Fachbereich Erziehungswissenschaft und Psychologie der Freien Universität Berlin

The role of mineralocorticoid receptors (MR) and N-methyl-D-aspartate receptors (NMDA-R) in steroid hormone secretion and social cognition in major depressive disorder

Dissertation
zur Erlangung des akademischen Grades

Doktor der Naturwissenschaften (Dr. rer. nat.)

vorgelegt von

M. Sc. Psych. Jan Nowacki

Berlin, 2022

Erstgutachterin (first reviewer):

Prof. Dr. Katja Wingenfeld

Zweitgutachter (second reviewer):

Prof. Dr. Malek Bajbouj

Tag der Disputation (day of thesis defense):

31.05.2022

ACKNOWLEDGEMENTS

For reasons of data protection, the acknowledgements are not published in this version of the dissertation.

Table of contents

English Summary

In reaction to stress, activation of the hypothalamic-pituitary-adrenal (HPA) axis leads to the secretion of the steroid hormone cortisol which binds upon mineralocorticoid (MR) and glucocorticoid receptors (GR). Patients with major depressive disorder (MDD) suffer from altered MR and GR functioning and there is evidence that this might contribute to alterations in steroid hormone systems (cortisol, aldosterone, DHEA-S) and to cognitive deficits that have been found in MDD. Interestingly, MR stimulation decreases cortisol secretion and enhances both memory and executive functioning in healthy individuals and patients with MDD. The current research project examined whether MR stimulation affects other steroid hormones and whether the beneficial effects of MR stimulation on cognition are extendable to processes of social cognition in MDD. Furthermore, MR stimulation leads to N-methyl-D-aspartate receptor (NMDA-R) mediated glutamatergic signal transmission and NMDA-R stimulation has cognitive-enhancing effects. Therefore, the current research project examined whether simultaneous NMDA-R stimulation might enhance the effects of MR stimulation in MDD. The central research question was: *What is the effect of MR and NMDA-R stimulation on steroid hormone secretion and social cognition in healthy individuals and patients with MDD?*

One hundred and sixteen MDD patients and 116 healthy individuals matched in age, sex, and education years were randomly assigned to one treatment condition: no stimulation (placebo + placebo), MR stimulation (0.4 mg fludrocortisone + placebo), NMDA-R stimulation (placebo + 250 mg D-cycloserine, DCS) and MR/NMDA-R stimulation (both drugs). Salivary steroid hormone concentrations (cortisol, aldosterone, DHEA-S) were assessed hourly, and participants conducted social cognition tasks to measure cognitive empathy (ability to understand another person's emotions), emotional empathy (ability to empathize with another person's emotions), recognition of emotional facial expressions, and selective attention to emotional stimuli.

The main observations are that: (1) separate MR stimulation decreases cortisol concentrations in healthy individuals and patients with MDD, (2) separate MR stimulation enhances cognitive empathy in both groups but has no effect on the other examined social cognitive processes, (3) simultaneous MR and NMDA-R stimulation has no effect on steroid hormone secretion and social cognition, and (4) separate NMDA-R stimulation decreases cognitive empathy in MDD patients and increases emotion recognition in both groups.

In conclusion, the current research project confirms the important role of MR in the psycho-neuro-endocrinological stress response in healthy individuals and patients with MDD. The observations emphasize that the beneficial effects of MR stimulation on cognition might be partially extendable to social cognition in healthy individuals and patients with MDD. Evidently, these cognitive-enhancing effects are not improvable by simultaneous NMDA-R stimulation, yet NMDA-R appear to be involved in social cognitive processes. Thus, the current research project emphasizes an involvement of MR and NMDA-R in social cognition in healthy individuals and patients with MDD. Future research should examine whether MR and NMDA-R might serve as potential treatment targets to improve (social) cognition in MDD.

Deutsche Zusammenfassung (German Summary)

Stress aktiviert die Hypothalamus-Hypophysen-Nebennierenrinden Achse (engl. HPA axis). Dies führt zur Ausschüttung von Cortisol, welches am Mineralocorticoid (MR) und Glucocorticoid Rezeptor (GR) bindet. Patienten mit einer Majoren Depression (engl. MDD) zeigen eine veränderte MR und GR Funktion. Es gibt Hinweise darauf, dass diese Veränderungen im Zusammenhang mit Veränderungen im Steroidhormonsystem (Cortisol, Aldosteron, DHEA-S) und Veränderungen kognitiver Fähigkeiten stehen, welche auch bei MDD auftreten. Interessanterweise führt eine MR Stimulation zu einer verringerten Cortisolsekretion und verbesserten Gedächtnisfunktion und Exekutivfunktionen bei Gesunden und Patienten mit MDD. Das vorliegende Dissertationsprojekt untersuchte, inwiefern eine MR Stimulation die Ausschüttung anderer Steroidhormone beeinflusst und inwiefern die vorteilhaften Effekte einer MR Stimulation hinsichtlich Kognition auf soziale Kognition übertragbar sind. Eine Aktivierung des MR führt zu einer N-Methyl-D-Aspartat Rezeptor (NMDA-R) mediierten glutamatergen Signalübertragung und eine Aktivierung des NMDA-R zu einer Verbesserung kognitiver Fähigkeiten. Daher untersuchte die vorliegen Forschungsarbeit außerdem, ob die Effekte einer MR Stimulation durch gleichzeitige NMDA-R Stimulation verbessert werden können. Die zentrale Forschungsfrage lautete: *Was ist der Effekt einer MR und NMDA-R Stimulation auf die Steroidhormonsekretion und soziale Kognition bei Gesunden und Patienten mit MDD?*

