12	0.00	N/A											7.03	3.95	1.75	0.63
Kostoglou-Athanassiou et al.	1998b	8	100.00	N/A	20 hours	yes	0	0	0	0	1	1	1	blood	4.23	1.13	1.41	0.26
		7	100.00	N/A											5.44	1.06	1.68	0.19
Kreutz	2014	24	76.19	N/A	N/A	N/A	0	0	0	0	0	0	0	saliva	13.04	5.59	2.48	0.41
Krüger et al.	2003	10	0.00	25.20	N/A	N/A	0	0	0	0	0	0	0	blood	76.03	31.84	4.25	0.40
Krüger et al.	2006	10	0.00	27.00	5:45pm	N/A	0	0	0	0	0	0	0	CSF	7.72	1.14	2.03	0.15
		10	0.00	28.50											8.52	2.31	2.11	0.27
Kujath et al.	2015	74	100.00	28.40	N/A	no	0	0	0	0	0	0	0	blood	346.20	324.20	5.53	0.79

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Kumaresan et al.	1983	11	100.00	24.00	9:00am - 10:00am	N/A	0	0	0	0	0	0	0	blood	3.12	6.90	0.25	1.33
Laczi et al.	1998	6	100.00	N/A	8:00am	yes	1	0	1	1	0	1	0	blood	5.20	1.94	1.58	0.36
Lancaster et al.	2015	37	0.00	23.69	10:00am - 11:00am	N/A	0	0	0	0	0	0	0	blood	307.28	122.61	5.65	0.38
Landgraf	1985	3	0.00	26.67	10:00am - 12:00pm	yes	1	1	1	1	1	1	1	blood	4.12	0.49	1.41	0.12
Lawson et al.	2011	19	100.00	27.50	12 hours	yes	1	1	1	1	0	1	1	blood	32.03	22.40	3.27	0.63
Lawson et al.	2012	13	100.00	22.00	9:00am	yes	1	1	1	1	1	1	0	blood	16.40	8.29	2.68	0.48
Le Mellédo et al.	2001	12	100.00	27.00	N/A	N/A	0	0	0	0	0	0	0	blood	1.28	0.37	0.21	0.28
Leake, Weitzman & Fisher	1980	6 6	0.00 100.00	N/A N/A	N/A	N/A	0	0	0	0	0	0	0	blood	1.33 1.33	0.81 0.42	0.13 0.24	0.56 0.31
Leake, Buster & Fisher	1984	9 5	100.00 0.00	N/A N/A	10:00am - 2:00pm	N/A	0	0	0	0	0	0	0	blood	2.00 2.17	0.99 0.38	0.58 0.76	0.47 0.17
Legros et al.	1984	6	0.00	N/A	9:30am	yes	1	0	0	0	0	0	0	blood	6.52	3.62	1.74	0.52
Lien et al.	2016	96	57.29	N/A	N/A	N/A	0	0	0	0	0	0	0	blood	28.40	14.00	3.24	0.47
Light et al.	2005	19 19 16	100.00 100.00 100.00	51.70 49.60 54.10	N/A	yes	0	0	0	1	0	0	0	blood	1.36 1.37 2.22	0.57 0.57 0.76	0.23 0.24 0.74	0.40 0.40 0.33
Lischke et al.	2012a	23 24	0.00 0.00	25.78 26.38	N/A	yes	1	1	1	1	1	1	1	blood	24.63 27.18	18.97 16.61	2.97 3.14	0.68 0.56
Lui et al.	2010	20	0.00	30.50	N/A	N/A	0	0	0	0	0	0	0	blood	79.30	17.13	4.35	0.21
Marazziti et al.	2006	45	73.33	31.50	8:00am - 9:00am	yes	1	0	0	0	0	0	0	blood	1.53	1.18	0.19	0.68
Marazziti et al.	2012	20	60.00	35.00	8:00am - 9:00am	yes	1	1	1	1	0	1	0	blood	1.14	1.07	-0.18	0.79
Marazziti	2015a	44	47.73	28.30	8:00am - 9:00am	yes	1	0	0	0	0	0	0	blood	2.55	2.18	0.66	0.74
Marchesi et al.	1997	9	0.00	43.70	8:30am	yes	1	1	1	1	1	1	1	blood	2.24	0.15	0.80	0.07
Marsh et al.	2015	37 35	60.27 60.27	22.31 22.31	N/A	N/A	0	0	0	0	0	0	0	saliva	2.15 0.86	0.36 0.13	0.75 -0.16	0.17 0.15

Authors	Year	*n*[a]	% female[b]	Age *M*	Time of day[c]	Fast[d]	Fast[e]	Eat[e]	Drink[e]	Caffeine[e]	Smoking[e]	Alcohol[e]	Exercise[e]	Specimen	Oxy *M*[f]	Oxy*SD*[f]	Oxy *M*log[g]	Oxy *SD*log[g]
Martin et al.	1998	6	100.00	25.00	8:00am - 10:00am	yes	1	0	0	0	0	0	1	CSF	5.14	0.91	1.62	0.18
Mascaro, Hackett & Riling	2014	83	0.00	33.20	7:30am - 3:25pm	no	0	0	0	0	0	0	0	blood	8.80	4.35	2.07	0.47
		48	0.00	30.40											6.60	3.47	1.77	0.49
McQuaid et al.	2016	67	100.00	19.37	1:00pm - 5:30pm	yes	1	1	1	1	1	1	0	blood	11.20	4.75	2.33	0.41
Mennella & Pepino	2006	8	100.00	25.00	8:45am	yes	1	0	0	0	1	0	0	blood	19.03	8.23	2.86	0.41
Miaskiewicz, Stricker & Verbalis	1989	14	7.14	N/A	N/A	yes	1	0	0	0	1	1	0	blood	2.68	0.90	0.93	0.33
Miller et al.	2009	10	100.00	35.90	afternoon or early evening	N/A	0	0	0	0	0	0	0	blood	33.50	37.30	3.11	0.90
		10	0.00	38.30											77.70	93.00	3.91	0.94
Mitchell et al.	1981	6	100.00	N/A	N/A	N/A	0	0	0	0	0	0	0	blood	13.32	2.47	2.57	0.18
Mitchell et al.	2013	21	0.00	44.95	N/A	N/A	0	0	0	0	0	0	0	urine	5.78	2.38	1.67	0.40
Mohiyeddini, Opacka-Juffry & Gross	2014	90	0.00	27.70	N/A	N/A	0	0	0	0	0	0	0	blood	366.22	2150.76	4.12	1.89
Mohiyeddini & Opacka-Juffry	2015	73	0.00	28.00	2:00pm - 5:00pm	yes	1	1	1	1	1	1	0	blood	380.00	24.60	5.94	0.06
Monde	2014	15	66.60	30.47	12:00pm - 5:00pm	yes	1	1	1	1	1	1	0	saliva	4.26	4.03	1.42	0.24
		53	38.18	18.00	1:00pm - 5:00pm										6.56	4.00	1.88	0.08
Monteleone et al.	2016	19	100.00	27.70	N/A	yes	1	0	0	0	0	0	0	blood	21.39	7.94	3.00	0.36
Montgomery et al.	1991	9	0.00	N/A	4:00am	yes	0	0	0	0	1	1	1	blood	8.67	1.50	2.15	0.17
Moons, Way & Taylor	2014	55	65.45	21.00	N/A	N/A	0	0	0	0	0	0	0	blood	5.26	0.52	1.66	0.10
		104	58.65	21.00											5.43	0.54	1.69	0.09

Authors	Year	*n*[a]	% female[b]	Age *M*	Time of day[c]	Fast[d]	Fast[e]	Eat[e]	Drink[e]	Caffeine[e]	Smoking[e]	Alcohol[e]	Exercise[e]	Specimen	Oxy *M*[f]	Oxy*SD*[f]	Oxy *M*log[g]	Oxy *SD*log[g]
Morhenn, Beavin & Zak	2012	65	52.30	21.36	N/A	N/A	0	0	0	0	0	0	0	blood	190.37	122.04	5.08	0.59
		30	53.30	21.36											249.93	173.51	5.32	0.63
Motoki et al.	2016	27	0.00	20.63	2:00pm - 5:00pm	yes	1	1	0	1	1	1	1	blood	21.77	7.25	3.03	0.32
		24	100.00	20.17											20.01	3.93	2.98	0.19
Munro et al.	2013	15	100.00	21.70	10:00am - 3:00pm	yes	0	0	0	0	1	1	0	blood	183.60	77.50	5.13	0.40
Murphy et al.	1987	13	0.00	N/A	10:30am	yes	0	0	0	0	1	1	1	blood	1.41	1.80	0.11	0.68
Murphy et al.	1990	8	0.00	N/A	2:30pm	yes	0	0	0	0	1	1	1	blood	1.11	0.57	-0.01	0.48
Nagasawa et al.	2015	20	80.00	36.60	N/A	yes	1	1	1	1	0	1	0	urine	20.10	25.66	2.52	0.98
		8	87.50	36.60											12.10	13.30	2.10	0.89
		11	45.45	N/A											20.62	5.21	3.00	0.25
Newman et al.	1999	8	0.00	24.97	9:00am	yes	1	1	1	1	1	1	1	blood	10.78	41.01	1.01	1.66
		6	0.00	61.42											5.90	4.92	1.51	0.73
		7	100.00	27.86											10.07	28.42	1.21	1.48
		9	100.00	58.56											13.31	30.30	1.68	1.35
North	1991	25	40.00	N/A	9:00am - 11:00am	yes	1	1	1	1	1	1	1	blood	2.25	1.70	0.59	0.67
Nussey et al.	1986	10	40.00	N/A	8:00am - 11:00am	yes	1	0	1	1	1	1	0	blood	1.15	0.63	0.01	0.51
Nussey et al.	1988a	9	55.56	N/A	N/A	N/A	0	0	0	0	0	0	0	blood	1.61	1.20	0.26	0.66
Nussey et al.	1988b	6	0.00	1.91	N/A	yes	1	0	0	0	0	0	0	blood	1.91	2.47	0.15	0.99
Ohlsson et al.	2002	8	100.00	37.00	8:00am	yes	1	1	1	1	1	1	0	blood	1.01	0.30	-0.03	0.29
Ohlsson, Rehfeld & Forsling	2004	11	100.00	34.40	morning	yes	1	0	0	0	1	0	0	blood	1.31	0.33	0.24	0.25
Opacka-Juffry & Mohiyeddini	2012	90	0.00	27.70	2:00pm - 5:00pm	yes	1	1	1	1	0	1	0	blood	377.60	226.74	5.78	0.55
Ottesen et al.	1988	6	100.00	N/A	N/A	no	0	0	0	0	0	0	0	blood	2.40	0.69	0.84	0.28
Ozsoy, Esel & Kula	2009	32	62.50	39.78	8:00am	yes	1	0	0	0	0	0	0	blood	13.11	7.54	2.45	0.49
Park	2007	51	100.00	22.30	10:00am	no	0	0	0	0	0	0	0	blood	235.60	114.20	5.36	0.46
		45	0.00	22.30											179.50	159.40	4.90	0.76
Parker et al.	2010	19	47.37	34.26	6:00pm	no	0	0	0	0	0	0	0	blood	0.96	0.31	-0.04	0.07

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Peskind et al.	1987	7	0.00	25.90	1:00am - 3:00pm	yes	0	0	0	1	1	0	1	blood	2.22	1.69	0.57	0.68
		7	0.00	25.90										CSF	10.29	1.83	2.32	0.18
Pitts et al.	1995	18	50.00	N/A	4:00pm	yes	0	0	0	0	0	0	1	CSF	13.86	6.92	2.52	0.47
Prehn et al.	2013	23	0.00	25.78	9:00am - 6:00pm	yes	1	1	1	1	1	1	1	blood	24.60	19.00	2.97	0.68
		24	0.00	26.38											27.20	16.60	3.14	0.56
Quintana et al.	2015	16	0.00	23.81	throughout the day	no	0	0	0	0	0	0	0	blood	6.07	5.88	1.47	0.81
Radant et al.	1992	7	0.00	28.00	9:30am	yes	1	0	0	0	1	1	0	blood	2.22	2.65	0.79	0.12
		4	0.00	26.00											2.93	0.72	1.05	0.24
Rapaport, Schetter & Bresee	2010	24	59.00	30.70	3:00pm - 7:00pm	yes	0	0	0	1	1	1	1	blood	188.39	101.96	5.11	0.51
		22	50.00	33.30											218.30	165.11	5.16	0.67
Raskind et al.	1986	7	0.00	68.00	early afternoon	yes	0	0	1	1	1	1	0	CSF	17.89	2.65	2.87	0.15
		8	0.00	25.00											16.66	3.17	2.80	0.19
Reyes	2014	44	0.00	25.02	10:00am - 2:00pm	N/A	0	0	0	0	0	0	0	blood	32.48	8.56	3.45	0.26
Rubin et al.	2010	31	100.00	27.05	55% in the afternoon	N/A	0	0	0	0	0	0	0	blood	322.93	187.05	5.63	0.54
		27	0.00	27.93											293.19	210.46	5.47	0.64
Rubin et al.	2013	14	100.00	28.43	N/A	N/A	0	0	0	0	0	0	0	blood	342.98	217.30	5.67	0.58
		24	0.00	27.58											427.28	259.01	5.90	0.56
Rubin et al.	2014	66	57.58	37.18	at least 87% before noon	N/A	0	0	0	0	0	0	0	blood	5.94	0.89	1.77	0.15
		61	75.41	42.77											5.72	0.86	1.73	0.15
		43	67.44	39.79											5.69	0.85	1.73	0.15
		91	64.84	41.01											5.86	1.05	1.75	0.18
Rubin et al.	2017	36	100.00	34.67	N/A	N/A	0	0	0	0	0	0	0	blood	6.15	0.89	1.81	0.14
		24	0.00	36.96											6.10	0.66	1.80	0.11
Saito et al.	2014	12	0.00	26.08	2:00pm - 6:00pm	yes	1	1	1	0	0	1	1	urine	344.38	196.18	5.70	0.53
Salonia et al.	2005	20	100.00	33.80	8:00am - 10:00am	yes	0	0	0	0	1	0	0	blood	2.41	1.12	0.78	0.44
		10	100.00	32.40											1.98	0.79	0.61	0.39

Authors	Year	*n*[a]	% female[b]	Age *M*	Time of day[c]	Fast[d]	Fast[e]	Eat[e]	Drink[e]	Caffeine[e]	Smoking[e]	Alcohol[e]	Exercise[e]	Specimen	Oxy *M*[f]	Oxy*SD*[f]	Oxy *M*log[g]	Oxy *SD*log[g]
Sanders, Freilicher & Lightman	1990	10	100.00	29.40	N/A	N/A	0	0	0	0	0	0	0	blood	2.97	5.76	0.31	1.25
		10	100.00	26.10											1.37	1.01	0.10	0.66
		10	0.00	32.60											1.05	0.44	-0.03	0.40
		10	0.00	30.90											1.74	1.52	0.30	0.72
		10	100.00	24.00											1.22	0.38	0.15	0.30
		10	100.00	26.80											1.24	0.41	0.16	0.32
		21	100.00	23.20											1.96	1.37	0.47	0.63
		21	100.00	22.30											1.92	0.78	0.58	0.39
Schneiderman et al.	2012	53	100.00	22.84	mid-afternoon hours	N/A	0	0	0	0	0	0	0	blood	509.83	228.67	6.14	0.43
		60	0.00	25.03											480.76	219.28	6.08	0.43
		23	100.00	24.63											263.76	240.68	5.27	0.78
		20	0.00	24.63											250.98	245.10	5.19	0.82
Schneiderman et al.	2014	60	100.00	22.84	mid-afternoon hours	yes	1	1	0	0	0	0	0	blood	459.05	140.26	6.08	0.30
		60	0.00	25.03											459.05	140.26	6.08	0.30
		40	52.50	24.63											257.82	239.92	5.24	0.79
Seckl et al.	1988a	9	0.00	N/A	N/A	yes	0	0	0	1	1	1	0	blood	1.91	0.30	0.63	0.16
Seckl et al.	1988b	6	0.00	N/A	N/A	yes	1	1	0	1	1	1	0	blood	1.51	0.73	0.31	0.46
Seckl, Johnson & Lightman	1989	6	0.00	N/A	N/A	yes	0	0	0	1	1	1	0	blood	1.18	0.88	-0.06	0.67
Shukovski, Healy & Findlay	1989	4	100.00	N/A	N/A	N/A	0	0	0	0	0	0	0	blood	10.78	7.96	2.20	0.60
Silber et al.	1987	20	100.00	28.00	morning	yes	1	0	0	0	0	0	0	blood	10.20	5.19	2.21	0.48
Silber, Larsson & Uvnäs-Moberg	1991	20	100.00	26.40	morning	yes	1	0	0	0	0	0	0	blood	14.16	2.68	2.63	0.19
		20	100.00	28.00											16.22	6.45	2.71	0.38
Smith et al.	2013	180	0.00	29.30	early evening; some late afternoon	yes	0	0	0	1	1	1	0	CSF	2.02	1.56	0.47	0.68
		180	100.00	27.90											1.92	1.67	0.37	0.75
Steinwall et al.	1998	8	100.00	N/A	7:00am - 9:00am	N/A	0	0	0	0	0	0	0	blood	2.63	1.10	0.89	0.40
Stock et al.	1989	9	77.77	35.00	N/A	yes	1	0	0	0	0	0	0	blood	27.00	30.00	2.89	0.90

Authors	Year	*n*[a]	% female[b]	Age *M*	Time of day[c]	Fast[d]	Fast[e]	Eat[e]	Drink[e]	Caffeine[e]	Smoking[e]	Alcohol[e]	Exercise[e]	Specimen	Oxy *M*[f]	Oxy*SD*[f]	Oxy *M*log[g]	Oxy *SD*log[g]
Stock, Silber & Uvnäs-Moberg	1989	20	100.00	N/A	morning	yes	1	0	0	0	0	0	0	blood	10.00	2.52	2.27	0.25
Stock, Bremme, Uvnäs-Moberg	1991	15	100.00	N/A	N/A	yes	1	0	0	0	0	0	0	blood	12.00	7.67	2.31	0.59
Stock, Karlsson & von Schoultz	1994	5	100.00	25.40	10:00pm	yes	1	0	0	0	0		0	blood	31.00	8.25	3.40	0.26
Strauss et al.	2015b	22	31.82	43.14	9:00am - 5:00pm	no	0	0	0	0	0	0	0	blood	19.66	5.86	2.94	0.29
Tabak et al.	2011	35	100.00	19.26	6:00pm - 7:30pm	yes	1	1	1	1	0	1	1	blood	1.61	2.78	-0.21	1.18
Taylor, Saphire-Bernstein & Seeman	2010	32	0.00	21.60	mid-afternoon	N/A	0	0	0	0	0	0	0	blood	273.45	234.36	5.34	0.74
		53	100.00	21.60											248.75	180.01	5.31	0.65
Tops et al.	2013	57	100.00	20.51	12:00pm - 3:00pm	yes	0	0	0	1	1	1	1	saliva	8.18	5.36	1.92	0.60
Tseng et al.	2014	41	0.00	33.50	8:00am - 10:00am	yes	1	0	0	0	0	0	0	blood	26.14	6.13	3.24	0.23
		55	100.00	33.54											25.37	5.69	3.21	0.22
Turan et al.	2013	24	41.67	34.42	8:00am - 9:00am	yes	0	0	0	0	0	1	0	blood	108.59	65.70	4.53	0.56
Turner et al.	1999	25	100.00	28.12	9:15am	yes	1	0	0	0	0	1	0	blood	4.61	0.27	1.53	0.06
Turner et al.	2002	32	100.00	N/A	8:00am	N/A	0	0	0	0	0	0	0	blood	3.16	3.95	0.68	0.97
Ückert et al.	2003	12	0.00	26.00	afternoon	yes	0	0	0	1	0	0	1	blood	71.10	41.20	4.12	0.54
Ulmer-Yaniv et al.	2016	46	50.00	N/A	3:00pm - 8:00pm	N/A	0	0	0	0	0	0	0	blood	529.41	300.27	6.13	0.53
		32	51.43	N/A											250.58	91.12	5.46	0.35
Uvnäs-Moberg et al.	1989	18	100.00	38.00	8:00am	yes	1	1	0	0	1	0	0	blood	9.06	7.64	1.94	0.73
Uvnäs-Moberg et al.	1991	10	N/A	N/A	N/A	yes	1	0	0	0	0	0	0	blood	16.42	10.66	2.62	0.59

Authors	Year	*n*[a]	% female[b]	Age *M*	Time of day[c]	Fast[d]	Fast[e]	Eat[e]	Drink[e]	Caffeine[e]	Smoking[e]	Alcohol[e]	Exercise[e]	Specimen	Oxy *M*[f]	Oxy*SD*[f]	Oxy *M*log[g]	Oxy *SD*log[g]
van IJzendoorn et al.	2012	18	100.00	19.77	9:00am	yes	0	0	0	1	1	1	1	saliva	2.44	2.46	0.54	0.84
		10	100.00	19.77											2.60	2.59	0.61	0.83
		18	100.00	19.77											2.77	2.76	0.67	0.83
van Londen et al.	1997	30	54.05	41.20	8:00am	yes	0	0	0	0	1	1	1	blood	1.28	3.12	-0.72	1.39
Varga & Kekecs	2014	12	0.00	29.62	11:00am - 3:30pm	yes	1	1	1	1	1	1	1	saliva	1.97	2.14	0.29	0.88
		4	0.00	N/A											3.03	1.84	0.95	0.56
Walss-Bass et al.	2013	20	30.00	39.65	morning	yes	1	1	1	1	0	1	0	blood	199.33	119.42	5.14	0.55
Weisman et al.	2012	35	0.00	29.70	1:00pm - 5:00pm	yes	1	1	0	1	1	1	0	saliva	20.91	22.84	2.65	0.89
Weisman et al.	2013	192	0.00	27.50	1:00pm - 7:00pm	N/A	0	0	0	0	0	0	0	blood	399.91	183.65	5.90	0.44
Williams et al.	1985	4	100.00	N/A	9:00am	N/A	0	0	0	0	0	0	0	blood	1.01	0.20	-0.01	0.20
Williams et al.	1986	5	0.00	N/A	N/A	yes	1	0	0	0	0	0	1	blood	1.61	0.67	0.40	0.40
		6	66.67	N/A	N/A	N/A	0	0	0	0	0	0	0		1.63	1.32	0.24	0.71
Wolff et al.	2006	29	43.30	25.00	N/A	N/A	0	0	0	0	0	0	0	blood	2.11	1.18	0.61	0.52
Zhong et al.	2010	1135	50.40	21.10	N/A	N/A	0	0	0	0	0	0	0	blood	214.00	230.00	4.98	0.88

Note. The table shows the data basis for the meta-regressions. As some studies reported data separately for different subsamples, those were analyzed separate-ly, as well, in order to include as many participants as possible into the meta-analytic procedure. N/A indicates that the information was not extractable from the paper. [a] Number of participants of whom valid oxytocin values were available. If outliers were removed by primary study authors, they were not included into the present analysis, either. [b] Percentage of female participants per sample. [c] If a time period was reported instead of a timepoint, the mean was used for the meta-regressions, if the period did not exceed 6 hours. If it exceeded 6 hours, this information was considered as too imprecise and treated as missing. 12a.m. indicates midnight and 12p.m. noon. [d] Indicates whether any fasting instructions were given to the participants (yes or no). [e] Indicates whether we considered that participants were (1) or were not (0) instructed to refrain from eating, drinking, consuming caffeine, smoking, consuming alcohol, exercising, or from any of those behaviors ("fast"). Missing values in the fasting variables were imputed to 0, assuming that if no instructions were reported in the paper, they were presumably not given. [f] Values represent pg/ml. g Values represent log-scaled pg/ml and were transformed according to the formulas provided in the ONLINE SUPPLEMENTARY MATERIAL 7.1.

7.3.3. Confounders of basal endogenous oxytocin concentrations

7.3.3.1 Baseline regression model

The results of the baseline regression model are shown in TABLE 7.2 and illustrated in FIGURE 7.2.

TABLE 7.2. Baseline regression model.

Predictor	β (*SEM*)	*t*	*p*	CI
Intercept	5.62 (0.31)	18.38	<.01	5.02: 6.22
Year	0.34 (0.09)	3.74	<.01	0.16; 0.51
Risk of bias	-0.00 (0.01)	-0.41	.68	-0.03; 0.02
SEM	-2.30 (0.27)	-8.37	<.01	-2.84; -1.76
Specimen				
Saliva	-4.03 (0.07)	-58.35	<.01	-4.16; -3.89
Urine	-1.76 (1.18)	-1.49	0.14	-4.09; 0.57
CSF	-2.77 (0.79)	-3.50	<.01	-4.32; -1.21
Extraction	-4.06 (0.10)	-42.45	<.01	-4.25; -3.89
Extraction*Specimen				
Extraction*Saliva	3.61 (0.14)	25.49	<.01	3.34; 3.89
Extraction*Urine	2.78 (1.19)	2.34	.02	0.45; 5.12
Extraction*CSF	4.06 (0.80)	5.08	<.01	2.49; 5.63

Note. The model is based on 339 subsamples. Study- and assay-related heterogeneity were considered as variance components. Sample-level regressors: year (reference: 2 000 years AD, scale: 10 years), risk of bias and appropriateness score (0 - 30 points), *SEM* of oxytocin value reported in the primary study in order to adjust for bias using the PET-PEESE method (Stanley & Doucouliagos, 2014), specimen (reference: blood) and extraction (reference: unextracted samples). CI = confidence interval. CSF = cerebrospinal fluid.