Insgesamt wurden 116 MDD Patienten und 116 Gesunde, vergleichbar hinsichtlich Alter, Geschlecht und Bildungsjahre, randomisiert einer Behandlungsbedingung zugewiesen: keine Stimulation (Placebo + Placebo), MR Stimulation (0.4 mg fludrocortisone + Placebo), NMDA-R Stimulation (Placebo + 250 mg D-cycloserine, DCS) und MR/NMDA-R Stimulation (beide Präparate). Steroidhormonkonzentrationen (Cortisol, Aldosteron, DHEA-S) wurden stündlich im Speichel gemessen und Teilnehmer führten sozial kognitive Aufgaben durch, um kognitive Empathie (Fähigkeit die Emotionen einer anderen Person zu verstehen), emotionale Empathie (Fähigkeit mit einer anderen Person mitzufühlen), Emotionserkennung emotionaler Gesichter und selektive Aufmerksamkeit hinsichtlich emotionaler Stimuli zu messen.

Die zentralen Beobachtungen waren, dass (1) eine einfache MR Stimulation die Cortisolkonzentrationen bei Gesunden und Patienten mit MDD verringerte, dass (2) eine einfache MR Stimulation die kognitive Empathie bei beiden Gruppen erhöhte, jedoch keinen Einfluss auf die anderen untersuchten sozial kognitiven Prozesse hatte, dass (3)

gleichzeitige MR und NMDA-R Stimulation keinen Effekt auf die Steroidhormonsekretion und soziale Kognition hatte und dass (4) eine einfache NMDA-R Stimulation die kognitive Empathie bei MDD Patienten verringerte und zu einer verbesserten Emotionserkennung bei beiden Gruppen führte.

Zusammengefasst bestätigt die vorliegende Forschungsarbeit, dass MR eine bedeutende Rolle in der psycho-neuro-endokrinen Stressreaktion bei Gesunden und Patienten mit MDD spielen. Die Beobachtungen machen deutlich, dass die vorteilhaften Effekte einer MR Stimulation hinsichtlich Kognition auf soziale Kognition bei Gesunden und Patienten mit MDD partiell übertragbar sind. Anscheinend können diese vorteilhaften Effekte nicht durch gleichzeitige NMDA-R Stimulation verbessert werden. Dennoch scheint der NMDA-R in sozial kognitiven Prozessen involviert zu sein. Die Beobachtungen betonen eine Beteiligung von MR und NMDA-R in sozialer Kognition bei Gesunden und Patienten mit MDD und zukünftige Forschung sollte das Potential der Modulation beider Rezeptoren hinsichtlich der Verbessrung (sozial) kognitiver Prozesse in MDD weiter untersucht.

1 Theoretical and Empirical Rationale

"Psychological stress is a particular relationship between the person and the environment that is appraised by the person as taxing or exceeding his or her resources and endangering his or her well-being"
(Lazarus & Folkman, 1984, p. 19)

In psychological stress research, the work of Lazarus and Folkman (1984) represents a pioneering attempt to conceive the complex nature of stress. According to this theory, stress is the product of a transaction between an individual and their environment. Once the environmental demands are appraised as challenging, threatening, or harming by the individual, a stress reaction is initiated. Coping mechanisms help the individual to deal with the stressor and, in the long-term, to restore the equilibrium of the individual-environment transaction (Lazarus, 1990; Lazarus & Folkman, 1984). Interestingly, Lazarus (1974) emphasized very early that understanding the complex transaction between the individual and their environment is crucial to understanding "diseases that have psychological determinants" (Lazarus, 1974). In line with this idea, the current research project aims to gain further insight into the important role of stress in health and disease.

Central to the human stress response is the *hypothalamic-pituitary-adrenal* (HPA) axis. In reaction to stress, the HPA axis gets activated, which regulates the secretion of the steroid hormone *cortisol*. Cortisol acts via *mineralocorticoid receptors* (MR) and *glucocorticoid receptors* (GR) in the human body (de Kloet et al., 2019; McEwen et al., 2015). Furthermore, patients with *major depressive disorder* (MDD) show dysregulations in HPA axis activity, which contribute to altered steroid hormone concentrations (Otte et al., 2016). Studies revealed that MDD patients show higher cortisol concentrations than healthy individuals (Stetler & Miller, 2011), suffer from impairments in several cognitive domains (Rock et al., 2014), and show an association between altered cortisol secretion and cognitive impairments (Hinkelmann et al., 2009). Interestingly, research emphasizes that MR stimulation decreases cortisol secretion, improving both verbal memory and executive functioning in healthy individuals and patients with MDD (Otte et al., 2015a). Considered together, the observations suggest that MR are involved cortisol secretion and that MR stimulation has cognitive-enhancing effects in MDD. Whether MR stimulation affects other steroid hormones and whether the beneficial effects of MR stimulation are

extendable to social cognition in MDD remains largely unknown. Therefore, the first aim of the current research project was to explore the effect of MR stimulation on steroid hormone secretion and social cognition in MDD.