There were significant differences in predicted oxytocin concentrations between specimens. In addition, there was a significant main effect of extraction, as well as significant interactive effects of specimen and extraction. The intercept represents the predicted mean oxytocin concentration within unextracted blood samples. It indicates that within unextracted blood samples, oxytocin was 275.61 pg/ml, as determined by the factor exp (5.62). Within extracted blood samples, mean predicted oxytocin was only 4.75 pg/ml. Within unextracted saliva samples, mean predicted oxytocin was 4.92 pg/ml and within extracted saliva samples, it was 3.15 pg/ml. Within unextracted urine samples, mean predicted oxytocin was 47.42 pg/ml and within extracted urine samples, it was 13.20 pg/ml. Mean predicted oxytocin was 17.31 pg/ml within unextracted CSF samples and within extracted CSF samples, it was 17.29 pg/ml. Our Open Science Framework repository contains the exact calculations of predicted oxytocin concentrations.

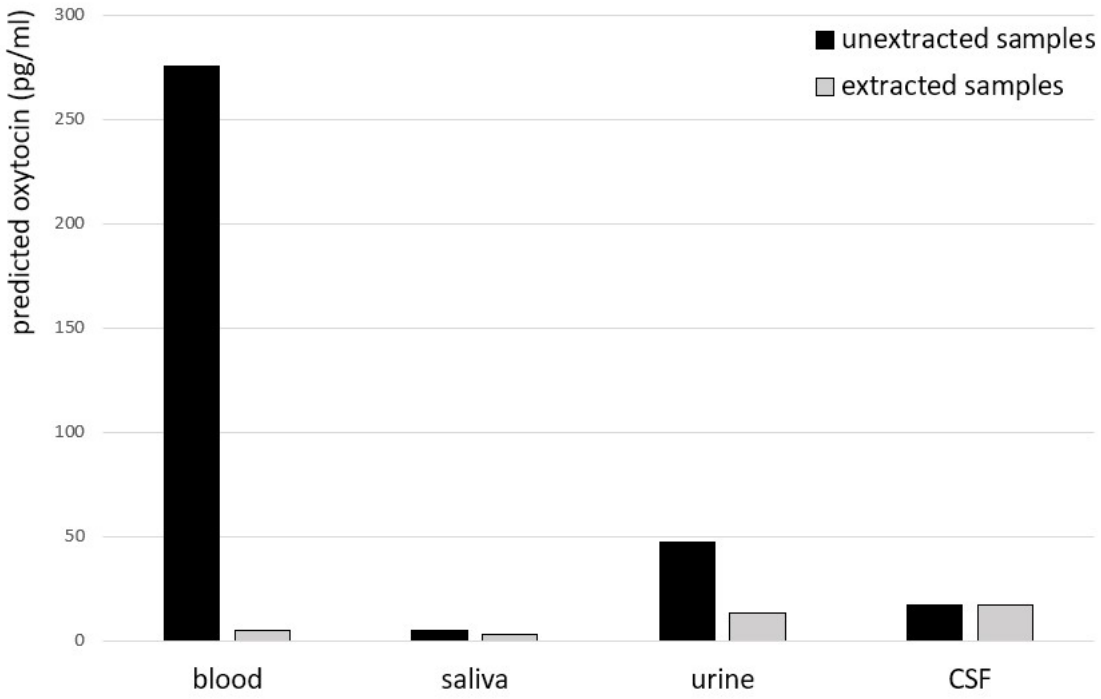

FIGURE 7.2. Predicted endogenous oxytocin concentrations in extracted vs. unextracted blood, saliva, urine and cerebrospinal fluid (CSF) samples.

7.3.3.2 Impact of confounders

TABLE 7.3 shows the results of the subsequent inclusion of the potentially confounding variables in the regression model, across all studies. With increasing percentages of females and mean age of the samples, reported oxytocin concentrations also increased significantly. A significant impact of time of day was detected. Instructions to refrain from smoking prior to sample collection also led to significantly higher oxytocin concentrations. Instructions to refrain from eating, drinking, caffeine or alcohol consumption, exercising, as well as the combined fasting variable (i.e., considering any of the fasting instructions) did not exert a significant effect.

TABLE 7.3. Investigation of potential confounders.

Predictor	Number of subsamples[a]	β (*SEM*)	*t*	*p*	CI
Main analyses across unextracted and extracted samples					
Sex	330	0.00 (0.00)	7.28	<.01	0.00; 0.00
Age	243	0.03 (0.01)	2.00	<.05	0.00; 0.06
Time of day	219	0.08 (0.02)	4.04	<.01	0.04; 0.11
Eat	339	0.04 (0.06)	0.70	.48	-0.08; 0.17
Drink	339	0.01 (0.07)	0.22	.83	-0.12; 0.15
Caffeine	339	0.02 (0.07)	0.33	.74	-0.11; 0.15
Smoking	339	0.09 (0.04)	2.28	.02	0.01; 0.18
Alcohol	339	0.01 (0.07)	0.22	.83	-0.12; 0.15
Exercise	339	0.02 (0.17)	0.13	.90	-0.31; 0.35
Fast	339	0.03 (0.06)	0.43	.66	-0.10; 0.16
Subgroup analyses within unextracted samples					
Sex	90	0.00 (0.00)	2.60	.01	0.00; 0.00
Age	83	-0.01 (0.02)	-0.59	.56	-0.05; 0.02
Time of day	71	0.10 (0.02)	4.88	<.01	0.06; 0.13
Eat	95	0.18 (0.29)	0.63	.53	-0.40; 0.76
Drink	95	0.61 (0.37)	1.66	.10	-0.12; 1.33
Caffeine	95	0.43 (0.31)	1.37	.17	-0.19; 1.06
Smoking	95	0.23 (0.05)	4.33	<.01	0.12; 0.33
Alcohol	95	0.47 (0.31)	1.49	.14	-0.15; 1.09
Exercise	95	0.60 (0.48)	-1.26	.21	-1.56; 0.35
Fast	95	0.44 (0.28)	1.58	.12	-0.11; 0.99
Subgroup analyses within extracted samples					
Sex	240	0.00 (0.00)	6.67	<.01	0.00; 0.00
Age	160	-0.04 (0.02)	-1.73	.09	-0.09; 0.01
Time of day	148	0.23 (0.09)	2.50	.01	0.05; 0.42
Eat	244	-0.08 (0.06)	-1.20	.23	-0.20; 0.05
Drink	244	-0.07 (0.07)	-0.98	.33	-0.21; 0.07
Caffeine	244	-0.11 (0.07)	-1.60	.11	-0.24; 0.02
Smoking	244	-0.06 (0.07)	-0.89	.37	-0.20; 0.08
Alcohol	244	-0.09 (0.07)	-1.22	.22	-0.22; 0.05
Exercise	244	0.15 (0.18)	0.82	.41	-0.21; 0.51
Fast	244	-0.10 (0.07)	-1.43	.15	-0.23; 0.04

Note. Potential confounders were subsequently added to the baseline regression model. Study- and assay-related heterogeneity were considered as variance component. Additionally, the sample-level regressors year (reference: 2 000 years AD, scale: 10 years), risk of bias and appropriateness score (0 - 30 points), SEM of oxytocin value reported in the primary study in order to adjust for bias using the PET-PEESE method (Stanley & Doucouliagos, 2014), specimen (reference: blood) and extraction (reference: unextracted samples) were considered. The following sample-level regressors were tested: sex (percentage of women per sample), age (reference: 30 years, scale: 10 years), time of day (reference: 12a.m./midnight, scale: 3 hours), instructions not to eat, drink, consume caffeine, smoke, consume alcohol, exercise or any fasting instructions, considered as given (1) or not given/not reported (0). Missing values on fasting variables were imputed to 0. CI = confidence interval. [a] Numbers vary as for some predictors, information was not reported in all primary studies.

7.3.3.3 Subgroup analyses within studies using unextracted and extracted samples

The differential impact of confounders within studies using unextracted and extracted samples is also shown in TABLE 7.3. The effect of sex was replicated within unextracted and extracted samples, respectively. The effect of age was no longer significant in neither of the subgroups, which might be explained by the reduced statistical power and indicate that this effect was relatively small. The effect of time of day was also confirmed within both subgroups. With regard to instructions not to smoke, extraction was a moderator. Within unextracted samples, instructions not to smoke were associated with higher oxytocin measurements, whereas this effect was not replicated within extracted samples. In line with the results of the main analyses, none of the remaining fasting exerted significant effects in neither of the subgroups.

7.3.3.4 Diurnal rhythm of endogenous oxytocin concentrations

The significant impact of time of day suggested a possible diurnal variability of endogenous oxytocin concentrations. To test for time-related fluctuations, we performed cosinor-based rhythmometry (Cornelissen, 2014). In this model, two linear predictors, reflecting different parameters of circadian change, jointly moderated the outcome (see ONLINE SUPPLEMENTARY MATERIAL 7.5). Therefore, diurnal fluctuations of endogenous oxytocin can be assumed. Predicted oxytocin concentrations tended to decrease during the nighttime, reaching a nadir at 8 a.m., before steadily increasing during the day, with peak concentrations between 7 p.m. and 8 p.m. However, it is worth noting that the actual data basis for this prediction only covered a 16h period from 6 a.m. to 10 p.m.. The course of oxytocin concentrations during the remaining hours was estimated based on our model and has no actual data basis. Therefore, a valid conclusion can only be made with regard to the increase of endogenous oxytocin concentrations from morning to afternoon.

7.4. Discussion

7.4.1 Summary of evidence

This is the first systematic review and meta-analysis to address the impact of different kinds of specimen, extraction, participants' sex and age, as well as time of day of sampling, and fasting instructions in 326 studies assessing basal endogenous oxytocin. We applied meta-regressions to test whether sample-level differences in these variables explained variability in reported oxytocin concentrations. We were able to estimate the confounders' impact based on a large database of 229 primary studies comprising 339 subsamples and 12 741 participants, even though the studies were not explicitly designed to address this scientific question.

Previous research has discussed specimen (Hoffman et al., 2012; Valstad et al., 2017) and extraction (Szeto et al., 2011) as reasons for heterogeneity in oxytocin concentrations. Our results confirmed that oxytocin was differentially reflected in blood, saliva, urine, and CSF. This is in line with previous research challenging an assumed correlation between basal concentrations in different specimens (Hoffman et al., 2012; Valstad et al., 2017) and emphasizes that future research is required to understand the exact pathways and possible interindividual differences of oxytocin distribution through the human body. Moreover, we detected a specimen-specific impact of extraction. In line with Szeto et al. (2011), we showed that the difference between oxytocin concentrations derived from unextracted and extracted samples was high in blood samples. On the other hand, our results show a weaker impact of extraction in urine and, in line with previous research (Jong et al., 2015), no impact of extraction in saliva or CSF samples. Overall, this study confirms that attention to specimen and extraction is of utmost importance when interpreting and comparing results from clinical studies on endogenous oxytocin (Jong et al., 2015; Leng & Sabatier, 2016; McCullough et al., 2013; Szeto et al., 2011).

With regard to confounders, our results showed that oxytocin levels were higher in samples with higher percentages of female participants. This seems unsurprising, as oxytocin has often been associated with typically female life events and behaviors, such as giving birth (Macdonald & MacDonald, 2010), affectionate parenting styles (Feldman, Weller, Zagoory-Sharon, & Levine, 2007), or tend-and-befriend

stress reactions (Taylor et al., 2000). Moreover, estrogens seem to promote oxytocin synthesis (Gabor, Phan, Clipperton-Allen, Kavaliers, & Choleris, 2012; Lim & Young, 2006).

The meta-analysis also showed that oxytocin concentrations were higher in samples with a higher mean age, supporting the idea that oxytocin-related events, such as bond formations, parenting, or grand-parenting, which accumulate throughout the lifespan, exert an impact on the oxytocin system (Huffmeijer et al., 2013). However, this effect was not replicated within the subgroups of studies using unextracted and extracted samples, respectively, and therefore, it can be assumed that the effect is relatively small, if existent.

While a diurnal rhythm of oxytocin release has already been discussed (Macdonald & Feifel, 2013), its course seems surprising. Our results indicated that concentrations were lower when oxytocin was measured in the morning higher when it was measured in the afternoon. Five of the included primary studies conducted within-subjects measurements of endogenous oxytocin concentrations over day- and night-cycles and detected either no significant fluctuations (Amico, Tenicela, Johnston, & Robinson, 1983; Graugaard-Jensen, Hvistendahl, Frøkiaer, Bie, & Djurhuus, 2014) or different patterns than those detected by our study (Forsling, Montgomery, Halpin, Windle, & Treacher, 1998; Kling et al., 1994; Landgraf, Häcker, & Buhl, 1982). However, as these studies were based on small sample sizes, they do not permit a fair comparison with our results or allow for speculation about factors that might have contributed to the diverging findings. To complement our study and to gain an understanding of the opposing results of previous preliminary research, we encourage the investigation of diurnal rhythms using large samples and longitudinal within-subjects designs. Such studies should also take possible moderators of diurnal variability into account. For instance, as cortisol, which is known to interact with oxytocin (Brown, Cardoso, & Ellenbogen, 2016), displays a clear awakening response (Pruessner et al., 1997), it might be worthwhile to consider awakening time.

Lastly, our study showed that instructions to refrain from smoking led to higher oxytocin concentrations in unextracted blood samples. This extraction-specific effect is in line with evidence indicating that unextracted samples are more sensitive to influences of the sampling protocol (Robinson et al., 2014). Therefore, and in accordance with McCullough et al. (2013) who concluded that assays measuring

oxytocin in extracted compared with unextracted blood are in general better validated, it can be argued that in blood samples, extraction should be the method of choice.

7.4.2 Limitations and future directions

It is worth noting that the fasting variables indicated whether or not authors reported that they instructed participants to refrain from certain behaviors before sampling. By this means, we created a proxy to estimate the impact of these behaviors on oxytocin concentrations. However, it was not possible to gather information concerning the actual compliance with these instructions. Therefore, the provision of instructions seemed to be the best estimation for the behavior of interest. Nevertheless, it needs to be noted that these variables only reflect assumed rather than actual behavior. This might explain discrepancies between our meta-analysis' results and findings from previous empirical studies that specifically investigated the impact of certain behaviors on oxytocin secretion. For instance, studies using within-subjects designs showed increased oxytocin measurements after physical exercise (Jong et al., 2015; Landgraf et al., 1982), whereas our meta-analysis did not detect between-studies differences depending on instructions to rest before the collection of baseline samples. These discrepancies emphasize that our results do not definitely prove that eating, drinking, caffeine consumption and exercise are irrelevant confounders of endogenous oxytocin measurements. Instead, we recommend conducting more studies with adequate designs that specifically address this question. Therefore, researchers that measure endogenous oxytocin concentrations to address scientific questions which are unrelated to these behaviors should at least record them to exclude any possible confounding impact on their data.

Moreover, we were unable to use a validated scale for the risk of bias rating. Due to the variety of included study designs, it was impossible to find a scale that suited the pool of heterogeneous studies. Our self-developed items ensured that studies with higher estimated study quality and precise fit to our study purposes were considered more strongly in our regression models. These items represent an approximation of these constructs but are not comparable with a validated scale.

It should be a matter of course that laboratories that offer to analyze endogenous oxytocin concentrations adequately validated their assays beforehand. However, it is worth noting that even though we used a variance component to control for assay-related heterogeneity, we were lacking insights into practices in each laboratory and were therefore unable to differentiate for quality standards.

Although we showed the impact of selected important confounders of endogenous oxytocin concentrations, this review should not be regarded as comprehensive. In fact, in another meta-analysis (Engel, Klusmann, Ditzen, Knaevelsrud, & Schumacher, 2019) we showed that oxytocin concentrations also fluctuate during the course of the menstrual cycle. In females, an impact of menopause, number of pregnancies and births, or hormonal contraception use might also be assumed. In this context, Bale and Epperson (2017) argue that female variability should not be used as an argument to exclude women from empirical studies. Instead, more research should be conducted to gain a deeper understanding of this variability. Other possible sex-unspecific confounders of endogenous oxytocin which were beyond the scope of this study are medication use, body mass index, habitual smoking, or relationship status. More studies and systematic overviews focusing on the impact of these possible confounders are warranted.

7.4.3 Conclusions

Endogenous oxytocin measurements can be a potent tool to assess possible dysregulations of the oxytocin system in mental disorders, as they are mostly non-invasive and allow repeated sampling over time and simultaneous measurements of different hormones from the same sample (Crockford et al., 2014). However, caution is required when interpreting and comparing results from clinical studies on endogenous oxytocin based on different specimens. Particularly in blood samples, the impact of extraction needs to be taken into account, as well. Moreover, we encourage researchers to consider the confounders identified in this meta-analysis in future studies in order to minimize methodological noise and strengthen trust in measurements of endogenous oxytocin in the context of mental disorders. Specifically, participants' sex, age and smoking behavior as well as the time of day of sampling should be reported. Ideally, their impact should be controlled for statistically or by the study design.

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Conflicts of interest

Mrs. Engel, Mr. Laufer, Dr. Miller, Dr. Niemeyer, Prof. Dr. Knaevelsrud and Dr. Schumacher have no conflicts of interest to declare.

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*reference was included in systematic review.

Contributions

Sinha Engel and Sarah Schumacher designed the study. Helen Niemeyer provided methodological advice.

Sinha Engel performed the literature search, coordinated the data collection, and contacted the authors of all primary studies for which relevant data were missing. Ludwig Ohse supported the grey literature search and corresponded with authors of registered trials. Anya Deubel, Hannah Klusmann, and Annika Walinski assisted in gathering full-text articles.

Sinha Engel, Sebastian Laufer, Hannah Klusmann and Annika Walinski performed the study selection, data collection and inspection, as well as the risk of bias and appropriateness rating.

Robert Miller performed the statistical analysis and drafted the report of the results.

Sinha Engel and drafted the manuscript. All authors revised the manuscript for important intellectual content.

Sarah Mannion proofread the manuscript.

Sarah Schumacher and Christine Knaevelsrud supervised the study.

Online supplementary material

The following supplementary material is available online:

ONLINE SUPPLEMENTARY MATERIAL 7.1. Statistical formulas.

ONLINE SUPPLEMENTARY MATERIAL 7.2. Full reference list of included studies.

ONLINE SUPPLEMENTARY MATERIAL 7.3. Descriptives with regard to study information and potential confounders.

ONLINE SUPPLEMENTARY MATERIAL 7.4. Ratings of primary study quality and appropriateness for review aims.

ONLINE SUPPLEMENTARY MATERIAL 7.5. Results of the cosinor-based rhythmometry.

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CHAPTER 8

Menstrual cycle-related fluctuations in oxytocin concentrations: A systematic review and meta-analysis

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Menstrual cycle-related fluctuations in oxytocin concentrations: A systematic review and meta-analysis

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Abstract

Oxytocin affects physiological and psychological functions that are often expressed sex-specifically, suggesting interactions between oxytocin and sex hormones. As female sex hormone concentrations change during the menstrual cycle, oxytocin might fluctuate, too. This systematic review and meta-analysis investigated endogenous oxytocin concentrations across menstrual cycle phases in healthy women. Data from 13 studies (120 women) showed a significant increase of oxytocin concentrations from the early follicular phase to ovulation (g = 0.39 [0.25; 0.53], p < .001) and a significant decrease from ovulation to the mid-luteal phase (g = -0.50 [-0.81; -0.18], p < .001). There were no significant differences between the early follicular and mid-luteal phase (g = -0.19 [-0.70; -0.32], p = .471). These findings contribute to a deeper understanding of differences in normal and abnormal psychobiological processes in women. They highlight the necessity to consider the menstrual cycle phase in studies on oxytocin in women.

Keywords: Menstrual cycle; Oxytocin; Female; Estrogens; Estradiol; Progesterone; GnRH; FSH; LH

8.1 Introduction

Oxytocin is a neuropeptide that is synthesized in magnocellular neurons of the paraventricular and supraoptic nuclei and in parvocellular neurons of the paraventricular nucleus of the hypothalamus (Jurek & Neumann, 2018). Through axonal and somato-dendritic release, oxytocin acts centrally as neurotransmitter and neuromodulator (Jurek & Neumann, 2018; Landgraf & Neumann, 2004), targeting receptors in the amygdala, hippocampus, striatum, suprachiasmatic nucleus, bed nucleus of stria terminalis and brain stem (Gimpl & Fahrenholz, 2001; Meyer-Lindenberg, Domes, Kirsch, & Heinrichs, 2011). Furthermore, through projections of the hypothalamic magnocellular neurons (Jurek & Neumann, 2018), oxytocin is transmitted to the posterior pituitary, from where it is released into the peripheral bloodstream, acting as a hormone (Ludwig & Leng, 2006; Meyer-Lindenberg et al., 2011). Consequently, oxytocin's functions can be distinguished between its central role as a neurotransmitter and modulator and its peripheral role as a hormone. In humans, several options exist to measure endogenous oxytocin concentrations in different body fluids. At least some of oxytocin's central actions are assumed to be reflected in cerebrospinal fluid (CSF; Veening, Jong, & Barendregt, 2010). Alternatively, the physiological and psychological consequences of increased central oxytocin availability can be observed after intranasal administration of the peptide (Born et al., 2002). Its peripheral actions can be examined by measuring oxytocin concentrations in blood plasma and serum, urine or saliva (Rutigliano et al., 2016). Originating from research on oxytocin's physiological functions related to sexual behavior and reproduction, in recent years, the scientific interest in the neuropeptide has expanded into psychology. Oxytocin's impact on social behavior and stress reduction contributes to understanding the biological underpinnings of these basic psychological functions and makes it a promising candidate as a biomarker for mental disorders (Meyer-Lindenberg et al., 2011). Notably, these functions are often expressed sex-specifically and studies point towards interactions between oxytocin and sex hormones such as estrogens.

8.1.1 Sex-specificity of oxytocin's physiological and psychological functions

Concerning oxytocin's peripheral functions, it correlates with sexual stimulation, sexual arousal and muscular contractions during orgasm in both sexes (Carmichael, Warburton, Dixen, & Davidson, 1994;

Carter, 1992). In men, oxytocin plays a key role in the central regulation of the penile erection and in ejaculation (Carter, 1992; Filippi et al., 2003). For example, it enhances the contraction of the seminiferous tubule that is involved in ejaculation (Gimpl & Fahrenholz, 2001). In women, oxytocin release stimulates uterine contractions during birth and milk ejection during lactation (for an overview, see Macdonald & MacDonald, 2010). As breastfeeding is assumed to be highly relevant for the formation of a strong bond between a mother and her child (Carter, 1998), the role of oxytocin in interpersonal relationships attracted increasing interest. In mothers and fathers, a parenting-related increase of endogenous peripheral oxytocin concentrations was observed (Gordon, Zagoory-Sharon, Leckman, & Feldman, 2010).

Whereas in mothers, the oxytocin system is naturally activated due to birth and lactation, parental care activates it not only in mothers, but also in fathers (Feldman, 2012). Notably, parenting styles and behaviors associated with peripheral oxytocin differ between men and women and are thus illustrative, although not exclusive, examples for the sex-specificity of some of oxytocin's behavioral functions.

It has been suggested that mothers' parenting is often expressed by means of affectionate behaviors (Feldman, Weller, Zagoory-Sharon, & Levine, 2007), inducing feelings of predictability and safety (Feldman, 2012), whereas fathers tend to engage in arousing, exploratory, rough-and-tumble contact (Paquette, 2004), preparing for novelty and excitement (Feldman, 2012). Accordingly, peripheral oxytocin was correlated with maternal gaze, affect, vocalizations and affectionate touch in mothers, while in fathers, it was associated with positive arousal, object exploration and stimulatory touch (Gordon et al., 2010). These oxytocin-related parenting behaviors positively impact children's neurocognitive and attachment-related development (Feldman, 2003; Feldman & Eidelman, 2003).

As strong social bonds facilitate adaptive stress responses (Olff et al., 2013), the oxytocin system has also been discussed as possible biological underpinning of the buffering impact of social support on stress (Ditzen & Heinrichs, 2014). Indeed, intranasal administration of synthetic oxytocin has anxiolytic and stress-reducing effects (Acheson et al., 2013; Eckstein et al., 2015; Heinrichs, Baumgartner, Kirschbaum, & Ehlert, 2003) in men and women, but sex differences exist with regard to behavioral stress coping. On a behavioral level, men tend to show stress-induced fight-and-flight reactions that

were mainly attributed to testosterone, whereas in women, peripheral oxytocin was hypothesized to promote tend-and-befriend behaviors (Taylor et al., 2000). On a physiological level, intranasally administered oxytocin has been shown to decrease physiological stress parameters, such as alpha-amylase concentrations, in a partner conflict paradigm in women, while it increased alpha-amylase concentrations and emotional arousal in men (Ditzen et al., 2013).

Because of oxytocin's involvement in social behavior and stress regulation it is also relevant as a biomarker of psychological disorders. Alterations in basal peripheral oxytocin have been investigated in psychological disorders such as autism, schizophrenia or depression (for an overview, see Cochran, Fallon, Hill, & Frazier, 2013). Furthermore, the use of oxytocin nasal spray in some studies improved symptoms of autism (Andari et al., 2010; Parker et al., 2017) and lowered emotional stress responses in people with borderline personality disorder (Amad, Thomas, & Perez-Rodriguez, 2015).

8.1.2 Interactions between sex hormones and oxytocin

Sex-differences in the above described physiological and psychological functions suggests that the oxytocin system might interact with sex hormones. Most systematic research addressing this association, to date, has focused on the interactions between estrogens and oxytocin. On a behavioral level, experiments with rats and mice showed that estrogens intensify oxytocin-related stress reduction (McCarthy, McDonald, Brooks, & Goldman, 1996; Ochedalski, Subburaju, Wynn, & Aguilera, 2007). On a neurophysiological level, the oxytocin system is regulated by estrogens (Lim & Young, 2006), which promote the synthesis of oxytocin and oxytocin receptor mRNA and thereby increase the transcription rate of oxytocin receptor genes (Gabor, Phan, Clipperton-Allen, Kavaliers, & Choleris, 2012). To date, there is only one study in humans that specifically investigated these interactions: In female anorexia and bulimia patients as well as in healthy control women, pharmacological treatment with estrogen resulted in increased blood oxytocin concentrations (Chiodera et al., 1991). This preliminary evidence is supported by more detailed evidence from animal and in-vitro studies, indicating an estrogen-stimulated oxytocin release in neurons of the supraoptic hypothalamic nucleus (Wang, Ward, & Morris, 1995). Moreover, oxytocin production in the hypothalamic paraventricular nucleus of mice was mediated by estrogen receptor beta functioning (Patisaul, Scordalakes, Young, & Rissman,

2003). Exogenous administration of estrogens increased oxytocin receptor mRNA levels in rats by activating estrogen receptor alpha (Amico, Thomas, & Hollingshead, 1997; Breton & Zingg, 1997). This effect was also observed for a combined administration with progesterone, but not for progesterone as stand-alone drug (Amico et al., 1997; Breton & Zingg, 1997). However, studies on human tissues revealed correlations of both, estrogens and progesterone, with oxytocin in the corpus luteum, when measured in ovarian tissue and ovarian veins (Dawood & Khan-Dawood, 1986). Furthermore, progesterone and oxytocin seem to follow similar concentration patterns during the estrous cycle in sheep and in the mid-luteal phase in cows (Walters, Schams, & Schallenberger, 1984; Webb, Mitchell, Falconer, & Robinson, 1981).

In summary, there is only little systematic research on interactions between sex hormones and oxytocin in humans until now. Evidence that is mainly derived from animal studies indicates that estrogens might regulate and progesterone might co-vary with oxytocin release.

8.1.3 Hormonal fluctuations across the menstrual cycle

The menstrual cycle is a precisely orchestrated sequence of reciprocally interconnected hormone releases, preparing a possible pregnancy. A regular cycle lasts about 28 days (notably, only a minority of women experience regular menstrual cycles of exactly 28 days) and can be divided into different phases. The follicular phase begins with the first day of the menstruation. The ovulation marks a specific timeframe that initiates the luteal phase, which lasts until the next menstruation. Each of these three phases or timeframes is characterized by a specific hormonal constellation. Besides the steroid hormones estradiol and progesterone, hormonal regulation of the menstrual cycle involves the polypeptide hormones gonadotropin releasing hormone (GnRH), follicle stimulating hormone (FSH) and luteinizing hormone (LH; Bale & Epperson, 2017).

During the follicular phase, the hypothalamus secrets GnRH with increased frequency. This stimulates the pituitary gland to produce gonadotropins, more precisely, FSH and LH. FSH stimulates the growth of follicles in the ovary, which produce estradiol. Estradiol, in turn, prepares the uterus for a possible pregnancy and blocks FSH and LH release in the pituitary gland. Lacking possibilities to cause follicle

growth, FSH and LH accumulate there until they peak and finally cause ovulation i.e., the release of the mature follicle from the ovary into the fallopian tube. At this point, LH, FSH and estradiol reach their highest concentrations during the course of the menstrual cycle. In the subsequent luteal phase, the corpus luteum, which develops in the ovary where the follicle was released, produces progesterone. Progesterone, in turn, prepares the body for a possible pregnancy, for instance by inhibiting GnRH synthesis and thereby preventing further follicles from growing. If there is no fertilization, the corpus luteum demises and through menstrual bleeding the follicle is excreted along with the inner layer of the endometrium (Bale & Epperson, 2017; Reed & Carr).

8.1.4 Scientific gap and objectives

The precisely coordinated fluctuations of female sex hormones during the course of the menstrual cycle as well as their interactions with the oxytocin system suggest that oxytocin concentrations might also fluctuate between menstrual cycle phases. This hypothesis is supported by studies in rats demonstrating that oxytocin concentrations change during the estrous cycle (Ho & Lee, 1992; Sarkar, Frautschy, & Mitsugi, 1992). Empirical studies have addressed possible fluctuations of endogenous oxytocin concentrations during the course of the human menstrual cycle, as well. In their qualitatively oriented systematic review, Macdonald and Feifel (2013) pointed out that “data on fluctuations in plasma OT levels across the menstrual cycle are mixed, with studies in different healthy and clinical populations showing both variation and lack of variation in normally cycling women” (p. 10).

To date, no systematic review and meta-analysis exists that quantitatively summarized evidence on these possible fluctuations and tested heterogeneity of these effects. Therefore, we conducted a systematic review and meta-analysis to summarize evidence on differences in basal oxytocin concentrations between menstrual cycle phases. Specifically, we investigated whether differences exist between the early follicular phase, ovulation and the mid-luteal phase. We defined our main review question according to the PICOS framework, as recommended by the PRISMA group (Moher, Liberati, Tetzlaff, Altman, & PRISMA Group, 2009).In terms of study population, we included healthy, naturally cycling women. We did not include intervention studies. Comparisons were conducted within subjects, comparing the same women in at minimum two different cycle phases. Concerning the outcome, we

were interested in differences in endogenous oxytocin concentrations between the early follicular phase, ovulation and the mid-luteal phase, respectively. Accordingly, we included longitudinal within-subjects study designs.

8.2. Methods

8.2.1 Protocol and registration

This study is based on a subset of data from a larger methodological review that investigated potential confounders of basal endogenous oxytocin concentrations in healthy humans (Engel et al., 2019). It was pre-registered at PROSPERO (Registration number: CRD42017072306) on the 17th of July 2017. It is available online at:

http://www.crd.york.ac.uk/PROSPERO/display_record.php?ID=CRD42017072306.

8.2.2 Eligibility criteria

We applied the following inclusion and exclusion criteria: Study participants were required to be healthy women with a mean age of 18 years or older. Participants with physical diseases, injuries or mental disorders were excluded. Furthermore, women with irregular menstrual cycles were excluded, which also applied for women who took any kind of hormonal contraceptives and menopausal women. Due to the critical role of oxytocin during labor and lactation (Nissen, Lilja, Widström, & Uvnäs-Moberg, 1995), women who were pregnant or had given birth less than a year ago were excluded, as well. Studies were excluded if there was a psychological or medical intervention before oxytocin measurement. As necessary for the intended comparisons, the same women had to be assessed at least twice at different menstrual cycle phases. In terms of outcomes, we were interested in possibly differing basal endogenous oxytocin concentrations measured in blood, saliva, urine or cerebrospinal fluid. Studies were required to report such differences between the follicular phase, ovulation and the luteal phase, respectively or to provide sufficient data to calculate an effect size. Concerning study design, longitudinal, within-subjects studies were eligible. Cross-sectional studies and between-subjects designs were excluded unless there was a subgroup for which within-subjects comparisons were possible. We included quantitative,

empirical, published or unpublished studies in English or German language. Qualitative studies and reviews were excluded. We did not define an exclusion criterion with regard to publication year.

8.2.3 Identification and selection of studies

In order to identify all eligible primary studies, we conducted a systematic literature search which followed the strategies recommended by Lipsey and Wilson (2001). The electronic databases *PsycINFO, PubPsych, PsycARTICLES, PubMed, Web of Science, BIOSIS, ProQuest Dissertations* and Theses *Global and Clinicaltrials.gov* were searched up to 28th March 2017. Search terms were "oxytocin AND (blood OR plasma OR serum OR CSF OR cerebrospinal fluid OR urine OR urinary OR saliva OR salivary)". Additionally, we screened abstracts of conference contributions, posters and commentaries. A snowball search system was applied by screening reference lists of overview articles and by contacting experts in the field. All published and unpublished studies in English or German language were screened.

In order to select all relevant studies from the results of the database research, titles and abstracts of studies were screened at first. Secondly, full-text articles were read to decide whether studies were eligible for inclusion. These two steps were performed by one researcher (SE) and resulted in a pool of studies that assessed basal endogenous oxytocin concentrations in healthy humans. In a third step, all studies that met the precise inclusion criteria were identified from this pool. Studies that measured basal oxytocin concentrations in at least two phases of the menstrual cycle were included in the descriptive part of the review. Studies that provided all necessary data were also considered in the meta-analytic procedure. Step three was performed by two independent raters (HK and SE). In case of discrepancies, a third independent rater decided about study inclusion (SSch).

If full-texts or abstracts were not available, we contacted the corresponding authors via email and asked for access to the paper in order to take it into consideration. We also contacted authors of study registers and asked whether unpublished data was already available.

8.2.4 Data extraction and preparation

A standardized coding scheme was used to extract relevant information from the included studies. Two raters (SE, HK) extracted the data from each study independently. In case of discrepancies, a third rater (SSch) decided.

For the qualitative review, we extracted the number of days and in which cycle phases oxytocin was measured, the measurement method (e.g. blood or saliva), the kind of assay, whether extraction preceded the biochemical analysis, the time of day of sampling, and information about the participants' age.

For the quantitative synthesis, we labeled day 1 as the first day of the follicular phase, and day 14 as ovulation or LH peak. We extracted mean or median oxytocin concentrations of all available days during the course of the cycle. Standard deviations, standard errors and ranges were extracted as measures of variance. If studies provided oxytocin concentrations of the individual participants, exclusively, we calculated the mean and standard deviation. If oxytocin concentrations were provided by graphs, exclusively, we used a web based plot digitizer (Rohatgi, 2015) to extract them. To ensure accuracy, two raters (HK, SE) extracted the data from each study, independently. Additionally, we calculated the correlation (*r*) of the values that were extracted by the two raters assuring reliability. In case of discrepancies, a third rater (SSch) decided which value to use. As required for the statistical procedures, we estimated means and standard deviations from medians, standard errors or ranges with the formulas provided by Hozo, Djulbegovic, and Hozo (2005). Correlations and effect sizes of differences between the cycle phases were extracted, if available. If studies did not report within-subjects correlations but provided the individual concentrations of oxytocin for each participant at the respective cycle phases, we calculated them. For studies that neither provided correlations nor individual oxytocin concentrations, we estimated the correlation by calculating the mean correlation of the other included studies, using a set of formulas by Olkin and Pratt (1958) which was recommended for meta-analyses by Schulze (2004). Furthermore, sample sizes were extracted. If the exact sample size was not reported for each cycle phase or if it varied across phases, we used the smallest sample size for the meta-analytic calculations.

If a study did not provide all necessary information for the meta-analytic procedure, we contacted the authors via e-mail, if contact details were found, and asked for the information. In the case of no response we sent reminders after 10 days. If no response was received or the authors indicated that they no longer had access to the data, we considered the data unavailable.

8.2.5 Risk of bias of individual studies

Risk of bias was assessed for all studies that were included into the meta-analytic procedures by means of the Cochrane Risk of Bias Assessment Tool for interrupted time series studies (Higgins, Sterne, Savović, Page, & Hróbjartsson, 2016). In accordance with the scale, we rated risk of specific biases for interrupted time series studies (i.e., whether menstrual cycle-related changes of oxytocin concentrations occurred independently of other changes over time, whether the expected effect of menstrual cycle phases on oxytocin concentrations was pre-specified and whether the menstrual cycle itself was unlikely to affect data collection), risk of attrition bias (incomplete outcome data), risk of reporting bias (selective outcome reporting), and risk of other bias (i.e., whether exclusion of women who used hormonal contraception was explicitly stated). We decided not to rate the items for performance bias and detection bias, as we considered the respective items as inappropriate for the purposes of our study. Concerning performance bias, we considered it impossible to blind participants for their menstrual cycle phase. With regard to detection bias, as biochemical analyses are relatively objective and less prone to interpretation biases (e.g., in contrast to psychometric interviews), we considered it unnecessary to ensure blinding of laboratory workers. The ratings were performed by two independent researchers (SE, HK) and in case of disagreements, a third person (SSch) decided.

8.2.6 Descriptive presentation of menstrual-cycle related fluctuations of oxytocin concentrations

In order to provide a visualization of possible fluctuations of oxytocin concentrations during the course of the menstrual cycle, we created a line graph of standardized means. We selected all studies that provided oxytocin values for more than 10 days during the cycle and z-standardized the oxytocin concentration of the single days. These values were then averaged for each day across all studies.

8.2.7 Meta-analytic procedures

In order to test possible fluctuations during the course of the menstrual cycle statistically, we conducted within-studies comparisons between the early follicular phase, ovulation and the mid-luteal phase, respectively, under the random effects model. We used reference days to mark these phases. Day 4 was used as reference day for the follicular phase since the risk of bias caused by an early ovulation is low and the early follicular phase is frequently used as a reference point in the literature (Altemus, 2001; Rubin et al., 2015). Day 14 was used as a reference day for ovulation since it is considered as the average day of ovulation (Hoffbauer, 2005). Day 22 was used as a reference day for the luteal phase to exclude the risk of influence through a late ovulation or an early menstruation. The mid-luteal phase is also frequently used as a reference point for the luteal phase (Altemus, 2001; Rubin et al., 2015). If oxytocin concentrations were not provided at days 4, 14, or 22, we used the day closest to the reference day. If there were two days in the same distance to the reference day, we applied the following rules: If one of the two days provided data for more individuals, the data of this day were used. If the participant number was the same, the data of the day that was more distant from ovulation were chosen in order to exclude the risk of bias by hormonal change through ovulation.

We used Hedges' *g*, corrected for small sample sizes, to test differences in oxytocin concentrations between the three phases, as recommended by Borenstein, Hedges, Higgins, and Rothstein (2009). Specifically, we determined standardized mean differences between the early follicular phase and ovulation, between ovulation and the mid-luteal phase, as well as between the early follicular and mid-luteal phase. Furthermore, we calculated a synthesized effect size across the three phases as an indicator of overall fluctuations of oxytocin concentrations during the course of the menstrual cycle (Borenstein et al., 2009). In this synthesized effect size calculation, we used the early follicular phase as the baseline measurement. This implies that we investigated the contrast of the differences between the early follicular phase and ovulation and the differences between the early follicular and mid-luteal phase, as recommended by Borenstein et al. (2009)

Heterogeneity was examined using the *Q*-statistic and the I^2-index (Borenstein et al., 2009). Significant *Q* coefficients ($p < .05$) indicate heterogeneity. I^2 values of 25, 50, and 75 were interpreted as the

minimum for indicating low, moderate and high heterogeneity, respectively (Crombie & Davies, 2009). If these coefficients indicated heterogeneity, we applied subgroup analyses. As possible moderators, we pre-defined measurement method of oxytocin, age, and risk of bias.

To test for publication bias, Egger's regression test (Egger, Davey Smith, Schneider, & Minder, 1997) Begg and Mazumdar's rank correlation (Begg & Mazumdar, 1994), and Duval and Tweedie's trim-and-fill procedure (Duval & Tweedie, 2000) were applied to all homogeneous data sets including at least six primary studies (Ioannidis, 2005). All calculations were conducted with the Comprehensive Meta-Analysis software (Biostat, 2014).

8.3 Results

8.3.1 Included studies

FIGURE 8.1 visualizes the results of the literature search and study selection. We included 19 studies (228 participants) into the descriptive part of this systematic review. When reviewing the included studies, we contacted authors who were involved in multiple included studies and asked if data overlapped between the studies. Subsequently, two studies were excluded from the meta-analytic procedure (Rubin, 2009; Rubin et al., 2015), due to data overlap with Rubin et al. (2010). Four studies were excluded due to insufficient data for the meta-analysis. Finally, we were able to include 13 studies (120 participants) into the meta-analytic procedure.

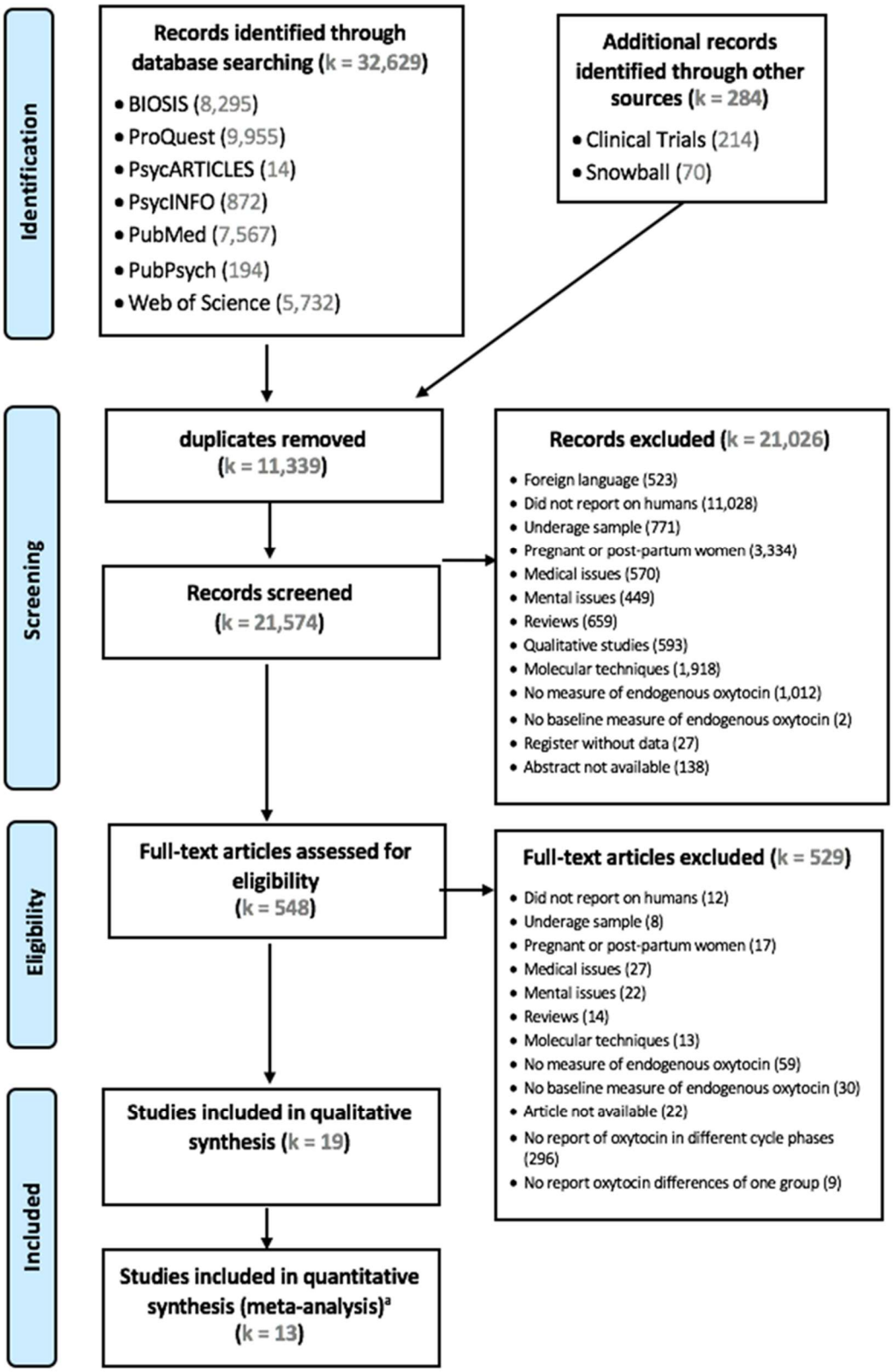

FIGURE 8.1 Flowchart illustrating the steps of the literature search. *k*=number of studies; [a] Four studies were excluded because of insufficient reported data and two studies because of overlapping data to another included study.

8.3.2 Qualitative review of included studies

The 19 studies included in the qualitative synthesis were published between the years 1981 and 2015 (see TABLE 8.1). The sample sizes consisted of three to 31 women per study. All included studies measured oxytocin in blood plasma. Studies measuring endogenous oxytocin in other fluids would have been eligible for inclusion, but such studies were not identified in the literature search. The studies reported oxytocin concentrations in two to 28 days and in three ($k = 7$) or two ($k = 8$) cycle phases. Four studies measured oxytocin in different cycle phases, but did not report the concentrations, explicitly. All studies analyzed endogenous oxytocin from blood samples. Fourteen studies analyzed oxytocin with radioimmunoassay, four studies with enzyme immunoassay and one study did not report which kind of assay was used. Extraction was performed in 10 studies, three studies analyzed unextracted samples and six studies did not provide information on this issue. The day time of sampling varied between studies. While seven studies measured oxytocin in the morning (between 6 am and 11:59 am) and three in the afternoon (between 12 and 5.59 pm), two studies used sampling protocols including morning and afternoon measurements. Seven studies did not report on time of day of sampling. The women's mean ages were between 20.40 and 39.00 years. The results of the risk of bias ratings are shown in the ONLINE SUPPLEMENTARY MATERIAL 8.1. Overall, the general study quality can be regarded as sufficient.

8.3.3 Descriptive presentation of oxytocin concentrations during the menstrual cycle

FIGURE 8.2 presents the fluctuation of oxytocin across the six studies that provided data for more than 10 days during the course of the menstrual cycle. The graph demonstrates that, on a descriptive level, mean oxytocin concentrations increased from the follicle phase to ovulation and decreased after ovulation. Furthermore, the graph shows fluctuations of the mean oxytocin concentrations not only between menstrual cycle phases but also between single days.

TABLE 8.1. Characteristics of included studies.

Author (year)	Number of days[a]	Number of phases[b]	Phases reported[c]	*n*	assay	extraction[d]	unit	age[e]	Sampling daytime[fg]
Altemus et al. (2001)	2	2	●●●	8	RIA	1	pmol/L	34.20 ± 1.80	morning
Amico et al. (1981)	14	3	●●●	5	RIA	1	µU/ml	26.00 ± 2.00	morning
Anderberg and Uvnäs-Moberg (2000)	2	2	●●●	16	RIA	1	pmol/l	-	morning
Challinor et al. (1994)	2	2	●●●	4	RIA	1	µU/ml	18.00-35.00[h]	morning - afternoon
Kostoglou-Athanassiou et al. (1998)	2	2	●●●	8	RIA	1	pmol/l	-	afternoon
Kostoglou-Athanassiou et al. (1996)	2	2	●●●	8	-	-	µU/ml	20 .00- 22.00[h]	afternoon
Kumaresan et al. (1983)	28	3	●●●	3-10	RIA	0	µU/ml	24.00 ± 10.80	morning
Leake et al. (1984)	2	2	●●●	9	RIA	-	-	25.00 - 45.00[h]	morning - afternoon
Le Mellédo et al. (2001)	-	-	●●●	-	RIA	-	pg/ml	27.00 ± 7.00	-
Liedman et al. (2008)	10	3	●●●	5	EIA	1	pg/ml	20.40 ± 0.50	-
Mitchell et al. (1981)	22	3	●●●	6	RIA	-	pg/ml	20.00 - 45.00[h]	-
Rubin (2009)	2	2	●●●	30	EIA	-	pg/ml	27.57 ± 6.79	-
Rubin et al. (2010)	2	2	●●●	31	EIA	0	pg/ml	27.55 ± 6.67	afternoon
Rubin et al. (2015)	2	2	●●●	31	EIA	0	pg/ml	27.55 ± 6.67	-
Salonia et al. (2005)	3	3	●●●	20	RIA	1	pg/ml	33.80 ± 0.50	morning
Shukovski et al. (1989)	27	3	●●●	4	RIA	1	pmol/l	-	-
Stock et al. (1991)	11	3	●●●	4-15	RIA	1	fmol ml^{-1}	22.00 - 39.00[h]	-
Uvnäs-Moberg et al. (1989)	28	3	●●●	14	RIA	-	pM	39.00 ± 1.70	morning
Williams et al. (1985)	28	3	●●●	4	RIA	1	pmol/l	20.00 - 22.00[h]	morning

Note. RIA = radioimmunoassay; EIA = enzyme immunoassay. [a] Number of days measured. [b] Number of phases measured. [c] Black points show phases with available values of oxytocin (either numbers or extractable from graphs with extraction tool), the first point represents the follicular phase, the second point the ovulation and the third point the luteal phase. [d] 1 = extraction, 0 = no extraction. [e] Reported as mean ± standard deviation. [f] Morning = 6:00am to 11:59 am; afternoon = 12 pm to 5.59 pm; evening = 6:00pm – 11:59pm; night = 12:00am – 5:59 am. [g] Oxytocin was measured in blood in all included studies, therefore, this the category measurement method is not stated in this table. [h] Mean age was not available; therefore, the age range was reported.

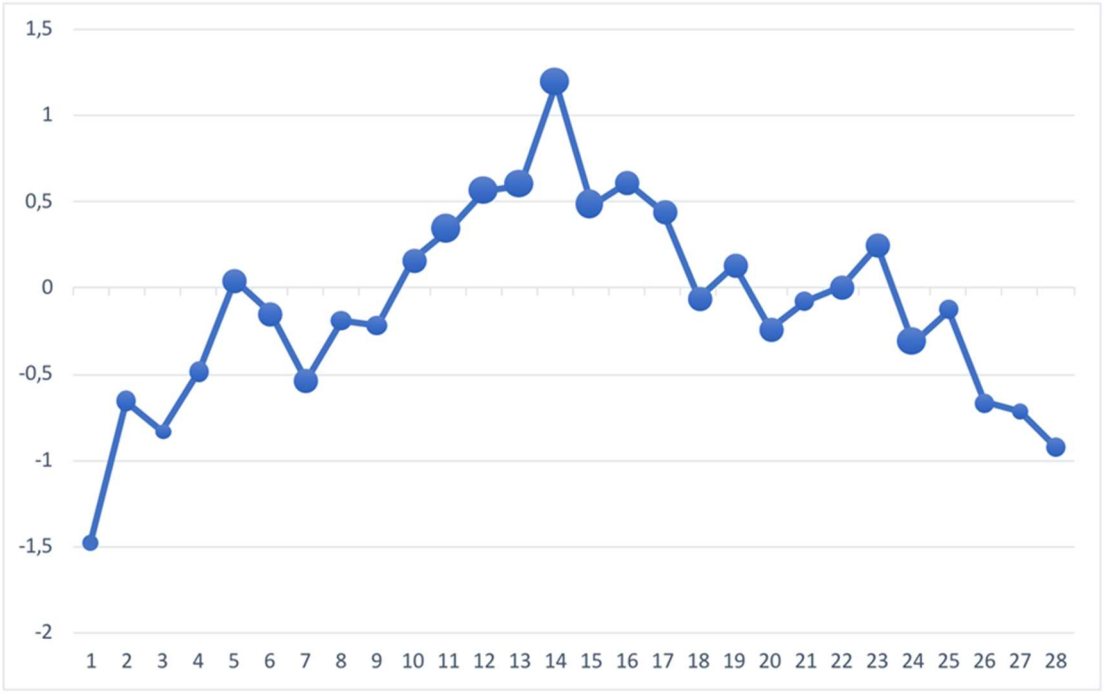

FIGURE 8.2 Descriptive analysis of mean z-standardized oxytocin concentrations during the menstrual cycle. y-axis: mean of z-standardized oxytocin concentrations for 6 studies that measured oxytocin on more than 10 days during the menstrual cycle. x-axis: days of the menstrual cycle starting with day one as the first day of bleeding. Size of points estimates the precision of the values, as sample sizes varied between n = 14 (smallest point) and n = 45 (biggest point): larger points indicate that more samples of oxytocin were provided on that day.

8.3.4 Meta-analytic results

The mean correlation for data extracted with an online plot digitizer (Rohatgi, 2015) by two raters was r = .99. Concerning the three cycle phases or timeframes, the maximum difference of the chosen days to the pre-specified reference days was two days to the follicular and luteal phase, and one day to ovulation. The calculation of effect estimates between early follicular phase, ovulation and the mid-luteal phase included data from 7 studies (48 participants) for each comparison. As 6 additional studies provided data for the follicular and the luteal phase only, they were used to replicate this comparison with a larger number of studies (120 participants) and thereby create more robust results. The overview

of the effects is listed in TABLE 8.2. The forest plots are reported in the ONLINE SUPPLEMENTARY MATERIAL 8.2.

TABLE 8.2. Overview of effect estimates.

Comparison	*k*	Hedges' *g*	*SEM*	s^2	CI (95%)	*Z*	*p*
Follicular phase/ Ovulation	7	0.39	0.07	0.01	0.25 - 0.53	5.31	< .001
Ovulation/ Luteal phase	7	-0.50	0.16	0.03	-0.81- (-0.18)	-3.06	<. 002
Follicular phase/ Luteal phase	7	-0.19	0.26	0.07	-0.70 - 0.32	-0.72	.471
Follicular phase/ Luteal phase	13	0.00	0.11	0.01	-0.21 - 0.22	0.03	.975

Note. k = number of studies, *SEM* = standard error, s^2 = variance, CI = Confidence interval

When comparing the early follicular phase with ovulation, a small but significant effect estimate was determined (g = 0.39 [0.25, 0.53], p < .001), indicating higher oxytocin concentrations around the time of ovulation than in the early follicular phase. The effect was homogeneous (Q = 5.13, p = .528, I^2 = 0.00). A significant, medium-sized effect estimate was found for changes from ovulation to the mid-luteal phase (g = -0.50 [-0.81, -0.18], p = .002), indicating higher endogenous oxytocin concentrations around the time of ovulation than in the luteal phase. Again, the effect was homogeneous (Q = 8.28, p = .222, I^2 = 27.07). Concerning changes in oxytocin concentrations from the early follicular to mid-luteal phase, no significant effect was detected (g = -0.19 [-0.7, 0.32] p = .471). This result was homogeneous (Q = 9.03, p = .172, I^2 = 33.57). This analysis was replicated by adding six studies that provided oxytocin concentrations in the follicular and luteal phase, exclusively. The previous result revealing no effect (g = 0.00 [-0.21, 0.22], p = .975; Q = 20.92, p = .052, I^2 = 42.64) was confirmed.

The synthesized effect size across the three time points was significant and of medium size (g = 0.51 [0.01, 1.01], p = .044), indicating that the change in oxytocin concentrations from the follicular phase to ovulation was larger than the change from the follicular to the luteal phase. Again, the effect was homogeneous (Q = 10.91, p = .091, I^2 = 45.02). The forest plots are illustrated in FIGURE 8.3.

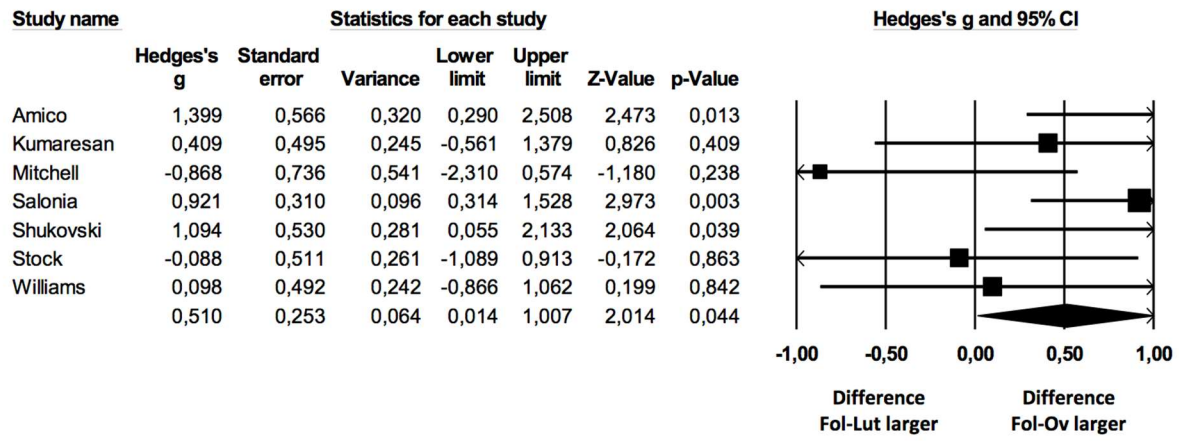

FIGURE 8.3. Synthesized effect size of the differences between the follicular and luteal phase and the follicular phase and ovulation.

8.3.5 Effect of sample extraction

There is ongoing debate about the comparability between measures of endogenous oxytocin in extracted and unextracted samples (Szeto et al., 2011). Therefore, we tested whether the effects remained stable when repeating our analyses only in those studies that explicitly stated that samples had been extracted before biochemical analysis. Accordingly, we performed the same comparisons of endogenous oxytocin concentrations between follicular phase, ovulation, and luteal phase, but excluded studies that clearly mentioned that samples were unextracted as well as those studies that did not report whether extraction was performed.

The comparison between the follicular phase and ovulation (across five studies, n = 37) still displayed a small, but now non-significant effect (g = 0.20 [-1.2, 0.53], p = .216). The direction of the effect remained the same, indicating higher oxytocin concentrations around the time of ovulation, and the effect was homogeneous (Q = 3.34, p = .503, I^2 = 0.00). The comparison between ovulation and mid-luteal phase (across five studies, n = 37) remained to be of medium size and significant. As in the previous analysis, it indicated that oxytocin concentrations were higher around the time of ovulation (g = -0.50 [-0.93, -0.06], p = .025). The effect was homogeneous (Q = 8.12, p = .870, I^2 = 50.71). The

comparison between the follicular phase and mid-luteal phase (across five studies, $n = 37$) now displayed a small, significant effect, indicating higher oxytocin concentrations during the follicular phase (g = -0.48 [-0.93, -0.03], $p = .038$). The effect was homogeneous ($Q = 2.90$, $p = .575$, $I^2 = 0.00$). However, when including the total number of available studies that extracted the samples prior to analysis ($k = 8$, $n = 57$), the significant difference between follicular and mid-luteal phase disappeared ($g = -0.15$ [-0.44, 0.15], $p = .330$). This effect was homogeneous, as well ($Q = 11.85$, $p = .106$, $I^2 = 40.92$).

The synthesized effect size across the three time points remained significant and of medium size (g = 0.69 [0.17, 1.21], $p = .009$). This result indicates that the change in oxytocin concentrations from the follicular phase to ovulation was larger than the change from the follicular to the luteal phase and that there is an overall change in oxytocin concentrations during the course of the menstrual cycle. The synthesized effect size was also homogeneous ($Q = 6.46$, $p = .157$, $I^2 = 38.07$).

8.3.6 Publication bias

None of the data sets of the main analyses indicated publication bias according to Begg and Mazumdar's rank correlation (Begg & Mazumdar, 1994) and Egger's regression test (Egger et al., 1997). The trim-and-fill-procedure (Duval & Tweedie, 2000) imputed one possible missing study in the comparison between the follicular and luteal phase on the larger pool of studies ($k = 13$). The resulting adjusted effect did not differ significantly from the original one. All other data sets showed no imputed studies when the trim-and-fill-procedure (Duval & Tweedie, 2000) was applied. The detailed results of the tests for publication bias can be seen in the ONLINE SUPPLEMENTARY MATERIAL 8.3.

8.4 Discussion

8.4.1 Summary of evidence

The results of this systematic review and meta-analysis indicate that endogenous oxytocin concentrations fluctuate during the course of the menstrual cycle. Data suggests that oxytocin concentrations peak around the time of ovulation. There seems to be no difference between the early follicular phase and the mid-luteal phase, but oxytocin increases from the early follicular phase to

ovulation and decreases again during the luteal phase. Most effects were based on a relatively small number of studies (k = 7). However, we were able to replicate the effect from the comparison between the early follicular and mid-luteal phase on a larger subset of studies (k = 13). All effects were homogeneous. Additional analyses for publication bias indicated that, despite a minor tendency toward bias, the findings on menstrual-cycle related fluctuations of oxytocin concentrations were robust. In subgroup analyses of all studies that definitely performed an extraction before the biochemical analysis, the directions of all effects remained the same. The loss of significance of the difference between follicular phase and ovulation might be attributable to the reduced number of included studies and participants, and accordingly, loss of statistical power. Interestingly, the difference between mid-luteal and follicular phase was significant within this smaller set of studies, but this effect disappeared when including the complete set of studies that extracted their samples, which was available for this specific comparison.

8.4.2 Integration of results and future directions

Our results hold several implications on both, a practical and a theoretical level. Our qualitative description of the current state of research provided a comprehensive evaluation of previous efforts to understand and consider the impact of the menstrual cycle on the oxytocin system. Practical implications can be derived from the data that might help to extend the generalizability and increase the reliability of future studies. Our quantitative summary provided a description and statistical test of the fluctuations of oxytocin concentrations during the course of the menstrual cycle. Based on our data, theoretical implications for promising future studies addressing for instance the sex-specificity of oxytocin's functions and the interactions between oxytocin and sex hormones can be given.

8.4.2.1 Generalizability and reliability of evidence on the oxytocin system

First, to date, only a small number of studies addressed menstrual cycle-related fluctuations of endogenous oxytocin concentrations, resulting in a small number of studies that were eligible for inclusion. Notably, the majority of studies was published in the 1980s and the 1990s and comprised small samples (n = 3 to n = 31). In general, the investigation of oxytocin's psychological functions, its

impact on psychopathology and therapeutic potential can be regarded as a growing field of research. Nevertheless, menstrual-cycle related variations, as a basic and important influence on the oxytocin system, have been neglected. Therefore, to confirm the robustness of our results, more studies are needed that repeatedly measure oxytocin concentrations during the course of the menstrual cycle using state-of-the-art biochemical assays.

As reviewed by Rutigliano et al. (2016), many results in research on oxytocin and psychiatric disorders are heterogeneous, which the authors attributed to a lack of consideration of confounders amongst other explanations. With regard to the results of our systematic review and meta-analysis, it seems likely that the menstrual cycle might be one of these influencing confounders. Our findings indicate that oxytocin concentrations fluctuate in a coordinated manner with other female sex hormones. Women in different phases of their menstrual cycle, women using hormonal contraceptives and menopausal women all differ with regard to the basal concentrations and the extent of fluctuations of these hormones. This suggests that any study on the oxytocin system, regardless of its specific scientific question, should take these variations into account to derive valid results and enabling to draw firm conclusions.

Moreover, our study revealed that oxytocin fluctuates not only between the menstrual phases, but also between single days. This implies a high overall variability of oxytocin concentrations and suggests a potentially high number of additional confounders. The assumed interactions between oxytocin and sex hormones suggest that it might be worthwhile to investigate the influence of contraceptives, the fertility status or number of pregnancies and births on endogenous oxytocin concentrations. However, other confounders are conceivable as well, and efforts should be taken to identify them ensuring appropriate confounder control in future studies. After all, this might contribute to less heterogeneous and more reliable study results.

8.4.2.2 Sex-specificity of oxytocin's functions and their cycle-related fluctuations

Second, the results of this review and meta-analysis contribute to a better understanding of oxytocin's sex-specific functions and their variations across the menstrual cycle. Sex-specific functions, such as oxytocin's influence on social behavior or stress response, might not only differ between men and

women but also within women, depending on their current cycle phase. It is possible that these functions are reinforced around the time of ovulation compared to the luteal or follicular phase, as already suggested by some findings. For instance, social perception seems to change during the course of the menstrual cycle. During the time of ovulation women seem to be able to categorize faces, especially male faces, more easily (Macrae, Alnwick, Milne, & Schloerscheidt, 2002). Furthermore, face characteristics that are perceived as more socially dominant are preferred around the time of ovulation (Penton-Voak et al., 2003). Besides, some studies show a fluctuation of certain social behaviors across the menstrual cycle. Buser (2012) reported that in a trust game, women scored highest during the late follicular and ovulatory phase. Additionally, sexual activity seems to be increased during the late-follicular and the ovulatory phase (Matteo & Rissman, 1984) which – according to our data - would be paralleled by the increase in oxytocin concentration during that time. From an evolutionary perspective, an increase in social behaviors and trust seems plausible since the ovulation phase is also the phase where fertilization is possible. With regard to stress and the menstrual cycle, studies investigated mainly physiological stress parameters, that seem to be decreased, when circulating estradiol is high (Kirschbaum, Kudielka, Gaab, Schommer, & Hellhammer, 1999). Subjective stress measures also differed in different cycle phases and women reported to feel less stressed at ovulation (Albert, Pruessner, & Newhouse, 2015). Combining these two aspects, recent data suggests that in women, preferences for masculine faces increased during ovulation while estradiol was high and, above this, preferences interacted with stress with a shift towards more feminine faces after stress (Ditzen, Palm-Fischbacher, Gossweiler, Stucky, & Ehlert, 2017).

As trust, stress and social behaviors are also relevant in the context of psychological disorders which have been associated with alterations in the oxytocin system, it can be speculated that oxytocin-related symptoms of psychological disorders also fluctuate during the course of the menstrual cycle in accordance with oxytocin concentrations. Specifically, symptoms might decrease during ovulation. In fact, studies with animals and healthy human samples indicate menstrual cycle-related variability in areas crucial for mental health such as stress and affect (Guo et al., 2018; Ross, Coleman, & Stojanovska, 2003). However, there is a lack of studies investigating menstrual-cycle related fluctuations of psychopathological symptoms in clinical populations. Further research in this field is highly relevant to

understand the exact role and the impact of oxytocin and other sex hormones in psychopathology. Furthermore, a lot of oxytocin-related functions such as stress regulation, further social behaviors or parental care are not yet examined in the context of the menstrual cycle. From a biological point of view further research is needed to determine why oxytocin fluctuates during the menstrual cycle. The question which hormone(s) influences oxytocin is still unanswered and the assumptions that are made in this study need to be further investigated.

8.4.2.3 Interactions between oxytocin and sex hormones

Third, the observed ovulation-related increase of oxytocin concentrations might inform theory on interactions between oxytocin and sex hormones that is, until today, mainly based on results from animal studies. Those provided preliminary evidence that estrogens stimulate oxytocin production by means of estrogen receptor beta functioning (Patisaul et al., 2003). The results of the current meta-analysis seem to confirm this finding, as estradiol concentrations reach their highest level during the menstrual cycle right before ovulation (Bale & Epperson, 2017). Accordingly, the ovulation-related peak of oxytocin might be thought of as a consequence of increased estradiol availability and estrogen receptor beta functioning. Furthermore, our results seem to be in line with evidence pointing towards an inverse interaction between oxytocin and progesterone (Dawood & Khan-Dawood, 1986; Walters et al., 1984; Webb et al., 1981). Whereas our results indicated that oxytocin concentrations are highest at ovulation, progesterone concentrations are still low at ovulation and reach their peak during the luteal phase (Bale & Epperson, 2017). This implies that the association between both hormones might be chronologically delayed. More studies investigating the dynamic interactions between estrogens, progesterone and oxytocin are needed, especially in human samples. Moreover, this field of research could be extended to other hormones regulating the menstrual cycle, specifically GnRH, FH, and LH. Evidence on interactions between oxytocin and these hormones is even sparser, even though associations might be assumed. Additionally, as the sex-specificity of oxytocin's functions suggests an interaction with female sex hormones, an interaction with testosterone might be assumed, as well. This is supported by the fact that many psychological functions of testosterone seem opposite to those that are usually ascribed to oxytocin (Macdonald, 2013). To sum up, our results clearly point towards interactions between oxytocin

and sex and cycle-related hormones. However, the lack of robust research on humans makes it difficult to integrate these findings into theory. This underlines the statement by Gimpl and Fahrenholz (2001) that "the regulation by gonadal and adrenal steroids is one of the most remarkable features of the oxytocin system and is, unfortunately, the least understood" (p.630).

8.4.3 Limitations

This systematic review and meta-analysis needs to be interpreted in consideration of its limitations. The most remarkable one refers to the small number of studies we were able to include. Higher numbers of studies and participants would be necessary to gain more robust meta-analytic effects. Nevertheless, it seems remarkable that even with relatively low statistical power, significant, homogeneous, and even across the subgroup analysis of studies that extracted the samples before analysis, relatively stable effects were found. Still, we cannot exclude the possibility that few studies or participants with especially pronounced fluctuations of oxytocin might have biased our findings. Therefore, more primary studies with larger samples are definitely needed. It might be worthwhile to update this meta-analysis if the number of eligible studies can be significantly extended in upcoming years.

Additionally, it should be considered that many of the included studies are rather old, only three were published after the year 2000. Especially in the field of biological research, this might be problematic since the methods and designs used to assess and determine oxytocin concentrations are subject to continuous development. Again, more primary studies using state-of-the-art biological assays, including extraction procedures, and a subsequent update of this meta-analysis are recommended.

Concerning our methodological approach, it needs to be critically mentioned that our analyses on oxytocin concentrations were partially based on data extracted from graphs. We considered a higher exclusion rate from the meta-analytic procedure and a resulting reduction of eligible studies as more problematic than possibly imprecise oxytocin values. It can be assumed that the extracted data is valid as the correlation between the extractions of the two independently rating researchers was very high (r = 0.99). However, a direct extraction of the exact values as reported by the authors would have been our method of choice.

It is worth noting that the menstrual cycle-related variability in endogenous oxytocin concentrations detected by this meta-analysis does not allow for conclusions about oxytocin's function as a neuromodulator (Lefevre et al., 2017). It remains unclear to which extent endogenous measurements reflect central processes (Valstad et al., 2017). Above this, oxytocin functions in the brain and in the body are determined through oxytocin receptor sensitivity and limited data is available on oxytocin receptor sensitivity over the course of the menstrual cycle to date (Einspanier, Bielefeld, & Kopp, 1998). It might be worthwhile to integrate according parameters into future studies to detect whether the menstrual cycle-related variability of peripheral oxytocin concentrations also extends to its central actions.

8.4.3 Conclusions

In conclusion, the available data suggests that peripheral oxytocin concentrations fluctuate during the course of the menstrual cycle. Specifically, an increase from the follicular phase to ovulation was detected as well as a decrease during the luteal phase compared to ovulation. It needs to be considered that peripheral oxytocin concentrations cannot give a valid picture of the neuropeptide's function in the brain or even specific brain areas. In addition, the small number of included studies and participants highlight the need for more studies with larger samples that underpin our findings. Furthermore, endogenous oxytocin concentrations might be influenced by a number of physiological factors. A comprehensive investigation of possible influencing factors such as sex, age, daytime, sleep or medication intake is warranted for the future. In this regard, our study can be viewed as a first step.

On the one hand, the results of this meta-analysis provide practical implications. They highlight the necessity for researchers to control for menstrual cycle phase when measuring oxytocin concentrations in women, in order to draw valid and unbiased conclusions. On the other hand, our results might provide theoretical implications. They indicate that oxytocin, which is related to important psychological functions might also interact with sex hormones. Therefore, it might have the potential to contribute to a better understanding of sex differences in psychological functions and symptoms. Considering that men and women differ regarding prevalence, symptomatology and course of mental disorders (Boyd et al., 2015) this study might have discovered one possible underpinning of these sex differences.

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Conflicts of interest

Mrs. Engel, Mrs. Klusmann, Prof. Dr. Ditzen, Prof. Dr. Knaevelsrud and Dr. Schumacher have no conflicts of interest to declare.

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*reference was included in systematic review.

Contributions

Sinha Engel, Hannah Klusmann, Christine Knaevelsrud and Sarah Schumacher designed the study.

Sinha Engel developed the search strategy and performed the literature research. Ludwig Ohse supported the grey literature research and corresponded with authors of registered trials.

Hannah Klusmann and Sinha Engel performed the study selection. Anya Deubel and Annika Walinski assisted in gathering full-text articles.

Sinha Engel and Hannah Klusmann performed the data collection and inspection, as well as the risk of bias rating. Sarah Schumacher acted as independent rater in the study selection, data collection and risk of bias rating

Hannah Klusmann contacted the authors of all primary studies for which data was missing.

Sinha Engel and Hannah Klusmann performed the statistical analyses. Johannes Bohn provided statistical advice.

Hannah Klusmann and Sinha Engel and drafted the manuscript. Sarah Schumacher, Christine Knaevelsrud and Beate Ditzen supervised the meta-analysis and revised the manuscript for important intellectual content.

Online supplementary material

The following supplementary material is available online:

ONLINE SUPPLEMENTARY MATERIAL 8.1. Results of the risk of bias rating

ONLINE SUPPLEMENTARY MATERIAL 8.2. Forest plots of meta-analytic results

ONLINE SUPPLEMENTARY MATERIAL 8.3. Results of the publication bias analyses

Available online at:

https://www.sciencedirect.com/science/article/pii/S0091302218300980?via%3Dihub

CHAPTER 9

Discussion

9.1 Summary of findings

The first arm of this dissertation investigated oxytocin's involvement along the stages of PTSD symptom development, manifestation, and remission due to pharmacological and psychotherapeutic interventions. **Studies 1** and **2** covered the stage of PTSD symptom development and its pharmacological prevention. **Study 1** demonstrated that the beneficial effects of repeated intranasal oxytocin administration early posttrauma on PTSD symptoms up to six months later in individuals with high acute PTSD symptoms (van Zuiden et al., 2017) were independent of sex and hormonal contraception use. This is an important finding because in **study 1**, it also became evident that women using hormonal contraception had improved recovery from traumatic stress from one and a half up to six months after the event. This effect was not observed when considering dichotomous sex (van Zuiden et al., 2017), indicating that different gonadal-steroids related statuses impact traumatic event processing. The differentiation between women using hormonal contraception and cycling women was also important in **study 2**. It showed that in women using hormonal contraception, higher blood oxytocin concentrations, as assessed early posttrauma, predicted higher PTSD symptoms one and a half, three and six months later. However, they were not prognostic for PTSD symptom development in men or cycling women. In line with previous evidence (Stock, Karlsson, & Schoultz, 1994), women using hormonal contraception also had the highest mean oxytocin concentrations, compared to both other groups. This indicates a positive association between synthetic estrogens intake and oxytocin. Further, in cycling women, endogenous oxytocin concentrations peaked around ovulation (**study 7**) and therefore, endogenous estradiol also appears to be positively correlated with oxytocin. In **study 2**, it was not possible to investigate the impact of menstrual cycle phases and gonadal steroids concentrations more closely. Therefore, it remains an interesting question to follow up, how exactly female gonadal steroids might explain the observed prognostic effects of endogenous oxytocin concentrations. **Study 2** further depicted that the preventive intervention's effects was not moderated by blood oxytocin concentrations, as assessed immediately before its initiation. Thereby, **studies 1** and **2** contribute to a field of research which, instead of assuming that intranasal oxytocin administration exerts the same effects in all individuals, takes a closer look at potential moderators (Bartz, Zaki et al., 2011; Olff et al., 2013; Shamay-Tsoory & Abu-Akel, 2016). At least the effects of repeated intranasal oxytocin administration early posttrauma on the mid- to long-

term recovery of early PTSD symptoms appear to be independent of individuals' endogenous oxytocin concentrations, sex and hormonal contraception use.

Study 3 investigated parameters of the endogenous oxytocin system in manifest PTSD and differentiated the effects of traumatic event exposure from those of PTSD symptoms. Based on a comprehensive description of all empirical studies in this field of research, meta-analyses revealed that endogenous oxytocin concentrations neither differed between trauma exposed and non-trauma exposed individuals, nor between individuals with and without PTSD diagnosis. Further, they were uncorrelated with the amount of traumatic event exposure and PTSD symptoms. However, except for the group comparison between individuals with and without PTSD, all effects were heterogeneous. Subgroup analyses successfully reduced heterogeneity, and homogeneous, also non-significant effects were found in subgroups of studies that were more comparable regarding methodological aspects, demographic characteristics of the sample, including sex, and traumatic event-related parameters. Yet, inconsistent results were detected when taking interactions between the type of biological specimen and extraction into account. Extraction is a step which takes place before analyzing oxytocin in a biological specimen, such as blood, saliva, urine or CSF. It enriches oxytocin concentrations and ensures that the assay does not measure matrix components or other, potentially interfering molecules (Szeto et al., 2011). Both, the non-significant group comparisons and correlations, as well as the observation that oxytocin was differently associated with the amount of traumatic event exposure, depending on the respective biochemical analysis, strongly questions an assumed eligibility of endogenous oxytocin concentrations as diagnostic biomarker for PTSD. **Study 3** further described the current state of research on the interactions between traumatic event exposure and PTSD symptoms on the one hand and oxytocin receptor gene DNA variation and methylation on the other hand, but the data that had been published so far were insufficient for valid conclusions.

Study 4 and **study 5** covered the stage of PTSD symptom remission by investigating oxytocin in TF-CBT. In line with the results from **study 3**, **study 4** detected no differences in blood oxytocin concentrations between three groups of male active and former German Armed Forces service members: such who were treatment seeking for PTSD, healthy trauma exposed individuals who were deployed

and healthy non-trauma exposed individuals who were never deployed. PTSD patients took part in a five-week internet-based TF-CBT (Niemeyer et al., 2020). Their blood oxytocin concentrations were described longitudinally, that is, before, immediately and three months after the internet-based TF-CBT and correlated with PTSD symptoms at each timepoint. Again, confirming the results from **study 3**, oxytocin was never correlated with PTSD symptoms. While PTSD symptoms remained unexpectedly stable over time, oxytocin did not change in mean, but was unstable within individuals. Thus, the present finding again strongly questions the eligibility of endogenous oxytocin concentrations as diagnostic, but also as prognostic, prevention- and treatment-related biomarker. **Study 5** demonstrated that higher blood oxytocin concentrations before onset of the internet-based TF-CBT were, at least on a descriptive level, associated with higher patient ratings of the therapeutic alliance. Also with regard to other psychotherapy process variables, the study indicated potential benefits of higher oxytocin concentrations, which might be stimulated by intranasal administrations (van IJzendoorn, Bhandari, van der Veen, Grewen, & Bakermans-Kranenburg, 2012). However, in the light of the findings from **study 4**, it must be critically questioned whether such prognostic value can actually be ascribed to endogenous oxytocin concentrations or whether these findings must be attributed to other confounding factors that change over time.

The impact of the biochemical analysis method (**study 3**) and the low temporal stability of endogenous oxytocin concentrations (**studies 4** and **5**) definitively challenge the assumption that they can serve as a PTSD-specific biomarker. As mentioned, it appears that factors other than PTSD symptoms impact endogenous oxytocin concentrations. Therefore, the second arm of this dissertation gathered systematic knowledge on such confounders.

Study 6 summarized all empirical studies that measured endogenous oxytocin concentrations in healthy humans. Supporting the results from **study 3**, the meta-analyses confirmed that observed oxytocin concentrations differed, depending on specimen and extraction, as well as on interactions between those two variables. In all empirical studies of this dissertation which measured endogenous oxytocin concentrations (**study 2**, **study 4** and **study 5**), extracted blood samples were analyzed. Therefore, the confounders that are relevant for these types of samples will be discussed here. In extracted blood

samples, oxytocin concentrations were higher within samples with a higher percentage of women, indicating an impact of sex. Further, time of day had a significant impact on oxytocin concentrations in extracted blood samples. As the figure which depicts the diurnal rhythm of oxytocin concentrations (published in the corresponding Open Science Framework repository) referred to unextracted blood samples, FIGURE 9.1 was added here to show the diurnal rhythm in extracted blood samples. In line with the pattern observed in unextracted samples, in extracted blood samples, predicted oxytocin concentrations decreased during the nighttime, reached a nadir in the morning, then increased during the course of the day and reached a peak in the late evening. Age, as well as instructions to refrain from eating, drinking, consuming caffeine, smoking, consuming alcohol, or exercising before sample collection did not impact measurements of oxytocin concentrations in extracted blood samples. Out of the pool of studies included in **study 6**, in **study 7**, those which reported endogenous oxytocin concentrations in cycling women at least twice during the course their menstrual cycle were picked out. Meta-analytically aggregating these data showed that endogenous oxytocin concentrations increased from the follicular phase to ovulation and subsequently decreased during the luteal phase. The analyses consistently produced significant and homogeneous effect sizes.

Thus, while the first arm of this dissertation showed that endogenous oxytocin concentrations are influenced by factors other than PTSD symptoms and are therefore, at least at the current state of research, are not eligible PTSD-specific biomarkers, the second arm indicated that controlling for participants' sex, time of day of sampling, and in women, the menstrual cycle phase, might reduce at least some of the confounding influences.

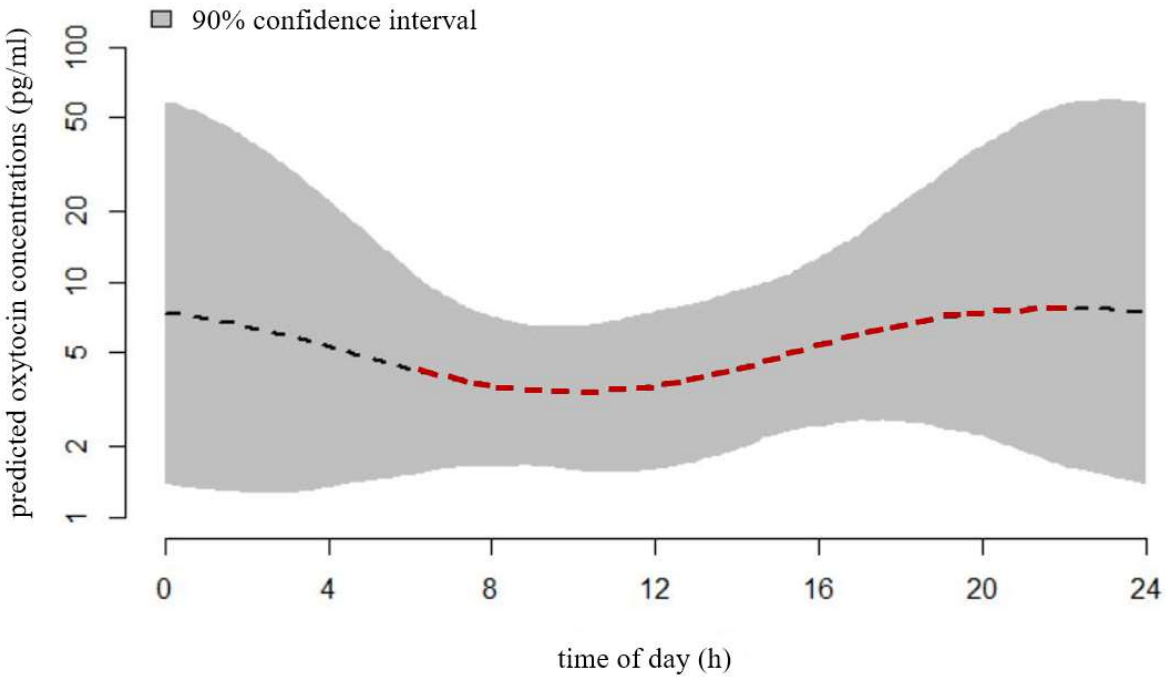

FIGURE 9.1. Diurnal fluctuations of endogenous oxytocin concentrations in extracted blood samples. The primary studies this analysis is based on measured endogenous oxytocin concentrations between 6:00am and 10:00pm. This 16-hour period is illustrated in red. The regression model predicted oxytocin concentrations between 10:01 pm and 05:59 am, as well, but this estimation is not supported by actual data. Therefore, as indicated by the large confidence interval, predicted fluctuations during this 8-hour period need to be interpreted cautiously.

TABLE 9.1 provides an overview on this dissertation's studies, including their specific scientific questions regarding oxytocin as a potential biomarker along the pathway from traumatic event exposure to PTSD symptom development, manifestation, and remission. It states whether the respective study is empirical or a systematic review, it includes a classification that explains in which function oxytocin was investigated, and further, it briefly summarizes the study's main findings.

TABLE 9.1. Overview on this dissertation's studies

Study Nr.	Title	Scientific question	Study type	Biomarker classification	Findings
1	Patterns of recovery from early posttraumatic stress symptoms after a preventive intervention with oxytocin: Hormonal contraception use is a prognostic factor	Is intranasal oxytocin administration early after traumatic event exposure as a preventive strategy for PTSD equally effective in men, women using hormonal contraception and cycling women?	Empirical study	Pharmacological agent	The effectiveness of intranasal oxytocin administration early after traumatic event exposure as a preventive strategy for PTSD did not differ between men, women using hormonal contraception and cycling women
2	Early posttraumatic autonomic and endocrine markers to predict posttraumatic stress symptoms after a preventive intervention with oxytocin	▪ Do blood oxytocin concentrations, as assessed early after traumatic event exposure, predict PTSD symptom development in men, women using hormonal contraception and cycling women? ▪ Do they moderate the effects of intranasal oxytocin administration as a preventive strategy for PTSD, in men, women using hormonal contraception and cycling women?	Empirical study	Prognostic biomarker; Prevention-related biomarker: Prescriptive marker	▪ In women using hormonal contraception, higher endogenous oxytocin concentrations, as assessed early after traumatic event exposure, were prognostic markers for higher PTSD symptoms one and a half, three and six months later ▪ They were neither prognostic markers in men nor in cycling women ▪ They did not moderate the effects of intranasal oxytocin administration as a preventive strategy for PTSD in men, women using hormonal contraception and cycling women
3	Trauma exposure, posttraumatic stress disorder and oxytocin: A meta-analytic investigation of endogenous concentrations and receptor genotype	▪ Do endogenous oxytocin concentrations, oxytocin receptor gene DNA variation and methylation differ between individuals with and without traumatic event exposure or with and without PTSD? ▪ Are these parameters correlated with the amount of traumatic event exposure or PTSD symptoms?	Systematic review and meta-analysis	Diagnostic biomarker	▪ Endogenous oxytocin concentrations did not differ between individuals with and without traumatic event exposure or with and without PTSD ▪ They were not correlated with the amount of traumatic event exposure and PTSD symptom ▪ Data on oxytocin receptor gene DNA variation and methylation were insufficient for valid conclusions
4	Oxytocin and vasopressin in internet-based cognitive-behavioral treatment for posttraumatic stress disorder	▪ Do blood oxytocin concentrations differ between male PTSD patients, male healthy trauma exposed individuals and male healthy non-trauma exposed individuals? ▪ Are they correlated with PTSD symptoms before, immediately and 3 months after TF-CBT? ▪ (How) do they change over time?	Empirical study	Diagnostic biomarker; Treatment-related biomarker: Outcome marker	▪ There were no group differences in endogenous oxytocin concentrations ▪ Endogenous oxytocin concentrations were not correlated with PTSD symptoms at any timepoint ▪ Endogenous oxytocin concentrations did not change in mean, but within individuals
5	Does oxytocin impact the psychotherapeutic process? An explorative investigation of internet-based cognitive-behavioral treatment for posttraumatic stress disorder	▪ Are blood oxytocin concentrations, as assessed before TF-CBT onset, associated with patients' therapeutic alliance ratings during and after the TF-CBT?	Empirical study	Treatment-related biomarker: Mechanisms of change indicator	▪ PTSD patients with higher endogenous oxytocin concentrations before TF-CBT onset provided higher therapeutic alliance ratings during and after the TF-CBT

Study Nr.	Title	Scientific question	Study type	Biomarker classification	Findings
6	Demographic, sampling- and assay-related confounders of endogenous oxytocin concentrations: A systematic review and meta-analysis	Do specimen type, extraction, sex, age, time of day, or fasting instructions influence measurements endogenous oxytocin concentrations?	Systematic review and meta-analysis	Prognostic biomarker; Diagnostic biomarker; Prevention- and treatment-related biomarker: Prescriptive marker; Outcome biomarker; Mechanisms of change indicator*	▪ Endogenous oxytocin concentrations measurements depended on specimen and extraction, as well as on their interactions ▪ In extracted blood samples, oxytocin concentrations were higher with increasing percentages of female participants and at later times of day. Age and fasting instructions did not impact the measurements
7	Menstrual cycle-related fluctuations in oxytocin concentrations: A systematic review and meta-analysis	(How) do endogenous oxytocin concentrations fluctuate during the course of the menstrual cycle?	Systematic review and meta-analysis	Prognostic biomarker; Diagnostic biomarker; Prevention- and treatment-related biomarker: Prescriptive marker; Outcome biomarker; Mechanisms of change indicator*	Endogenous oxytocin concentrations increased during the follicular phase, peaked at ovulation and decreased during the luteal phase

Note. * The studies of the second arm of this dissertation did not investigate oxytocin as such biomarker, but instead gathered systematic knowledge on factors that need to be taken into account when investigating endogenous oxytocin concentrations as prognostic, diagnostic, prevention- or treatment-related biomarker. PTSD = posttraumatic stress disorder; TF-CBT = trauma-focused cognitive behavioral therapy.

9.2 Discussion and interpretation of findings: Translational challenges

9.2.1 The translational research process

The present dissertation belongs to the field of translational research. Since the 1970s, which brought great advances in molecular biology, the gap between the basic and applied clinical sciences grew (Butler, 2008). Translational research, which has become increasingly acknowledged since about the year 2000 (Butler, 2008), tries to bridge that gap, in order to ensure that the knowledge that has been gained from the basic scientific disciplines results in improved patient care.

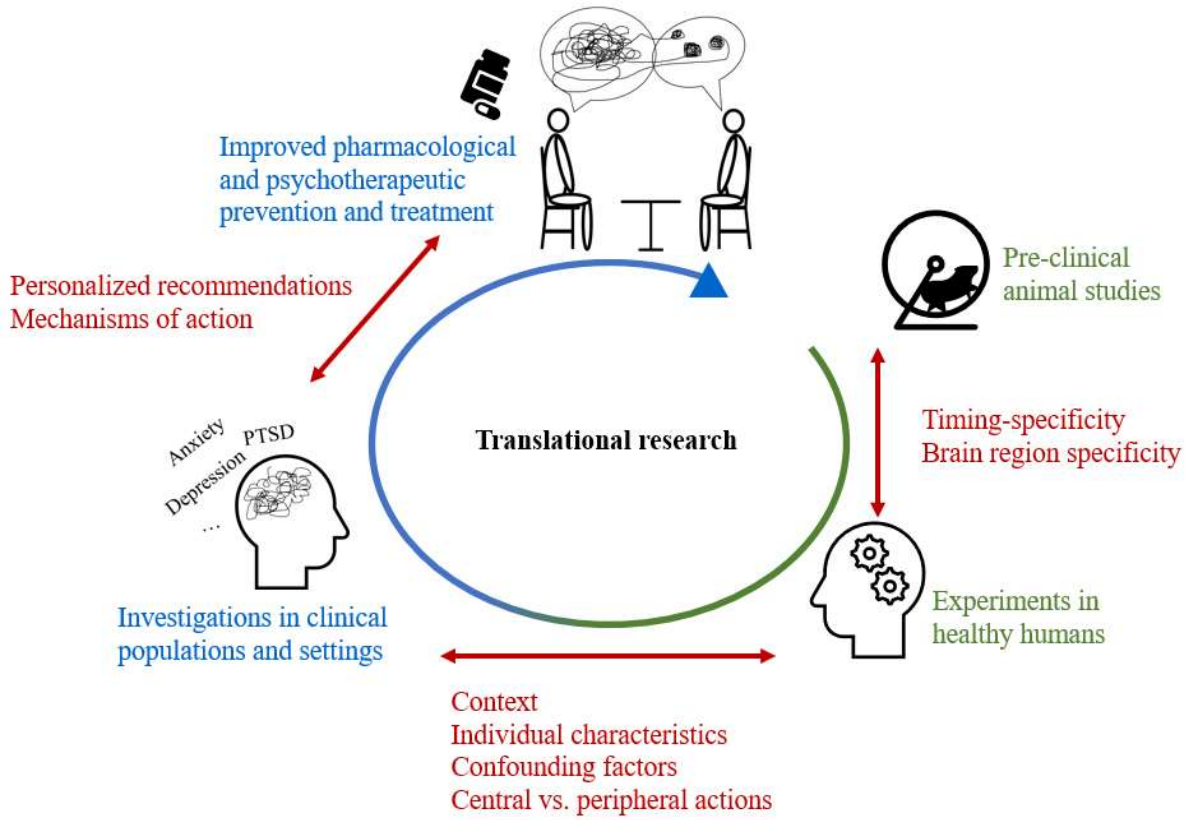

FIGURE 9.2. Schematic illustration of the translational research process. Research which can be classified into the basic scientific disciplines is shown in green, research which belongs to the applied clinical sciences is depicted in blue. The specific challenges and questions that arise when bridging the respective stages of the translational research process to investigate oxytocin's involvement in PTSD symptom development, manifestation, and remission, are listed in red. They are discussed in more detail in the text.

FIGURE 9.2 illustrates the translational research process, which is circular. For this dissertation, the basic sciences, and, more specifically, pre-clinical animal studies are set as a starting point. Translational scientists are interested in identifying and studying suitable animal models of mental disorders (Nestler & Hyman, 2010). As shown in CHAPTER 1, fear conditioning paradigms provide a good theoretical rationale for the development, manifestation, and remission of PTSD symptoms, and can therefore be used in pre-clinical animal studies of PTSD (Parsons & Ressler, 2013). Animal studies provide the advantage that the biological systems of interest are more accessible and can thus be studied more precisely. Accordingly, oxytocin's brain region-specific and timing dependent effects on fear and extinction learning were first explored in animal studies. These studies showed that the infusion of synthetic oxytocin into the central (Campbell-Smith et al., 2015; Roozendaal et al., 1992) and basolateral amygdala (Campbell-Smith et al., 2015) of rats before (Campbell-Smith et al., 2015) or during fear learning (Roozendaal et al., 1992) decreased fear acquisition (Roozendaal et al., 1992) and recall (Campbell-Smith et al., 2015). Further, oxytocin infusion into rats' basolateral amygdala before fear learning increased extinction acquisition (Campbell-Smith et al., 2015), However, oxytocin infusion into the central amygdala before extinction learning decreased extinction acquisition and recall (Campbell-Smith et al., 2015).

Following the translational research process further, oxytocin's effects on fear and extinction learning were then investigated in experimental studies in healthy humans. While fear conditioning paradigms can be applied to both, animals and humans, the methodological approaches to access oxytocin functioning are more restricted in humans. They range from studying the effects of intranasal oxytocin administration to measuring endogenous oxytocin concentrations and investigating oxytocin receptor gene functioning. As neither of these approaches provides a brain region-specific estimation of oxytocin's effects, knowledge about this influencing factor is lost along the pathway of translational research. What is won is that, in addition to studying oxytocin's effects on behaviors, its effects on psychological processes can be studied. In healthy humans, oxytocin's effects on extinction learning also appeared to be timing dependent, as increased extinction acquisition (Acheson et al., 2013; Eckstein et al., 2015) and recall (Acheson et al., 2013) were reported when oxytocin was administered intranasally after extinction learning, whereas intranasal oxytocin administration before fear learning did not impact

extinction acquisition (Cavalli et al., 2017). However, regarding the current state of research, the impact of the timing of oxytocin administration on extinction learning seems to be opposing in animals and humans, which certainly needs further investigation.

This dissertation is based upon findings from these basic sciences. It tried to transfer this knowledge to the clinical setting, to understand the underlying biological mechanisms of PTSD symptom development, manifestation, and remission more precisely. Investigating biomarkers for PTSD holds several promises: On a theoretical level, it can contribute to a more comprehensive understanding of traumatic event processing and PTSD symptom development and manifestation (Fischer & Ehlert, 2019; Insel, 2013). This is clinically relevant, as it might be helpful to identifying individuals with increased need for psychological or psychiatric care more precisely. On a practical level, investigating biomarkers for PTSD can improve PTSD prevention and treatment, by informing new interventions, augmenting existing ones, or deduce personalized intervention recommendations (Machado-Vieira, 2012). This is clinically relevant, as it might help to providing individuals at risk of developing or already suffering from PTSD symptoms with the most effective intervention.

9.2.2 Contextual and individual moderators of the effects of intranasal oxytocin administration as a basis for personalized interventions

However, transferring findings from basic sciences to clinical research brings further challenges with it. These translational challenges also became evident in this dissertations' studies. It is characteristic for applied clinical-psychological research that it observes patients in their everyday lives and thus, methodologically speaking, in an externally valid setting. In such a setting, several potentially confounding factors naturally occur, which can be controlled for in animal studies or in experimental studies in humans. For this reason, the internal validity in applied clinical-psychological studies is comparatively lower and potentially confounding contextual and individual factors need to be considered. Indeed, oxytocin's effects on fear processing, but also its effects on social functioning, do not appear to be unique, but instead depend on such contextual and individual factors (Bartz, Zaki et al., 2011; Olff et al., 2013; Shamay-Tsoory & Abu-Akel, 2016). Most relevant in this context, traumatic event exposure (Grimm et al., 2014), as well as psychopathology (Bartz, Simeon et al., 2011), were

identified as moderators of oxytocin's effects. The social salience hypothesis (Shamay-Tsoory & Abu-Akel, 2016) suggests that intranasal oxytocin administration, as evaluated as a preventive intervention for PTSD symptom development in **studies 1** and **2**, should be applied in a context which can be perceived as safe (Olff et al., 2013). In **studies 1** and **2**, the first sniff of oxytocin was applied at patients' homes or in the laboratory setting, either way supported by a healthcare professional and thus in a professional and standardized environment which can be regarded as safe. However, the following sniffs were applied at participants' homes and in their everyday lives, impeding standardization and making it impossible to control for potential contextual moderators. Whereas context could thus unfortunately not be considered as potential moderator, **studies 1** and **2** specifically focused on individual moderators. They indicated that neither sex, nor hormonal contraception use, nor different endocrine or autonomic states before intervention onset moderated its effects. However, it must be noted that dividing the participating women into two groups, differentiating between women using hormonal contraception and cycling women, substantially reduced the statistical power, which limits the informative value of these findings. At the same time, it must be assumed that this differentiation was still too broad to make precise conclusions about these women's underlying gonadal steroids-related statuses, which are determined by their menstrual cycle phases, as well as by the specific type, dosage, and intake phase of hormonal contraception. Further, due to their small number, postmenopausal women had to be excluded from the studies. Therefore, even though sex and hormonal contraception use were not relevant moderators of the effects of intranasal oxytocin administration, gonadal steroids-related statuses might still be influential.

Acute PTSD symptoms before intervention onset, which are a risk factor for subsequent PTSD symptom development (Harvey & Bryant, 1999), did moderate the effects of intranasal oxytocin administration (van Zuiden et al., 2017). Consequently, it appears that individuals with acute PTSD symptoms after traumatic event exposure are the ideal target group for this intervention. However, this RCT was the first trial to evaluate oxytocin as a preventive strategy for PTSD symptom development. Its findings clearly need to be confirmed in an independent sample. Such a replication study should consider gonadal-steroids-related statuses of women more precisely, by additionally taking fertility status, menstrual cycle phase, as well as hormonal contraception type, dosage and intake phase into account.

This also implies that subsequent studies need to increase the number of women they investigate. Up to now, women have too often been excluded from all stages of translational research on PTSD (Kornfield et al., 2018; Olff, 2017). As women's greater hormonal variability contributes to greater variability in findings, they are considered to be "messy" (Bale & Epperson, 2017). However, in line with an understanding of translational research as sex-sensitive and in line with the claim of increasingly personalizing interventions to those target groups who benefit the most from them (Bolea-Alamanac, Bailey, Lovick, Scheele, & Valentino, 2018; Ferretti, Santuccione-Chadha, & Hampel, 2019; Garcia & Delahanty, 2017), the variety of factors that influence women's gonadal steroids-related statuses must be accounted for, in order to specifically address and increasingly understand the "messiness" (Bale & Epperson, 2017). This does not exclusively apply to studies which evaluate intranasal oxytocin administration for PTSD prevention, but also to such which evaluate it in the context of PTSD treatment, as stand-alone or adjunctive drug.

9.2.3 Understanding the mechanisms of action of intranasal oxytocin administration as PTSD prevention or treatment

Next to personalizing recommendations for specific preventive or therapeutic interventions for PTSD, it is a current challenge to reveal the exact mechanisms of actions underlying translationally informed interventions, such as intranasal oxytocin administration. To date, a solid neurobiological explanation for oxytocin's effects is still lacking (van Zuiden et al., 2017). Landgraf and Neumann (2004) certified oxytocin with high scientific attractivity and justify this claim with its multiple modes of action. Indeed, as previously explained, oxytocin impacts fear processing (as summarized in Macdonald & Feifel, 2014 and in CHAPTER 1), social functioning (as summarized in Donaldson & Young, 2008; Feldman, 2012; Heinrichs et al., 2009; Macdonald & MacDonald, 2010 and in CHAPTER 1), as well as multiple physiological processes (as summarized in Boll et al., 2018; Gimpl & Fahrenholz, 2001; Lawson, 2017; Winter & Jurek, 2019; Yang et al., 2013 and in CHAPTER 1). This broad range of functions, however, poses a challenge when aiming at tracing back the effectiveness of intranasal oxytocin administration in PTSD prevention or treatment to one specific underlying mechanism of action, out of the many potentially assumable ones.

Preceding from a neurocognitive perspective, altered fear learning, as indicated by increased fear responsiveness and altered discrimination between threat-related and neutral stimuli, as well as decreased extinction learning underly PTSD symptom development, manifestation and non-remission (as summarized in Desmedt et al., 2015; Norrholm & Jovanovic, 2011, 2018; Parsons & Ressler, 2013; Rothbaum & Davis, 2003; Sheynin & Liberzon, 2017; Zuj & Norrholm, 2019; Zuj, Palmer, Lommen et al., 2016, as well as in CHAPTER 1). Oxytocin promoted the decline of healthy individuals' conditioned fear responses during extinction learning (Acheson et al., 2013; Eckstein et al., 2015; Hu et al., 2019). Therefore, it can be hypothesized that, if applied as preventive intervention, intranasal oxytocin administration promotes recovery from traumatic stress by improving extinction learning, that is, learning that stimuli that co-occurred with the traumatic event no longer indicate threat (Zuj & Norrholm, 2019). Likewise, as extinction learning is the central neurocognitive process underlying exposure-based treatments (Craske et al., 2014; Rothbaum & Davis, 2003; Smith et al., 2017; Stojek et al., 2018), oxytocin has been administered intranasally prior to exposure sessions, assuming that it can augment the effectiveness of TF-CBT (Flanagan et al., 2018). However, even though there is a solid theoretical rationale to assume that extinction learning is the mechanism of action by which oxytocin reduced PTSD symptoms in previous studies (Flanagan et al., 2018; van Zuiden et al., 2017), this has not been explicitly tested, yet. For this purpose, studies that investigate the effectiveness of PTSD prevention or treatment need to complement their clinical assessment by suitable measures of the assumed underlying mechanism of change. Regarding the question whether PTSD symptom remission due to intranasal oxytocin administration can indeed be ascribed to increased extinction learning, fear conditioning paradigms need to be added.

Fear and extinction learning paradigms are ideal for translational research, as their neurobiological underpinnings are precisely understood, as the corresponding behavioral and biological mechanisms are well conserved across species, including humans, and, of course, as they provide a solid theoretical explanation for the establishment of fear symptoms (Parsons & Ressler, 2013). Therefore, it is justifiable that the most influential neurocognitive models of PTSD symptom development, manifestation and remission mainly refer to these processes (Parsons & Ressler, 2013). Yet, it remains an open question in how far the described alterations are specific for PTSD. In fact, fear and extinction learning paradigms

have been used to model a variety of anxiety disorders, as well (Lissek et al., 2005; Milad & Quirk, 2012). Just as it has be argued that individuals with PTSD fail to learn that stimuli that previously co-occurred with a traumatic event do not predict current threat (Parsons & Ressler, 2013; Zuj & Norrholm, 2019), it has been argued that individuals with social phobia fail to learn that stimuli that previously co-occurred with a humiliating social experience do not predict current humiliation (Lissek et al., 2008; Mineka & Zinbarg, 1995) and that patients with panic disorder fail to learn that interoceptive sensations that previously co-occurred with a panic attack do not predict a serious medical condition (Cort et al., 2017; Wolpe & Rowan, 1988). In contrast to this apparent universal applicability of altered fear and extinction learning for a range of mental disorders, there are indications that impaired safety learning is more specific for PTSD. An investigation which compared fear conditioning mechanisms between individuals with PTSD and individuals suffering from general anxiety and depression symptoms indicated that impaired differential fear conditioning (i.e., impaired discrimination between the CS+ and the CS-) and impaired safety signal acquisition were specific for PTSD (Acheson et al., 2015). These processes both reflect the inability to detect stimuli that indicate safety, namely, the CS- and the safety signal, respectively. Furthermore, while a set of studies documented impaired safety learning in PTSD (Jovanovic et al., 2009; Jovanovic, Norrholm, Blanding, Davis et al., 2010; Jovanovic, Norrholm, Blanding, Phifer et al., 2010; Sijbrandij et al., 2013, as summarized in CHAPTER 1), this deficit was not observed in individuals with high trait anxiety (Kindt & Soeter, 2014) or in individuals with major depression (Jovanovic, Norrholm, Blanding, Davis et al., 2010), again suggesting specificity for PTSD. Although in recent years, safety learning paradigms, such as the conditional discrimination task, have been established in PTSD research (Jovanovic, Kazama, Bachevalier, & Davis, 2011), to date, they have never been combined with measures of oxytocin functioning. However, it might be highly interesting to investigate oxytocin along with a precise differentiation between extinction and safety learning deficits in PTSD: Based on evidence that social stimuli are particularly effective safety signals (Hornstein, Fanselow, & Eisenberger, 2016), Eckstein et al. (2019) suggested that oxytocin might promote social safety learning, particularly in individuals who experienced predominantly positive social interactions during their lifetimes, for whom social stimuli are generally positively connotated. In contrast, they argued, in individuals who experienced predominantly adverse social interactions, extinction learning

might be more important. This shows that those factors that are considered as important moderators of oxytocin's effects, such as positive or negative representations of others (Olff et al., 2013), also need to be taken into account when investigating the exact mechanisms of action that underly the effectiveness of intranasal oxytocin administration as PTSD prevention or treatment.

Next to fear, extinction and safety learning, other mechanisms by which oxytocin might exert potential preventive or therapeutic effects in trauma exposed individuals can be assumed. For instance, it has been suggested that intranasal oxytocin administration promotes the perception of social support in individuals suffering from PTSD symptoms (Nawijn et al., 2017). Based on that, oxytocin might help these individuals to benefit more from social support, to therefore increasingly seek positive social interactions and to finally break the vicious cycle of PTSD-related social isolation (Olff, 2012). Thus, improved activation of social resources provides an alternative and also solid theoretical rationale of the mechanisms of action underlying PTSD symptom remission by intranasal oxytocin administration (Flanagan et al., 2018; van Zuiden et al., 2017). Here too, it is imperative to complement the clinical assessments that are used in classical PTSD prevention or treatment evaluations by measures that reflect these social processes.

Study 5 took up this idea by testing whether blood oxytocin concentrations before onset of an internet-based TF-CBT were associated with patients' ratings of the therapeutic alliance during and after the intervention. Even though the study did provide some indications towards beneficial effects of higher pre-treatment oxytocin concentrations, its informative value is clearly limited by the low temporal stability of those concentrations that was also observed (as discussed in **study 4**). In combination with the results from **studies 3** and **6**, which detected a notable impact of the biochemical analysis method on the observed endogenous oxytocin concentrations (**study 6**), as well as on their correlation with traumatic event exposure (**study 3**), it needs to be critically asked: What exactly do endogenous oxytocin concentrations reflect?

9.2.4 Reducing the impact of state- and trait-related cofounders of endogenous oxytocin concentrations

Presumably because endogenous oxytocin concentrations represent a measure of oxytocin functioning which, in contrast to intranasal administration, does not involve the manipulation of a biological system, they have been used relatively frequently in clinical populations, among them, individuals with PTSD (Cochran, Fallon, Hill, & Frazier, 2013). However, when investigating endogenous oxytocin concentrations as potential prognostic, diagnostic, prevention- or treatment related biomarkers, it must be ensured that any potentially observed association can be ascribed to PTSD symptoms rather than to other confounding factors. Unfortunately, **study 4** revealed that PTSD patients' blood oxytocin concentrations at one timepoint were uncorrelated with the same individuals' blood oxytocin concentrations at another timepoint, while PTSD symptoms did not change. This strongly suggests that the oxytocin measurements were not influenced by trait-related factors, such as PTSD symptoms, but instead by state-related confounders which differed within individuals between the respective assessment timepoints. However, especially considering the high standardization of the measurements, these confounders remain unknown up to now. Whereas this problem only became evident due to the longitudinal design of **study 4**, it must be assumed that it also impacted the other empirical studies which related endogenous oxytocin concentrations to traumatic event exposure or PTSD symptoms and which were meta-analytically summarized in **study 3**. Therefore, to make more valid conclusions about endogenous oxytocin functioning in PTSD, it is imperative to increase the intraindividual stability of blood oxytocin concentrations. In the following, two possible solution approaches will be presented.

As investigations of endogenous oxytocin concentrations as biomarkers for mental disorders still represent a comparatively young field of research, a lot can be learned from looking at other biological systems that are already more established as indicators of pathological psychological functioning. The first possible solution approach can be derived from analyzing the existing research on hypothalamic-pituitary-adrenal (HPA) axis functioning in mental disorders. The HPA axis regulates the neuroendocrine response to stress by inducing a cascade of hormone releases which finally result in the release of cortisol from the adrenal glands (J. Andrews, Ali, & Pruessner, 2013; Chrousos & Gold,

1992). Cortisol can be measured in different body tissues, such as CSF, blood, saliva, urine, but also in hair and fingernails (Izawa et al., 2015; Kudielka & Wüst, 2010; Stalder & Kirschbaum, 2012). Besides responding to acute stress, the HPA axis follows a diurnal rhythm, depicting a strong increase of cortisol concentrations immediately after awakening with peak concentrations after around 30 to 45 minutes, which then constantly decrease during the course of the day and night (Clow, 2004; J. C. Pruessner et al., 1997). Therefore, single measures of cortisol concentrations are highly unstable within individuals, even within a single day. Addressing this observation, more sophisticated parameters, such as the cortisol awakening response, the diurnal slope, as well as areas under the curve, have been developed and established in cortisol research (Adam & Kumari, 2009; J. C. Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003). These parameters statistically merge several single measures of cortisol concentrations at a given timepoint and thereby provide more stable estimations of different aspects of basal and stress-induced cortisol release. However, as even these parameters are prone to intraindividual variability (Hellhammer et al., 2007), current guidelines recommend assessing them at two or more subsequent days (Stalder et al., 2016).

Just as increasing the number of measurements and merging them into more stable and meaningful biological parameters brought progress in the field of HPA axis research, oxytocin research might benefit from these advances. For instance, as **study 6** showed that oxytocin concentrations also follow a diurnal rhythm, albeit a different one than cortisol concentrations, the area under the curve with respect to ground might represent a more stable and informative measure of basal oxytocin functioning at a given day (J. C. Pruessner et al., 2003). In addition, just as the area under the curve with respect to increase is a viable indicator of the cortisol stress response (J. C. Pruessner et al., 2003), the oxytocin response to stress (Nishioka et al., 1998; Wotjak et al., 1998), but also other stimuli which cause oxytocin release, such as social support (Grewen et al., 2005), might be portrayed in this parameter. Thus, replacing single measurements of oxytocin concentrations which are highly unstable within individuals by more sophisticated summary measures of basal or stimulated oxytocin release might be useful. This balances a variety of state-related confounding factors and thereby enables a more precise estimation of trait-related factors, such as PTSD symptoms.

The second possible solution approach is to identify state- but also trait-related confounders of endogenous oxytocin concentrations and to systematically control for their impact, by means of standardizing measurement protocols or by means of statistical techniques. Here, again, the field of HPA axis research already made considerable progress in recent years, as a number of studies specifically devoted to detecting confounders of cortisol concentrations was conducted (Schlotz, 2011; Stalder et al., 2017). Further, their results were taken up in influential guidelines (Stalder et al., 2016). Considering confounders of endogenous oxytocin concentrations appears to be equally important. Therefore, **studies 6** and **7** quantified the impact of selected confounders, by means of meta-analytically testing their impact on reported endogenous oxytocin concentrations in many empirical studies in healthy humans. Interestingly, the most relevant confounders that were identified were such which are either stable within individuals or changing only on a yearly to daily basis, such as sex, age and menstrual cycle phase. Additionally, time of day was an important confounder. However, other state-related confounders, such as individuals' fasting or exercising behaviors before sample collection, were not influential. Considering that in **study 4**, the blood samples were always collected at the same time of day, all patients were male and their ages only negligibly changed between the measurement timepoints, it must be critically stated that the exact factors which caused the high intraindividual variability of the observed blood oxytocin concentrations are still unknown. At the same time, it must be assumed that these unknown factors were also influential, but not systematically considered in those other empirical studies on the association between endogenous oxytocin concentrations and traumatic event exposure or PTSD symptoms that were reviewed in **study 3.**

In addition, **study 3**'s findings clearly pointed out that characteristics of the biochemical analysis of endogenous oxytocin concentrations, namely, the type of biological specimen they were measured in and whether or not extraction was performed, influenced endogenous oxytocin concentrations. S**tudy 6** confirmed that these factors considerably impacted endogenous oxytocin concentrations of healthy humans. Therefore, the following section will critically reflect on the implications of these findings for translational studies, such as those included in this dissertation (**studies 2, 3, 4** and **5**), which integrated measures of endogenous oxytocin concentrations into applied clinical-psychological research on PTSD and other mental disorders.

9.2.5 Which aspects of oxytocin functioning do endogenous oxytocin reflect?

Regarding the methodological approach of intranasal oxytocin administration, in recent years, research has made considerable progress in understanding the central pathways by which it exerts its effects. This has been achieved by a precise description of the route of central oxytocin distribution following its intranasal administration (Quintana, Smerud, Andreassen, & Djupesland, 2018). However, regarding the methodological approach of measuring endogenous oxytocin concentrations, which is the predominantly applied one in clinical populations, it remains largely unknown which route of oxytocin distribution through the human body these concentrations correspond to. As explained in CHAPTER 1 (section 1.4.1), the synthesis of oxytocin in the hypothalamic nuclei is followed by its distribution via various, independent, central and peripheral pathways, targeting specific peripheral organs and specific brain regions, but also including diffuse spread of oxytocin via the CSF (Gimpl & Fahrenholz, 2001; Jurek & Neumann, 2018; Knobloch & Grinevich, 2014; Landgraf & Neumann, 2004; Song & Albers, 2018). It is an ongoing debate whether and how oxytocin concentrations, as measured in a given body fluid, can be traced back to central oxytocin functioning (Meyer-Lindenberg et al., 2011; Valstad et al., 2017). This debate is highly relevant for translational research, as oxytocin's behavioral and psychological functions are centrally mediated (Meyer-Lindenberg et al., 2011).

The current state of research indicates that endogenous oxytocin concentrations do reflect central oxytocin availability under certain conditions, albeit the exact underlying processes are not fully understood, yet. Increased central oxytocin availability, as induced by intranasal oxytocin administration, led to increased oxytocin concentrations in CSF (Striepens et al., 2013), blood (Striepens et al., 2013) and saliva (van IJzendoorn et al., 2012; Weisman, Zagoory-Sharon, & Feldman, 2012), presumably mediated via feedforward loops of oxytocin release (van IJzendoorn et al., 2012). Interestingly, these effects were characterized by differential temporal dynamics: While in blood and saliva, peaking oxytocin concentrations were observed within the first hour after administration (Striepens et al., 2013; van IJzendoorn et al., 2012; Weisman et al., 2012), CSF oxytocin concentrations only began to increase after 75 minutes (Striepens et al., 2013). It is also worth noting that oxytocin concentrations in CSF and blood were uncorrelated (Striepens et al., 2013). The correlation between

oxytocin concentrations in central and peripheral body fluids was more comprehensively studied in a meta-analysis: Valstad et al. (2017) found that central and peripheral oxytocin concentrations were uncorrelated under basal conditions, but positively correlated after intranasal oxytocin administration and after experimentally induced stress. Therefore, it can be concluded that endogenous oxytocin concentrations, as measured in CSF, blood, saliva or urine, do reflect central oxytocin functioning to some extent. However, the question which exact central processes are reflected in which body fluid after which time period still needs to be investigated more precisely.

Extending the debate on what exactly endogenous oxytocin concentrations reflect, critical concerns have been raised whether endogenous oxytocin concentrations can be validly measured at all or whether, on the contrary, at least some of the published studies reported nothing but "random numbers" (Leng & Sabatier, 2016). The central argument of this criticism is the extreme variability in endogenous oxytocin concentrations that were reported in different publications. Considerably higher values were detected in studies that analyzed unextracted, as compared with extracted samples (Leng & Sabatier, 2016; Szeto et al., 2011). This also became evident in **study 6**. Its meta-analysis confirmed a significant impact of extraction which was particularly relevant in blood samples: The mean oxytocin concentration in unextracted blood samples, as predicted by a meta-regression that summarized mean oxytocin concentrations of 339 independent subsamples, comprising 12,741 healthy individuals, was 275.61 pg/ml, as compared to 4.75 pg/ml in extracted blood samples. Notably, extracted and unextracted samples derived from the same original blood specimen were only weakly correlated (Szeto et al., 2011). These inconsistencies led some researchers to the conclusion that some biochemical analysis methods, namely, those applying extraction, are clearly superior to other, apparently invalid ones, namely, biochemical analyses of unextracted samples (Leng & Sabatier, 2016; McCullough, Churchland, & Mendez, 2013). Consequently, these researchers requested the introduction of a single, valid biochemical analysis method as the standard in oxytocin research (McCullough et al., 2013). In contrast, MacLean et al. (2019) argued that, instead of assuming that some biochemical analysis methods succeed in detecting endogenous oxytocin concentrations while others do not, they might all detect endogenous oxytocin concentrations, albeit in different states, for instance bound or unbound to other matrix components. The lowest common denominator of these opposing views is that the open question why

different biochemical analysis methods produce such highly variable values of endogenous oxytocin concentrations is yet to be resolved (MacLean et al., 2019).

With this still ongoing debate, the translational research process comes full circle. Thereby, an additional translational characteristic becomes evident, that is, the interdependency between the basic scientific and applied clinical-psychological disciplines. Findings from pre-clinical animal studies need to be transferred to humans and, finally, to clinical populations to gain clinical relevance by improving patient care. Alike, applied clinical-psychological research that is devoted to investigating biomarkers for mental disorders needs to rely on valid biochemical measurement methods, as developed in basic scientific studies, to adequately interpret its results. In this regard, this dissertations studies revealed that the following current translational challenges require continuous, interdisciplinary work: While intranasal oxytocin administration effectively reduced PTSD symptoms in some individuals (Flanagan et al., 2018; van Zuiden et al., 2017), the replicability and generalizability of these findings needs to be tested. In line with the latter challenge, the ideal target groups for intranasal oxytocin administration as preventive or therapeutic intervention need to be defined. Further, a precise description of the underlying mechanisms of action is necessary to optimize the intervention. In addition, targeted basic scientific research needs to develop meaningful parameters that reflect endogenous oxytocin functioning which can be validly assessed, and which are more stable within individuals than singles measures of endogenous oxytocin concentrations. This is a prerequisite to associate endogenous oxytocin functioning with trait-related factors, such as PTSD symptoms.

9.3 Strengths and limitations

Besides the specific strengths and limitations that were discussed in **studies 1 - 7**, respectively, this dissertation has several general strengths, but also some general limitations which must not be neglected. Some of the latter do not only apply to this dissertation but are instead relevant for the whole field of research on biomarkers for PTSD.

The comprehensiveness of this dissertation can certainly be regarded as a strength. Not only did it cover all stages of traumatic event processing, from PTSD symptom development to manifestation and remission (**studies 1 - 5**), but it also covered a wide range of biomarker functions, from investigating

oxytocin as a pharmacological agent (**studies 1** and **2**), to investigating it as a prognostic marker for PTSD symptom development (**study 2**), as a diagnostic biomarker for traumatic event exposure and PTSD symptoms (**studies 3** and **4**), as a prescriptive biomarker in a preventive intervention for PTSD (**study 2**), as well as an outcome biomarker (**study 4**) and a mechanisms of change indicator (**study 5**) in TF-CBT. At least some of the problems related to measurements of endogenous oxytocin concentrations that became apparent in the applied clinical-psychological studies were addressed in the more basic, biological-psychological studies that complement this dissertation (**studies 6** and **7**). In that sense, the translational meshing of different psychological disciplines, the more basic and the more applied ones, represents another strength of this dissertation. Finally, it combined primary empirical (**studies 1, 2, 4** and **5**) with meta-analytical research (**studies 3, 6** and **7**).

Based on this latter methodological distinction, firstly, the common limitations of this dissertation's empirical studies will be discussed, followed by, secondly, a discussion of the limitations that apply to its systematic reviews and meta-analyses. Lastly, this section will provide a more general appraisal of the validness of research on oxytocin, including this dissertation's studies.

An important limitation that affects all empirical studies included in this dissertation (**studies 1, 2, 4** and **5**) is that they encompassed secondary analyses of data from randomized controlled trials that were originally designed to test the effectiveness of a pharmacological or psychotherapeutic intervention for PTSD. Consequently, the resulting data were first and foremost evaluated with regard to their primary clinical outcome (Niemeyer et al., 2020; van Zuiden et al., 2017), that was also defined as such in the according pre-registrations (Australian Clinical Trials Registry ACTRN12616000956404; Frijling et al., 2014). Defining primary outcomes in pre-registrations, which specify the study's main scientific questions, hypotheses and methods before collecting or analyzing the data, is a prerequisite for conducting hypothesis-testing, as opposed to hypothesis-generating research (Kraemer et al., 2002; Nosek, Ebersole, DeHaven, & Mellor, 2018). It is thus a prerequisite for interpreting effects causally, that is, due to the respective intervention (Kraemer et al., 2002). Pre-registrations usually also involve an a-priori power analysis which is being conducted in order to identify to the sample size that is needed to detect a significant effect on the primary outcome (Lan & Lian, 2010). In the case of this dissertation's

empirical studies, the pre-defined sample size was targeted at detecting a possible main effect of the intervention on the primary outcome, that is, PTSD symptoms (Australian Clinical Trials Registry ACTRN12616000956404; Frijling et al., 2014). It was not additionally increased in order to take the impact of endogenous oxytocin concentrations into account, neither as a prognostic or prescriptive biomarker (**study 2**), nor as an outcome biomarker (**study 4**), nor as a mechanisms of change indicator (**study 5**). Further, it was not additionally increased in order to account for other prognostic or prescriptive factors, such as sex and hormonal contraception use (**study 1**), that are probably important for deriving personalized intervention recommendations (Bolea-Alamanac et al., 2018; Machado-Vieira, 2012).

It is comprehensible that the psychopathological symptoms have been pre-defined as the primary outcome, as they were the main targets of the interventions and most directly relevant for patients. It is also comprehensible that pre-defined sample sizes were not adapted for secondary outcomes or moderators, as this would have excessively increased the required numbers of individuals, and thus, the efforts associated with the clinical trials (Brookes et al., 2004). In this regard, methodological aspirations clearly need to be balanced with economic considerations, which are also important aspects of patient care. However, the consequence of this is that the results of this dissertation's empirical studies, but presumably also those of other studies on prevention- or treatment-related biomarkers for PTSD, can only generate, but not test hypotheses (Kraemer et al., 2002). Thus, their results need to be replicated within independent studies in independent samples, specifically targeted at detecting effects on a biological level, or specifically targeted at detecting moderation effects. Consequently, these types of empirical studies do not yet stand for themselves. Instead, the line of research that they have opened must be pursued further.

Biomarker research does have the potential to improve patient care, but unfortunately only indirectly, by understanding, informing, and thereby ultimately improving interventions. It is a relevant question whether funding agencies consider it attractive to fund research which understands and informs interventions or whether instead, it appears to be more attractive to fund research that directly evaluates interventions. The answer to it will impact the validness of applied clinical-psychological research on

biomarkers of PTSD in the future. At present, it can be stated that the majority of research on biological moderators or outcomes of interventions is presumably underpowered, as the according measurements have most frequently been added to existing intervention evaluation studies without adapting the pre-defined sample sizes,

A potent tool to address this problem are systematic reviews that include meta-analyses, as they summarize data from different primary empirical studies and thereby often significantly increase the total number of individuals investigated. However, there are at least two common problems with meta-analyses that also became evident in those included in this dissertation (**studies 3, 6** and **7**). Firstly, meta-analyses can only deliver valid results if the data that were published in the respective primary studies are valid. With respect to the debate on whether endogenous oxytocin concentrations can be validly measured at all (Leng & Sabatier, 2016), it becomes clear that the criticism addressed to the respective primary studies equally applies to meta-analyses that summarize them. Secondly, the impact of moderators can only be determined in subgroup analyses if they were measured and reported in the respective primary studies. This problem arose for example in **study 3**, when its pre-registration determined to investigate PTSD duration as potential moderator (PROSPERO CRD42018103036), but this was not possible because PTSD duration was reported in none of the studies. Further, it must be considered that moderators are influential on an individual level, but classical meta-analyses only aggregate summary measures of these individual data. This barrier can be overcome by more advanced meta-analytical approaches, such as individual patient data meta-analyses, which aggregate the individual instead of summary data observed in several independent studies (Ioannidis, 2017). Obviously, as compared to the classical meta-analytical approaches, such methods require higher efforts both for the team conducting individual patient data meta-analyses, as well as for the teams that provide the individual data. Nevertheless, it is encouraging to find that such approaches are already being implemented for biological data of patients with mental disorders in working groups such as ENIGMA (Zugman et al., 2020).

Finally, general criticism on the reproducibility, replicability, robustness, and generalizability of scientific findings stops neither at research on oxytocin as a biomarker for mental disorders, not at this

dissertation's studies. The most influential criticism in this regard has been voiced by Ioannidis (2005), stating that "most published research findings are false". Mostly, he referred to the problem of false positive results, which, as reflected in the discussion on publication bias (Ioannidis, Munafò, Fusar-Poli, Nosek, & David, 2014) are being disproportionately frequently published.

Publication bias also appears to be a relevant issue in the field of oxytocin research. This attracted attention when a research group that investigated the effects of intranasal oxytocin administration on social behaviors published a study in which they critically reviewed their own work, by comparing their expected with their observed results and by analyzing how this was reflected in their publications (Lane, Luminet, Nave, & Mikolajczak, 2016). They observed an unexpectedly high number of non-significant results: Testing 25 main effects of intranasal oxytocin administration, only one was significant and testing 25 interaction effects, depicting the differential effects of oxytocin, as compared with placebo administration, only five were significant. This was also reflected in a low publication rate which led the authors to conclude that oxytocin research is presumable considerably biased, as negative results are not being published. Similarly, a systematic review that specifically evaluated the credibility of findings on the effects of intranasal oxytocin administration on psychosocial outcomes detected several problems, such as lacking or unsuccessful replication of findings, particularly of interactive effects, inadequate power and, more generally, the explorative character of the published research (Mierop et al., 2020). When a well-powered registered replication of the famous and influential study by Kosfeld et al. (2005) did not successfully replicate its finding that intranasal oxytocin administration increased trust in healthy men (Declerck, Boone, Pauwels, Vogt, & Fehr, 2020), the replication crisis that is shaking the confidence in oxytocin research was even discussed in the German popular media (Schnabel, 2020).

This general problem of potential biases impacting oxytocin research is also relevant for the present dissertation, for two reasons: Firstly, because potentially biased findings inspired this dissertation's studies and secondly, because this dissertation's studies might potentially be biased themselves. It is worth noting that important measures have been implemented to minimize bias. For instance, pre-registration has been applied. However, as discussed before, both pre-registrations of the empirical

studies were rather unspecific with regard to secondary outcomes or moderators (Australian Clinical Trials Registry ACTRN12616000956404; Frijling et al., 2014). Additionally, regarding the meta-analyses, due to information and experience that was gained while conducting the study, as well as due to reviewers' comments, the final studies to some extent deviated from the way they were originally planned. For transparency, these deviations have been tracked in the original protocols (PROSPERO CRD42017072306 and CRD42018103036).

9.4 Implications for future research

Fortunately, there are various complementary approaches that are effective in increasing the reproducibility, replicability, robustness, and generalizability of science in general and oxytocin research in particular. Many of these are promoted by the Open Science movement (Foster & Deardorff, 2017; Open Science Collaboration, 2012). Indeed, research on oxytocin and other potential biomarkers for PTSD can benefit from implementing the measures these researchers suggest. They should be taken to heart in future studies.

As already mentioned, pre-registration is a potent tool to minimize systematic bias by a-priori specifying scientific questions, hypotheses and methods (Nosek et al., 2018; Nosek et al., 2019). The present dissertation's empirical studies were hypotheses-generating instead of hypotheses-testing (Kraemer et al., 2002). In order to actually test the hypotheses they generated, its positive findings, such as the significant prognostic effect of higher endogenous oxytocin concentrations early posttrauma, which predicted higher PTSD symptoms up to 6 months later (**study 2**), but also its negative findings, such as the non-significant correlation between PTSD symptoms and endogenous oxytocin concentrations (**studies 3** and **4**), require replication in an independent study, specifically designed for this purpose, and pre-registered accordingly. In this regard, it seems, but in fact, it is not needless to state that deviations from the pre-registration should be avoided as far as possible. This is emphasized so explicitly at this point, because previous research that analyzed the consistencies between pre-registrations and the resulting publications of empirical studies (Chan, Hróbjartsson, Haahr, Gøtzsche, & Altman, 2004), but also of systematic reviews and meta-analyses (Silagy, Middleton, & Hopewell, 2002), found that they were alarmingly low. This shows that studies, if they aim at testing hypotheses and interpreting

their findings causally, need to be planned realistically, pre-registered precisely and explicitly, conducted meticulously and reported comprehensively and transparently. As risks of biases occur at each of these steps, this is not a trivial task. Instead, this is what makes research challenging.

Conducting proper, pre-registered replication studies would be the next sensible step that can be derived from this dissertation's studies. However, considering the questionable validity of endogenous oxytocin concentrations, along with the open question which aspects of oxytocin functioning they reflect, it must be critically questioned whether it is already time to take this next step forward. It rather seems appropriate to take a step back in the translational research process, as the challenges related to the measurements of endogenous oxytocin concentrations cannot be solved by conducting more applied clinical-psychological research, but instead raise issues which can only be resolved by basic scientific research.

Concerning the previously reported findings on the effectiveness of intranasal oxytocin administration as a preventive intervention for PTSD symptom development, it does seem worthwhile to adequately replicate them in individuals with high acute PTSD symptoms, who appear to be the ideal target group (van Zuiden et al., 2017). Although **study 1** did not indicate that sex and hormonal contraception use moderated this intervention's effects, final conclusions about the impact of women's gonadal steroids-related statuses, as determined by their fertility status, menstrual cycle phase or the specific type, dosage and intake phase of hormonal contraception, could not be drawn. Studies which showed that gonadal steroids influence fear and extinction learning (Glover et al., 2012; Milad et al., 2010), PTSD symptoms (Nillni et al., 2015) and interact with oxytocin (**study 7**, Stock et al., 1994) suggest that they might be moderators, which were potentially not considered accurately enough. Therefore, factors that determine women's gonadal steroids-related statuses should be accounted for more precisely in future studies, in order to conduct sex-sensitive translational research and thereby open up new options for personalized interventions (Bolea-Alamanac et al., 2018; Ferretti et al., 2019; Garcia & Delahanty, 2017). Even though this dissertation's studies particularly focused on gonadal steroids, this is neither the only, nor the most important factor that deserves more attention. Just as translational research on PTSD should become fairer with regard to the sexes, it should become fairer with regard to gender, culture, nationality,

ethnicity and other socio-economic factors that might determine individuals' responses to preventive or therapeutic interventions (Asnaani & Hall-Clark, 2017; Christiansen & Berke, 2020; Dixon, Ahles, & Marques, 2016). Consequently, increasing effort should to be put into recruiting, investigating and providing treatment to populations who represent a minority or have previously been neglected (Dixon et al., 2016; A. L. Roberts, Gilman, Breslau, Breslau, & Koenen, 2011).

At the same time, it seems unrealistic to comprehensively consider all these factors as potential moderators of the effects of intranasal oxytocin administration in a single empirical study. This would excessively increase the required number of individuals and take high efforts and expertise to recruit the targeted samples. Again, it becomes clear that the ideal conceptions of translational research need to be balanced with economic interests and priorities of funding agencies. Fortunately, the limitations which single empirical studies are subject to can be overcome by another measure that is being promoted by the Open Science movement (Foster & Deardorff, 2017; Open Science Collaboration, 2012), namely, data sharing. There are several ways by which data can be responsibly shared, for instance by making non-identifiable data available via online repositories such as the Open Science Framework ("OSF"), by fostering collaborations between research groups, such as the ENIGMA (Thompson et al., 2020), or by providing primary data to researchers who conduct (individual patient data) meta-analyses (Ioannidis, 2017). As pre-registrations can also be applied when investigating previously analyzed data (Mertens & Krypotos, 2019), data sharing can even be used to conduct hypotheses-testing research. However, it can only shed new light to questions that cannot be answered by primary studies if the parameters of interest, in this case, the potential gonadal steroids-related and socio-economic moderators of intranasal oxytocin administration, are measured in these primary studies, ideally in a standardized manner. Therefore, the development of standards and guidelines for the measurement of these parameters, their implementation in empirical studies and aggregation in collaborative or meta-analytical research can further advance research on oxytocin as a biomarker for PTSD.

9.5 Conclusion

This dissertation's starting point were reflections on the implementation of a translational research perspective on PTSD, by investigating oxytocin as a potential biomarker for this disorder. PTSD was

considered as an ideal disorder for this purpose. It is the only mental disorder with a known common cause, namely, the traumatic event (Pitman et al., 2012). Furthermore, the psychopathological processes of PTSD symptom development, manifestation and remission correspond to neurocognitive processes of fear, extinction, and safety learning. These processes are well conserved across species and their neurobiological underpinnings are precisely understood (Parsons & Ressler, 2013). However, regarding the overall state of research on biomarkers for PTSD, even though it has now been conducted for several decades (Kandel, 1998; Yehuda et al., 2006), and even though there has been a line of promising new prognostic and diagnostic markers (Galatzer-Levy, Ma, Statnikov, Yehuda, & Shalev, 2017; Liberzon & Abelson, 2016; van Zuiden, Kavelaars, Geuze, Olff, & Heijnen, 2013) and a line of promising new agents for treatment augmentation (Kleine et al., 2013; Stojek et al., 2018), a clear breakthrough has been achieved in neither of the domains.

This suggests that certain translational challenges still need to be resolved before this line of research reaches its goal, that is, improved patient care. Some of these challenges became evident in this dissertation, which investigated oxytocin's involvement along the pathway from traumatic event exposure to PTSD symptom development, manifestation, and remission. In doing so, it applied two distinct methodological approaches to oxytocin functioning, that is, intranasal oxytocin administration and measurements of endogenous oxytocin concentrations.

Concerning intranasal oxytocin administration, it became clear that the exact mechanisms of action by which this intervention led to reduced PTSD symptoms in previous studies (Flanagan et al., 2018; van Zuiden et al., 2017) are not yet understood. They require further, targeted investigation, for instance by using experimental paradigms. Furthermore, the effects of intranasal oxytocin administration depend on a variety of contextual and individual moderators (Bartz, Zaki et al., 2011; Olff et al., 2013; Shamay-Tsoory & Abu-Akel, 2016). They might be one of the reasons underlying the poor replicability of main effects that were reported in previous studies on intranasal oxytocin administration (Lane et al., 2016; Mierop et al., 2020). Thus, moderators of the effects of intranasal oxytocin administration also need further, targeted investigations. Taking a large set of potentially relevant moderators into account, to make translational research fairer and more personalized, while maintaining a reasonable research effort

requires collaborations between individual research groups. In this regard, future research on biomarkers for PTSD can strongly benefit from the ideas promoted by the Open Science movement (Foster & Deardorff, 2017; Open Science Collaboration, 2012). An additional prerequisite for further progress in this line of research is to transfer findings from the large number of previous exploratory, hypothesis-generating studies to methodologically stricter, hypotheses-testing studies.

Concerning endogenous oxytocin concentrations, this dissertation's studies observed that they are highly unstable within individuals and that they strongly depend on the biochemical analysis method. Thereby, this dissertation raised strong concerns regarding the eligibility of endogenous oxytocin concentrations as a PTSD-specific biomarker. Controlling for relevant state- and trait-related confounders, as well as developing more robust parameters that aggregate several single measurements might to some extent address these problems. However, the questionable validity of endogenous oxytocin concentrations cannot merely be resolved by conducting more applied clinical-psychological research. Instead, it shows how in the translational research process, the applied clinical-psychological disciplines critically depend on knowledge from the basic sciences. Given the current state of knowledge, it can be concluded that while PTSD is an ideal disorder for implementing a translational research perspective, endogenous oxytocin concentrations are apparently not an ideal biomarker.

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SUPPLEMENTARY MATERIAL

The following material supplements CHAPTER 1:

SUPPLEMENTARY MATERIAL 1. Fear learning in posttraumatic stress disorder

SUPPLEMENTARY MATERIAL 2. Extinction learning in posttraumatic stress disorder

SUPPLEMENTARY MATERIAL 3. Safety learning in posttraumatic stress disorder

SUPPLEMENTARY MATERIAL 4. The effects of intranasal oxytocin administration on fear and extinction learning in humans

The following material supplements CHAPTER 5:

SUPPLEMENTARY MATERIAL 5. Means and standard deviations of posttraumatic stress disorder symptoms, oxytocin and vasopressin concentrations over time

SUPPLEMENTARY MATERIAL 6. Correlations between posttraumatic stress disorder symptoms, oxytocin and vasopressin concentrations over time: All cases

SUPPLEMENTARY MATERIAL 7. Correlations between posttraumatic stress disorder symptoms, oxytocin and vasopressin concentrations over time: Complete cases

SUPPLEMENTARY MATERIAL 1. Fear learning in posttraumatic stress disorder (PTSD)

Study	Design	Stimulus type	Subjects	Sex	PTSD group	Comparison group(s)	US	CS+/ CS-	Dependent variable	Learning process	Results
Acheson et al. (2015)	C-s	Cue	US American Marines and Navy corpsmen	♂	Individuals with PTSD symptoms (*n*=42)	Healthy individuals (*n*=923); Individuals with anxiety symptoms (*n*=37); Individuals with depression symptoms (*n*=12), all trauma exposed and non-trauma exposed	Air blast to the larynx	Blue or yellow circles or squares	Eyeblink reflex to acoustic startle probes; Subjective anxiety ratings	Acquisition	Deficient differential conditioning (Deficient increase in startle response to the CS+, as compared with the CS-) in individuals with PTSD compared with all comparison groups; No group difference in subjective anxiety ratings
Blechert et al. (2007)	C-s	Cue	Community sample	♀♂	Individuals with PTSD (*n*=36)	Healthy trauma exposed individuals (*n*=21); Non-trauma exposed individuals (*n*=34)	Electric shock	Two Rorschach inkblots	Skin conductance; Subjective valence ratings	Acquisition	Increased skin conductance responses to the CS+ and the CS- in individuals with PTSD compared with non-trauma exposed individuals; No group difference in valence ratings; No group difference between individuals with PTSD and healthy trauma exposed individuals
Garfinkel et al. (2014)	C-s	Cue	Operation Enduring Freedom and Operation Iraqi Freedom US American veterans	♂	Individuals with PTSD (*n*=14)	Healthy trauma exposed individuals (*n*=14)	Electric shock on fingers	Pictures of pink or blue lamps	Skin conductance	Acquisition	▪ Acquisition: No group difference
Grillon and Morgan (1999)	C-s	Cue	US American Gulf War veterans	♂	Individuals with PTSD (*n*=12)	Healthy trauma exposed individuals (*n*=12)	Electric shock on the wrist	Blue or green lights	Eyeblink reflex to acoustic startle probes	Acquisition; Recall	▪ Acquisition: Deficient differential conditioning in individuals with PTSD ▪ Recall: Increased in healthy trauma-exposed controls in session 1; No group difference in session 2

Study	Design	Stimulus type	Subjects	Sex	PTSD group	Comparison group(s)	US	CS+/ CS-	Dependent variable	Learning process	Results
Grillon and Morgan (1999)	C-s	Context	US American Gulf War veterans	♂	Individuals with PTSD (*n*=12)	Healthy trauma exposed individuals (*n*=12)	Electric shock on the wrist	Placement of electrode	Eyeblink reflex to acoustic startle probes	Recall	No group difference
Guthrie and Bryant (2006)	Prog	Cue	Firefighters	♂	Trauma exposed individuals with different amount of PTSD symptoms up to 24 months after traumatic event exposure (*n*=67)	/	Electric shock on finger	Red, blue, green or yellow circles	Skin conductance; Left corrugator electromyogram	Acquisition	Increased differential conditioning (increased electromyogram responses to the CS+, as compared with the CS-) pretrauma correlated with increased PTSD symptoms after traumatic event exposure; No effect of skin conductance responses pretrauma
Jovanovic et al. (2009)	C-s	Cue	US American veterans (majority of subjects)	♂	Vietnam veterans with PTSD (*n*=27), with high symptoms (*n*=13) and with low symptoms (*n*=14)	Health individuals (*n*=28)	Air blast to the larynx	Green, purple, orange or blue lights	Eyeblink reflex to acoustic startle probes	Acquisition	No group difference
Jovanovic, Norrholm, Blanding, Davis et al. (2010)	C-s	Cue	Community sample	♀♂	Individuals with PTSD (*n*=14); Individuals with PTSD and major depression (*n*=22)	Healthy individuals (*n*=53); Individuals with major depression (*n*=17)	Air blast to the larynx	Blue, black or purple star, triangle or square	Eyeblink reflex to acoustic startle probes	Acquisition	Deficient differential conditioning in individuals with PTSD compared with individuals without PTSD
Jovanovic, Norrholm, Blanding, Phifer et al. (2010)	C-s	Cue	Community sample	♀♂	Individuals with PTSD (*n*=29)	Healthy trauma exposed individuals (*n*=61)	Air blast to the larynx	Blue, black or purple star, triangle or square	Eyeblink reflex to acoustic startle probes	Acquisition	Deficient differential conditioning in individuals with PTSD
Milad et al. (2008)	C-s	Cue	US American monozygotic twins, discordant for combat exposure in Vietnam War	♂	Individuals with PTSD (*n*=7)	Healthy trauma exposed individuals (*n*=7); Non-trauma exposed individuals (*n*=14)	Electric shock on fingers	Pictures of pink or blue lamps	Skin conductance	Acquisition	No group difference

Study	Design	Stimulus type	Subjects	Sex	PTSD group	Comparison group(s)	US	CS+/ CS-	Dependent variable	Learning process	Results
Milad et al. (2009)	C-s	Cue	Community sample	♀♂	Individuals with PTSD (*n*=16)	Healthy trauma exposed individuals (*n* = 15)	Electric shock on fingers	Pictures of pink or blue lamps	Skin conductance	Acquisition	No group difference
Norrholm et al. (2011)	C-s	Cue	Community sample	♀♂	Individuals with PTSD (*n*=49)	Healthy trauma exposed individuals (*n*=78)	Air blast to the larynx	Differently colored shapes	Eyeblink reflex to acoustic startle probes	Acquisition	Increased startle responses to the CS+ and the CS- in individuals with PTSD
Orr et al. (2000)	C-s	Cue	Community sample	♀♂	Individuals with PTSD (*n*=15)	Healthy trauma exposed individuals (*n*=18)	Electric shock on fingers	Red, blue, white or green circles	Skin conductance; Heart rate; Left corrugator electromyogram	Acquisition	Increased differential conditioning in individuals with PTSD
Peri et al. (2000)	C-s	Cue	Community sample	♀♂	Individuals with PTSD (*n*=36)	Healthy trauma exposed individuals (*n*=20); Non-trauma exposed individuals (*n*=30)	Bursts of white noise	Differently colored slides	Skin conductance; Heart rare	Acquisition	No group difference
Sijbrandij et al. (2013)	C-s	Cue	Dutch Royal Army soldiers	♀♂	Individuals who were deployed to Afghanistan and experienced war-zone related stressors with different amounts of posttraumatic stress symptoms 2 months after deployment (*n*=144)	/	Electric shock on fingers	Blue, black or purple star, triangle or square	Eyeblink reflex to acoustic startle probes	Acquisition	No effect of startle response to the CS+ and differential conditioning (startle responses to the CS+, as compared with the CS-) on posttraumatic stress symptoms
Sijbrandij et al. (2013)	Prog	Cue	Dutch Royal Army soldiers	♀♂	Individuals who were deployed to Afghanistan and experienced war-zone related stressors 2 months before with different amounts of	/	Electric shock on fingers	Blue, black or purple star, triangle or square	Eyeblink reflex to acoustic startle probes	Acquisition	No effect of startle response to the CS+ and differential conditioning (startle responses to the CS+ compared with the CS-) 2 months after deployment on posttraumatic stress

Study	Design	Stimulus type	Subjects	Sex	PTSD group	Comparison group(s)	US	CS+/ CS-	Dependent variable	Learning process	Results
					posttraumatic stress symptoms 9 months after deployment (*n*=144)						symptoms 9 months after deployment
Steiger et al. (2015)	C-s	Cue	Community sample	♀♂	Individuals with PTSD (*n*=12)	Healthy trauma exposed individuals (*n*=14); Non-trauma exposed individuals (*n*=11)	Electric shock on finger	White and light-blue squares	Subjective valence and arousal ratings	Acquisition	No group difference
Steiger et al. (2015)	C-s	Context	Community sample	♀♂	Individuals with PTSD (*n*=12)	Healthy trauma exposed individuals (*n*=14); Non-trauma exposed individuals (*n*=11)	Electric shock on finger	Pictures of different rooms	Subjective valence and arousal ratings	Acquisition; Recall	▪ Acquisition: No group difference ▪ Recall: Increased in individuals with PTSD compared with healthy trauma exposed individuals
Thome et al. (2018)	C-s	Cue	Community sample	♀	Individuals with PTSD (*n*=30)	Healthy trauma exposed individuals (*n*=30); Non-trauma exposed individuals (*n*=30)	Electric shock	Circles of gradually increasing size	Eyeblink reflex to acoustic startle probes	Acquisition; Generalization	▪ Acquisition: Decreased startle responses to the CS+ in individuals with PTSD compared with non-trauma exposed individuals ▪ Generalization: No group difference
Wessa and Flor (2007)	C-s	Cue	Community sample	♀♂	Individuals with PTSD related to the air show disaster in Ramstein, Germany (*n*=14)	Healthy survivors of the air show disaster in Ramstein, Germany (*n*=15); Non-trauma exposed individuals (*n*=15)	Traumatic event-related picture	Circle or rhombus	Skin conductance; Heart rate; Eyeblink reflex to acoustic startle probes; Subjective valence and arousal ratings	Acquisition	Increased differential conditioning in individuals with PTSD and healthy trauma exposed individuals compared with non-trauma exposed individuals (skin conductance, startle responses, negative valence); no group differences in differential conditioning (heart rate, arousal)

Study	Design	Stimulus type	Subjects	Sex	PTSD group	Comparison group(s)	US	CS+/ CS-	Dependent variable	Learning process	Results
											Increased ratings of the CS+ in individuals with PTSD compared with both comparison groups (negative valence, arousal); no group difference (skin conductance, heart rate, startle)
Zuj, Palmer, Hsu et al. (2016)*	C-s	Cue	Community sample	♀♂	Individuals with PTSD (*n*=15)	Healthy trauma exposed individuals (*n*=33); Non-trauma exposed individuals (*n*=22)	Electric shock on hand	Red or blue circles	Skin conductance	Acquisition	No group difference
Zuj, Palmer, Malhi et al. (2017)*	C-s	Cue	Community sample	♀♂	Individuals with high posttraumatic stress symptoms (*n*=18)	Healthy trauma exposed individuals (*n*=33); Non-trauma exposed individuals (*n*=27)	Electric shock on hand	Red or blue circles	Skin conductance	Acquisition	No group difference
Zuj, Palmer, Gray et al. (2017)*	C-s	Cue	Community sample	♀♂	Individuals with PTSD (*n*=21)	Healthy trauma exposed individuals (*n*=33)	Electric shock on hand	Red or blue circles	Skin conductance	Acquisition	No group difference
Zuj et al. (2018)*	C-s	Cue	Community sample	♀♂	Individuals with PTSD (*n*=21)	Healthy trauma exposed individuals (*n*=36); Non-trauma exposed individuals (*n*=27)	Electric shock on hand	Red or blue circles	Skin conductance	Acquisition	Deficient differential conditioning in individuals with PTSD and healthy trauma exposed individuals compared with non-trauma exposed individuals

Note. Overview of experimental studies relating fear learning to PTSD symptoms or comparing this neurocognitive process between individuals with PTSD and healthy (non-)trauma exposed individuals. *Studies' samples overlap; C-s=cross-sectional; Prog=prognostic; US=unconditioned stimulus; CS+= fear context or cue; CS-= neutral context or cue.

SUPPLEMENTARY MATERIAL 2. Extinction learning in posttraumatic stress disorder (PTSD)

Study	Design	Stimulus type	Subjects	Sex	PTSD group	Comparison group(s)	US	CS+/CS-	Dependent variable	Learning process	Results
Acheson et al. (2015)	C-s	Cue	US American Marines and Navy corpsmen	♂	Individuals with PTSD symptoms (n=42)	Healthy individuals (n=923); Individuals with anxiety symptoms (n=37); Individuals with depression symptoms (n=12), all trauma exposed and non-trauma exposed	Air blast to the larynx	Blue or yellow circles or squares	Eyeblink reflex to acoustic startle probes; Subjective anxiety ratings	Acquisition	Increased fear responses to the CS+ in individuals with PTSD symptoms compared with healthy individuals
Blechert et al. (2007)	C-s	Cue	Community sample	♀♂	Individuals with PTSD (n=36)	Healthy trauma exposed individuals (n=21); Non-trauma exposed individuals (n=34)	Electric shock	Two Rorschach inkblots	Skin conductance; Subjective valence ratings	Acquisition	Increased fear responses to the CS+ in individuals with PTSD compared with non-trauma exposed individuals; No difference between individuals with PTSD and healthy trauma exposed individuals
Garfinkel et al. (2014)	C-s	Cue	Operation Enduring Freedom and Operation Iraqi Freedom US American veterans	♂	Individuals with PTSD (n=14)	Healthy trauma exposed individuals (n=14)	Electric shock on fingers	Pictures of pink or blue lamps	Skin conductance	Acquisition; Recall	▪ Acquisition: No group difference ▪ Recall: Decreased (increased fear responses to the CS+) in individuals with PTSD

Study	Design	Stimulus type	Subjects	Sex	PTSD group	Comparison group(s)	US	CS+/CS-	Dependent variable	Learning process	Results
Guthrie and Bryant (2006)	Prog	Cue	Firefighters	♂	Trauma exposed individuals with different amount of PTSD symptoms up to 24 months after traumatic event exposure (n=67)	/	Electric shock on finger	Red, blue, green or yellow circles	Skin conductance; Left corrugator electromyogram	Acquisition	Increased differential conditioning (increased electromyogram responses to the CS+ compared with the CS-) pretrauma correlated with increased PTSD symptoms after traumatic event exposure; No effect of skin conductance responses pretrauma
Lommen et al. (2013)	Prog	Cue	Dutch Royal Army soldiers	♀♂	Individuals who were deployed to Afghanistan and experienced war-zone related stressors with different amounts of posttraumatic stress symptoms 2 months after deployment (n=249)	/	Electric shock on fingers	Pictures of human faces that were evaluated as neutral	US expectancy	Acquisition	Increased fear responses to the CS+ before deployment correlated with increased posttraumatic stress symptoms after deployment
Milad et al. (2008)	C-s	Cue	US American monozygotic twins, discordant for combat exposure in Vietnam War	♂	Individuals with PTSD (n=7)	Healthy trauma exposed individuals (n=7); Non-trauma exposed individuals (n=14)	Electric shock on fingers	Pictures of pink or blue lamps	Skin conductance	Acquisition; Recall	▪ Acquisition: No group difference ▪ Recall: Decreased in individuals with PTSD compared with both comparison groups
Milad et al. (2009)	C-s	Cue	Community sample	♀♂	Individuals with PTSD (n=16)	Healthy trauma exposed individuals (n=15)	Electric shock on fingers	Pictures of pink or blue lamps	Skin conductance	Acquisition; Recall	▪ Acquisition: No group difference ▪ Recall: Decreased in individuals with PTSD

Study	Design	Stimulus type	Subjects	Sex	PTSD group	Comparison group(s)	US	CS+/CS-	Dependent variable	Learning process	Results
Norrholm et al. (2011)	C-s	Cue	Community sample	♀♂	Individuals with PTSD (n=49)	Healthy trauma exposed individuals (n=78)	Air blast to the larynx	Differently colored shapes	Eyeblink reflex to acoustic startle probes	Acquisition	Increased startle responses to the CS+ in individuals with PTSD; Increased startle responses to the CS+ and the CS- in individuals with PTSD during early and mid extinction phase; No group difference during late extinction phase
Orr et al. (2000)	C-s	Cue	Community sample	♀♂	Individuals with PTSD (n=15)	Healthy trauma exposed individuals (n=18)	Electric shock on fingers	Red, blue, white or green circles	Skin conductance; Heart rate; Left corrugator electromyogram	Acquisition	Increased differential conditioning (skin conductance) in individuals with PTSD; Increased skin conductance responses to the CS+ and the CS- in individuals with PTSD; No group difference in heart rate and electromyogram responses
Peri et al. (2000)	C-s	Cue	Community sample	♀♂	Individuals with PTSD (n=36)	Healthy trauma exposed individuals (n=20); Non-trauma exposed individuals (n=30)	Bursts of white noise	Differently colored slides	Skin conductance; Heart rare	Acquisition	Increased skin conductance responses to the CS+ and the CS- and increased heart rate responses to the CS+ in individuals with PTSD compared with both comparison groups
Steiger et al. (2015)	C-s	Cue	Community sample	♀♂	Individuals with PTSD (n=12)	Healthy trauma exposed individuals (n=14); Non-trauma exposed individuals (n=11)	Electric shock on finger	White and light-blue squares	Subjective valence and arousal ratings	Acquisition; Recall	▪ Acquisition: Increased fear responses to the CS+ in individuals with PTSD compared with both comparison groups ▪ Recall: Reduced in individuals with PTSD compared with both comparison groups

Study	Design	Stimulus type	Subjects	Sex	PTSD group	Comparison group(s)	US	CS+/CS-	Dependent variable	Learning process	Results
Steiger et al. (2015)	C-s	Context	Community sample	♀♂	Individuals with PTSD (n=12)	Healthy trauma exposed individuals (n=14); Non-trauma exposed individuals (n=11)	Electric shock on finger	Pictures of different rooms	Subjective valence and arousal ratings	Acquisition; Recall	▪ Acquisition: Increased arousal ratings of the CS+ in individuals with PTSD compared with non-trauma exposed individuals; no group difference in valence ratings. ▪ Recall: Reduced in individuals with PTSD compared with both comparison groups (valence ratings) and non-trauma exposed individuals (arousal ratings)
Wicking et al. (2016)	C-s	Cue	Community sample	♀♂	Individuals with PTSD (n=18)	Healthy trauma exposed individuals (n=18); Non-trauma exposed individuals (n=18)	Electric shock on finger	Square or rhombus	Skin conductance; Subjective valence, unpleasantness and intensity ratings	Recall	Increased differential conditioning (skin conductance) during early extinction recall in individuals with PTSD compared with both comparison groups; No group difference in subjective ratings
Wessa and Flor (2007)	C-s	Cue	Community sample	♀♂	Individuals with PTSD related to the air show disaster in Ramstein, Germany (n=14)	Healthy survivors of the air show disaster in Ramstein, Germany (n=15); Non-trauma exposed individuals (n=15)	Traumatic event-related picture	Circle or rhombus	Skin conductance; Heart rate; Eyeblink reflex to acoustic startle probes; Subjective valence and arousal ratings	Acquisition	Increased differential conditioning in individuals with PTSD compared with non-trauma exposed individuals (negative valence) and compared with both comparison groups (skin conductance, arousal); No group difference in heart rate and startle

Study	Design	Stimulus type	Subjects	Sex	PTSD group	Comparison group(s)	US	CS+/CS-	Dependent variable	Learning process	Results
Zuj, Palmer, Hsu et al. (2016)*	C-s	Cue	Community sample	♀♂	Individuals with PTSD (n=15)	Healthy trauma exposed individuals (n=33); Non-trauma exposed individuals (n=22)	Electric shock on hand	Red or blue circles	Skin conductance	Acquisition	No group difference in the early extinction phase; Increased fear responses to CS+ and CS- in individuals with PTSD compared with both comparison groups in the late extinction phase
Zuj, Palmer, Gray et al. (2017)*	C-s	Cue	Community sample	♀♂	Individuals with PTSD (n=21)	Healthy trauma exposed individuals (n=33)	Electric shock on hand	Red or blue circles	Skin conductance	Acquisition	Slower in individuals with PTSD in the early extinction phase; No group difference in late extinction phase
Zuj, Palmer, Malhi et al. (2017)*	C-s	Cue	Community sample	♀♂	Individuals with high posttraumatic stress symptoms (n=18)	Healthy trauma exposed individuals (n=33); Non-trauma exposed individuals (n=27)	Electric shock on hand	Red or blue circles	Skin conductance	Acquisition	Slower in individuals with high posttraumatic stress symptoms compared with both comparison groups in the early extinction phase; No group difference in the late extinction phase
Zuj et al. (2018)*	C-s	Cue	Community sample	♀♂	Individuals with PTSD (n=21)	Healthy trauma exposed individuals (n=36); Non-trauma exposed individuals (n=27)	Electric shock on hand	Red or blue circles	Skin conductance	Acquisition	Slower in individuals with PTSD compared with both comparison groups in the early extinction phase; No group difference in late extinction phase

Note. Overview of experimental studies relating extinction learning to PTSD symptoms or comparing this neurocognitive process between individuals with PTSD and healthy (non-)trauma exposed individuals. *Studies' samples overlap; C-s=cross-sectional; Prog=prognostic; US=unconditioned stimulus; CS+= fear context or cue; CS-=neutral context or cue.

SUPPLEMENTARY MATERIAL 3. Safety learning in posttraumatic stress disorder (PTSD)

Study	Design	Learning paradigm	Subjects	Sex	PTSD group	Comparison group(s)	US	CS+/ CS-	Safety signal	Dependent variable	Learning process	Results
Garfinkel et al. (2014)	C-s	Context	Operation Enduring Freedom and Operation Iraqi Freedom US American veterans	♂	Individuals with PTSD (*n*=14)	Healthy trauma exposed individuals (*n*=14)	Electric shock on fingers	Pictures of pink or blue lamps	Pictures of different rooms	Skin conductance	Acquisition; Recall	▪ Acquisition: No group difference ▪ Recall: Decreased in individuals with PTSD
Jovanovic et al. (2009)	C-s	Cue	US American Veterans (majority of subjects)	♂	Vietnam veterans with PTSD (*n*=27), with high symptoms (*n*=13) and with low symptoms (*n*=14)	Healthy individuals (*n*=28)	Air blast to the larynx	Green, purple, orange or blue lights	Green, purple, orange or blue lights	Eyeblink reflex to acoustic startle probes	Acquisition; Recall	▪ Acquisition: Increased startle responses to safety signal in individuals with PTSD and high symptoms compared with both comparison groups ▪ Recall: Decreased in individuals with PTSD and high symptoms compared with both comparison groups
Jovanovic, Norrholm, Blanding, Davis et al. (2010)	C-s	Cue	Community sample	♀♂	Individuals with PTSD (*n*=14); Individuals with PTSD and major depression (*n*=22)	Healthy individuals (*n*=53); Individuals with major depression (*n*=17)	Air blast to the larynx	Blue, black or purple star, triangle or square	Blue, black or purple star, triangle or square	Eyeblink reflex to acoustic startle probes	Acquisition; Recall	▪ Acquisition: Increased startle responses to safety signal in individuals with PTSD compared with individuals without PTSD ▪ Recall: Decreased in individuals with PTSD compared with individuals without PTSD
Jovanovic, Norrholm, Blanding, Phifer et al. (2010)	C-s	Cue	Community sample	♀♂	Individuals with PTSD (*n*=29)	Healthy trauma exposed individuals (*n*=61)	Air blast to the larynx	Blue, black or purple star, triangle or square	Blue, black or purple star, triangle or square	Eyeblink reflex to acoustic startle probes	Acquisition; Recall	▪ Acquisition: Increased startle responses to safety signal in individuals with PTSD ▪ Recall: Decreased in individuals with PTSD

Study	Design	Learning paradigm	Subjects	Sex	PTSD group	Comparison group(s)	US	CS+/ CS-	Safety signal	Dependent variable	Learning process	Results
Milad et al. (2008)	C-s	Context	US American monozygotic twins, discordant for combat exposure in Vietnam War	♂	Individuals with PTSD (*n*=7)	Healthy trauma exposed individuals (*n*=7); Non-trauma exposed individuals (*n*=14)	Electric shock on fingers	Pictures of pink or blue lamps	Pictures of different rooms	Skin conductance	Acquisition; Recall	▪ Acquisition: No group difference ▪ Recall: Decreased in individuals with PTSD compared with both comparison groups
Milad et al. (2009)	C-s	Context	Community sample	♀♂	Individuals with PTSD (*n*=16)	Healthy trauma exposed individuals (*n*=15)	Electric shock on fingers	Pictures of pink or blue lamps	Pictures of different rooms	Skin conductance	Acquisition; Recall	▪ Acquisition: No group difference ▪ Recall: Decreased in individuals with PTSD

Note. Overview of experimental studies relating safety learning to PTSD symptoms or comparing this neurocognitive process between individuals with PTSD and healthy (non-)trauma exposed individuals. C-s=cross-sectional; Prog=prognostic; US=unconditioned stimulus; CS+= fear context or cue; CS-=neutral context or cue.

SUPPLEMENTARY MATERIAL 4. The effects of intranasal oxytocin administration on fear and extinction learning in humans.

Study	Randomization	Learning paradigm	Subjects	Oxytocin administration	US	CS+/ CS-	Dependent variable	Learning process	Results
Acheson et al. (2013)	Semi-randomized (balancing for fear acquisition and sex)	Cue	Healthy men and women (*n*=44)	24IU immediately after fear learning; 45 min before extinction learning	Electric shock on wrist	Blue or yellow circles	Eyeblink reflex to acoustic startle probes; Subjective anxiety ratings	Extinction acquisition; Extinction recall	▪ Extinction acquisition: Higher startle responses to the CS+ in oxytocin condition during early extinction, no group differences in startle responses during late extinction; no group differences in subjective anxiety ratings ▪ Extinction recall: Increased (decreased startle responses to the CS+) in oxytocin condition; no group differences in subjective anxiety ratings
Cavalli et al. (2017)	Randomized	Cue	Healthy women and men (*n*=52)	24IU 45 min before fear learning	Loud scream	Square or diamond	Skin conductance; Subjective valence and arousal ratings	Fear acquisition; Extinction acquisition	▪ Fear acquisition: No group differences in subjective arousal during early acquisition; Increased subjective arousal ratings of the CS+ and the CS- during late acquisition in oxytocin condition; No group differences in subjective valence and skin conductance responses ▪ Extinction acquisition: No group differences
Cavalli et al. (2017)	Randomized	Context	Healthy men and women (*n*=52)	24IU 45 min before fear learning	Loud board scratch	Orange or blue spatial contexts	Skin conductance; Subjective valence and arousal ratings	Fear acquisition; Extinction acquisition	▪ Fear acquisition: No group differences in subjective arousal during early acquisition; Increased subjective arousal ratings of the CS+ and the CS- during late acquisition in oxytocin condition; No group differences in subjective valence and skin conductance responses ▪ Extinction acquisition: No group differences
Eckstein et al. (2015)	Randomized	Cue	Healthy men (*n*=36)	24IU immediately after fear learning; 30 min before extinction learning	Electric shock	Different neutral faces or houses	Skin conductance	Extinction acquisition	Increased fear responses to the CS+ and the CS- in oxytocin condition during early extinction; decreased fear responses to the CS+ and the CS- in oxytocin condition during late extinction
Eckstein et al. (2016)	Randomized	Cue	Healthy men (*n*=37)	24IU 30 min before fear learning	Electric shock	Different neutral faces or houses	Skin conductance	Fear acquisition	Increased fear responses to the CS+ and the CS- in oxytocin condition during early and late acquisition; Increased fear responses to the CS+ in oxytocin condition during late acquisition

Study	Randomization	Learning paradigm	Subjects	Oxytocin administration	US	CS+/ CS-	Dependent variable	Learning process	Results
E. Hoge et al. (2019)	Randomized	Cue	Healthy women and men (*n*=30)	30IU immediately after fear learning, one day before fear recall	Electric shock on fingers	Yellow circle or white square	Skin conductance	Fear recall	No group differences
Hu et al. (2019)	Semi-randomized (balancing for sex)	Cue	Healthy men and women (*n*=61)	40IU one day after fear learning and one day before extinction learning	Electric shock on wrist	Purple or blue cylinders	Skin conductance	Extinction acquisition	Decreased differential conditioning (no difference in skin conductance responses to the CS+ and the CS-) in oxytocin condition during late extinction, if individuals were exposed to a non-reinforced CS+ immediately before intranasal oxytocin administration
Petrovic et al. (2008)	Randomized	Cue	Healthy men (n=30)	32IU immediately after fear learning; 45 min before extinction learning	Electric shock	Faces of different identities, with direct or averted gaze	Skin conductance; Subjective valence ratings	Extinction acquisition	Subjective valence ratings: Decreased differential conditioning in oxytocin condition; No group differences in skin conductance responses

Note. All studies were cross-sectional, oxytocin or placebo were administered in a double-blind manner and only healthy subjects were investigated. The effects of intranasal oxytocin administration on safety learning in humans have not yet been studied in humans and therefore, no corresponding studies are listed here. US=unconditioned stimulus; CS+= fear context or cue; CS-=neutral context or cue.

SUPPLEMENTARY MATERIAL 5. Means and standard deviations of posttraumatic stress disorder (PTSD) symptoms, oxytocin and vasopressin concentrations over time.

Variable	T	Complete cases					All cases			
		n	*M*	*SD*	Min	Max	*n*	*M*	*SD*	Min	Max
CAPS	T1	16	32.56	15.79	15.00	62.00	37	33.54	14.88	6.00	62.00
	T1-T2	16	-1.12	8.85	-18.00	16	21	-1.76	9.69	-20.00	16.00
	T2	16	31.44	14.77	4.00	53.00	21	30.95	15.88	4.00	53.00
	T2-T3	16	-2.00	6.95	-17.00	8.00	19	-2.16	6.99	-17.00	8.00
	T3	16	29.44	17.81	2.00	61.00	19	27.74	17.66	2.00	61.00
Oxytocin	T1	16	0.73	0.09	0.58	0.84	35	0.71	0.09	0.53	0.85
	T1-T2	16	-0.03	0.10	-0.18	0.13	19	-0.01	0.10	-0.18	0.15
	T2	16	0.70	0.05	0.62	0.78	20	0.71	0.06	0.62	0.85
	T2-T3	16	-0.02	0.10	-0.15	0.27	17	-0.02	0.10	-0.15	0.27
	T3	16	0.68	0.10	0.59	0.96	18	0.68	0.09	0.59	0.96
Vasopressin	T1	16	3.47	0.58	2.45	4.41	36	3.59	0.60	2.45	4.66
	T1-T2	16	-0.24	0.57	-1.55	0.87	21	-0.32	0.57	-1.55	0.87
	T2	16	3.23	0.50	2.22	3.96	21	3.27	0.48	2.22	3.96
	T2-T3	16	0.17	0.65	-0.86	1.35	19	0.17	0.61	-0.86	1.35
	T3	16	3.40	0.43	2.81	4.13	19	3.43	0.41	2.81	4.13

Note. Cases were considered as complete if Clinician-Administered PTSD Scale for DSM-5 (CAPS) scores, oxytocin and vasopressin data were available for the assessments (T) T1, T2 and T3, respectively. Change scores (T1-T2 and T2-T3) were calculated by subtracting values of one assessment (T1 and T2, respectively) from values of the following assessment (T2 and T3, respectively). Consequently, positive change scores indicate an increase, negative change scores indicate a decrease in the respective parameter.

SUPPLEMENTARY MATERIAL 6. Correlations between posttraumatic stress disorder (PTSD) symptoms, oxytocin and vasopressin concentrations over time: All cases.

		1. CAPS					2. Oxytocin					3. Vasopressin				
Variable		**1.T1**	**1.T1-T2**	**1.T2**	**1.T2-T3**	**1.T3**	**2.T1**	**2.T1-T2**	**2.T2**	**2.T2-T3**	**2.T3**	**3.T1**	**3-T1-T2**	**3.T2**	**3.T2-T3**	**3.T3**
1. CAPS	**T1**	1.00 *n* = 37	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	T1-T2	-.32 (.16) *n* = 21	1.00 *n* = 21	-	-	-	-	-	-	-	-	-	-	-	-	-
	T2	.81 (<.01) *n* = 21	.29 (.20) *n* = 21	1.00 *n* = 21	-	-	-	-	-	-	-	-	-	-	-	-
	T2-T3	.14 (.56) *n* = 19	-.27 (.26) *n* = 19	-.02 (.94) *n* = 19	1.00 *n* = 19	-	-	-	-	-	-	-	-	-	-	-
	T3	.83 (<.01) *n* = 19	.23 (.33) *n* = 19	.92 (<.01) *n* = 19	.38 (.11) *n* = 19	1.00 *n* = 19	-	-	-	-	-	-	-	-	-	-
2. Oxytocin	**T1**	-.22 (.21) *n* = 35	.04 (.85) *n* = 20	-.27 (.25) *n* = 20	-.04 (.86) *n* = 18	-.22 (.37) *n* = 18	1.00 *n* = 35	-	-	-	-	-	-	-	-	-
	T1-T2	.30 (.20) *n* = 19	-.03 (.89) *n* = 19	.30 (.20) *n* = 19	.28 (.28) *n* = 17	.31 (.23) *n* = 17	-.81 (<.01) *n* = 19	1.00 *n* = 19	-	-	-	-	-	-	-	-
	T2	.18 (.45) *n* = 20	.09 (.72) *n* = 20	.24 (.31) *n* = 20	.28 (.27) *n* = 18	.27 (.27) *n* = 18	-.02 (.95) *n* = 19	.60 (.01) *n* = 19	1.00 *n* = 20	-	-	-	-	-	-	-
	T2-T3	-.16 (.53) *n* = 17	-.04 (.87) *n* = 17	-.19 (.46) *n* = 17	-.30 (.25) *n* = 17	-.29 (.26) *n* = 17	-.00 (.99) *n* = 16	-.19 (.49) *n* = 16	-.39 (.12) *n* = 17	1.00 *n* = 17	-	-	-	-	-	-
	T3	-.11 (.66) *n* = 18	-.03 (.89) *n* = 18	-.13 (.60) *n* = 18	-.13 (.60) *n* = 18	-.17 (.50) *n* = 18	.03 (.91) *n* = 17	.09 (.75) *n* = 16	.17 (.52) *n* = 17	.84 (<.01) *n* = 17	1.00 *n* = 18	-	-	-	-	-
3. Vasopressin	**T1**	.07 (.66) *n* = 36	.16 (.49) *n* = 21	.21 (.36) *n* = 21	.12 (.63) *n* = 19	.21 (.40) *n* = 19	.25 (.15) *n* = 35	-.06 (.79) *n* = 19	.11 (.64) *n* = 20	-.09 (.72) *n* = 17	-.05 (.83) *n* = 18	1.00 *n* = 36	-	-	-	-
	T1-T2	-.02 (.94) *n* = 21	-.17 (.46) *n* = 21	-.21 (.60) *n* = 21	-.09 (.70) *n* = 19	-.14 (.55) *n* = 19	-.08 (.73) *n* = 20	-.02 (.92) *n* = 19	-.14 (.56) *n* = 20	.15 (.57) *n* = 17	-.02 (.95) *n* = 18	.64 (<.01) *n* = 21	1.00 *n* = 21	-	-	-
	T2	.11 (.63) *n* = 21	-.01 (.96) *n* = 21	.10 (.66) *n* = 21	.03 (.90) *n* = 19	.08 (.74) *n* = 19	.07 (.75) *n* = 20	-.10 (.69) *n* = 19	-.03 (.89) *n* = 20	.06 (.81) *n* = 17	-.09 (.73) *n* = 18	.41 (.06) *n* = 21	.44 (.04) *n* = 21	1.00 n = 21	-	-
	T2-T3	-.19 (.43) *n* = 19	.13 (.59) *n* = 19	-.11 (.65) *n* = 19	-.16 (.51) *n* = 19	-.17 (.50) *n* = 19	.03 (.90) *n* = 18	.12 (.65) *n* = 17	.29 (.25) *n* = 18	-.34 (.18) *n* = 17	-.20 (.43) *n* = 18	-.09 (.71) *n* = 19	.53 (.02) *n* = 19	-.74 (<.01) *n* = 19	1.00 *n* = 19	-
	T3	-.10 (.69) *n* = 19	.02 (.92) *n* = 19	-.08 (.75) *n* = 19	-.20 (.40) *n* = 19	-.15 (.53) *n* = 19	.12 (.63) *n* = 18	-.09 (.73) *n* = 17	.12 (.65) *n* = 18	-.43 (.09) *n* = 17	-.39 (.11) *n* = 18	.37 (.12) *n* = 19	-.33 (.16) *n* = 19	.06 (.81) *n* = 19	.63 (<.01) *n* = 19	1.00 *n* = 19

Note. Table shows *r* (*p*). Change scores (T1-T2 and T2-T3) were calculated by subtracting values of one assessment (T1 and T2, respectively) from values of the following assessment (T2 and T3, respectively). Consequently, positive change scores indicate an increase, negative change scores indicate a decrease in the respective parameter. CAPS = Clinician-Administered PTSD Scale for DSM-5.

SUPPLEMENTARY MATERIAL 7. Correlations between posttraumatic stress disorder (PTSD) symptoms, oxytocin and vasopressin concentrations over time: Complete cases.

		1. CAPS					2. Oxytocin					3. Vasopressin				
Variable		**1.T1**	**1.T1-T2**	**1.T2**	**1.T2-T3**	**1.T3**	**2.T1**	**2.T1-T2**	**2.T2**	**2.T2-T3**	**2.T3**	**3.T1**	**3-T1-T2**	**3.T2**	**3.T2-T3**	**3.T3**
1. CAPS	**T1**	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	T1-T2	-.39 (.13)	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-
	T2	.83 (<.01)	.18 (.50)	1.00	-	-	-	-	-	-	-	-	-	-	-	-
	T2-T3	.28 (.29)	-.09 (.72)	.25 (.36)	1.00	-	-	-	-	-	-	-	-	-	-	-
	T3	.80 (<.01)	.11 (.68)	.93 (<.01)	.59 (.01)	1.00	-	-	-	-	-	-	-	-	-	-
2. Oxytocin	**T1**	-.19 (.47)	-.07 (.79)	-.25 (.35)	-.05 (.86)	-.23 (.40)	1.00	-	-	-	-	-	-	-	-	-
	T1-T2	.23 (.38)	.02 (.95)	.26 (.33)	.28 (.30)	.33 (.22)	-.85 (<.01)	1.00	-	-	-	-	-	-	-	-
	T2	.12 (.64)	-.08 (.76)	.08 (.76)	.44 (.08)	.24 (.36)	.04 (.89)	.50 (<.05)	1.00	-	-	-	-	-	-	-
	T2-T3	-.15 (.58)	.05 (.86)	-.13 (.62)	-.41 (.11)	-.27 (.31)	-.00 (.99)	-.19 (.49)	-.35 (.18)	1.00	-	-	-	-	-	-
	T3	-.09 (.75)	.00 (.99)	-.09 (.73)	-.18(.50)	-.15 (.58)	.02 (.95)	.087 (.75)	.19 (.47)	.85 (<.01)	1.00	-	-	-	-	-
3. Vasopressin	**T1**	.11 (.68)	.25 (.36)	.27 (.32)	.22 (.42)	.30 (.25)	.22 (.41)	-.21 (.43)	-.05 (.86)	-.03 (.92)	-.05 (.84)	1.00	-	-	-	-
	T1-T2	.08 (.77)	-38 (.14)	-.14 (.59)	-.19 (.49)	-.19 (.47)	-.14 (.60)	.01 (.97)	-.22 (.42)	.10 (.70)	-.01 (.97)	-.63 (<.01)	1.00	-	-	-
	T2	.22 (.41)	-.15 (.57)	.14 (.60)	.04 (.89)	.13 (.62)	.09 (.74)	-.24 (.37)	-.30 (.25)	.09 (.74)	-.08 (.78)	.44 (.08)	.42 (.10)	1.00	-	-
	T2-T3	-.25 (.34)	.07 (.80)	-.23 (.39)	-.11 (.67)	-.23 (.38)	.02 (.93)	.12 (.66)	.26 (.32)	-.33 (.22)	-.19 (.47)	-.12 (.67)	-.54 (.03)	-.75 (<.01)	1.00	-
	T3	-.13 (.63)	-.07 (.78)	-.18 (.63)	-.13 (.63)	-.20 (.45)	.14 (.61)	-.09 (.73)	.05 (.85)	-.39 (.13)	-.38 (.14)	.34 (.20)	-.33 (.21)	.01 (.98)	.65 (<.01)	1.00

Note. Table shows *r* (*p*). Cases were considered as complete if Clinician-Administered PTSD Scale for DSM-5 (CAPS) scores, oxytocin and vasopressin data were available for T1, T2 and T3, respectively. 16 cases were considered as complete. Change scores (T1-T2 and T2-T3) were calculated by subtracting values of one assessment (T1 and T2, respectively) from values of the following assessment (T2 and T3, respectively). Consequently, positive change scores indicate an increase, negative change scores indicate a decrease in the respective parameter.

Eigenständigkeitserklärung

Hiermit versichere ich, dass ich die vorgelegte Arbeit selbstständig verfasst und keine anderen als die angegebenen Hilfsmittel verwendet habe. Die Arbeit ist in keinem früheren Promotionsverfahren angenommen oder abgelehnt worden.

Berlin, 2020

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