Mineralocorticoid receptors are closely related to the *glutamate system* and *N-methyl-D-aspartate receptors* (NMDA-R). Studies show that stimulation of MR induces the release of *glutamate*, which binds upon NMDA-R in the brain (Joëls et al., 2018; Mikasova et al., 2017; Popoli et al., 2012). Furthermore, research emphasizes that NMDA-R are involved in the pathophysiology of MDD (Murrough et al., 2017) and that NMDA-R stimulation has cognitive-enhancing effects, by improving learning, memory, and decision making in healthy individuals for example (Feld et al., 2013; Onur et al., 2010; Scholl et al., 2014). Considered together, the observations suggest that MR and NMDA-R are closely related and that NMDA-R stimulation has cognitive-enhancing effects. Whether the beneficial effects of MR stimulation are improvable by simultaneous NMDA-R stimulation is far from certain. Therefore, the second aim of the current research project was to explore the effect of simultaneous MR and NMDA-R stimulation on steroid hormone secretion and social cognition in MDD. The central research question was:

What is the effect of MR and NMDA-R stimulation on steroid hormone secretion and social cognition in healthy individuals and patients with MDD?

The introduction of the current research project provides information on the psycho-neuro-endocrinological stress response. The introduction starts with a short description of the neuroendocrine stress response (section 1.1) and the steroid hormones central to this research project (section 1.2). Subsequently, information on MR and GR will be provided (section 1.3 & section 1.4), before explaining the relationship of both receptors to the glutamate system and NMDA-R (section 1.5). Afterwards, the role of MR and NMDA-R in (social) cognition (section 1.6) will be addressed, before an outline of the clinical picture, the pathophysiology of MDD, as well as the role of MR and NMDA-R in (social) cognition in MDD (section 1.7) will be provided. The introduction ends with a summary of the theoretical and empirical rationale of this research project (section 1.8).

1.1 The neuroendocrine stress response

Individuals who are confronted with a stressor experience stress. This experience can be divided into three phases: the perception and appraisal of the stressor, the processing of

the stressful information, and the stress response (de Kloet et al., 2019; Levine, 2005). After the stressor is perceived by the individual and the information is processed by the hypothalamus, one important brain region among others, several neuronal circuits are activated which prepare the individual to react. One of these neuronal circuits is the *sympathetic nervous system*; its activation leads to the expression of *noradrenaline* from widespread synapses and *adrenaline* from the adrenal medulla (de Kloet et al., 2005; Joëls et al., 2018).

After a short delay, the HPA axis is activated. The hypothalamus initiates corticotropin-releasing hormone (CRH) and vasopressin secretion in the paraventricular nucleus (PVN). The neuropeptides, in turn, contribute to the secretion of the adrenocorticotrophic hormone (ACTH) in the anterior pituitary (AP). Subsequently, stimulation of the adrenal cortex (AC) by ACTH initiates *glucocorticoid* secretion (de Kloet et al., 2005; Joëls & Baram, 2009; Pariante & Lightman, 2008). Glucocorticoids take several actions in the human organism, by binding upon MR and GR in the brain and in peripheral body parts (section 1.2 & section 1.3). One important function of MR and GR in the human stress response is the regulation of the HPA axis activity. In reaction to stress, increasing levels of corticosteroids lead to increased occupation of MR and GR, which induce inhibitory feedback loops. For instance, by binding upon GR in the PVN and AP, corticosteroids control the synthesis and secretion of CRH and ACTH respectively (de Kloet & Joëls, 2020; de Kloet et al., 2005; Pariante & Lightman, 2008). Furthermore, there is evidence that MR and GR in other areas of the brain contribute to HPA axis regulation. For instance, MR and GR in the hippocampus are involved in controlling the activity of the PVN (de Kloet & Joëls, 2020; Oitzl et al., 1997) and there is evidence that GR knockout in the prefrontal cortex of rats disturbs HPA axis functioning (de Kloet & Joëls, 2020; McKlveen et al., 2013). The interplay between MR and GR is important for a well-functioning HPA axis and a balanced neuroendocrine reaction to stress. While MR mainly regulate the onset and basal control of the HPA axis, GR are primarily involved in the termination of the neuroendocrine response to stress (de Kloet et al., 2018). In the following, information on HPA axis activity regulation via MR and GR and on brain areas involved in the stress response is summarized (Figure 1).

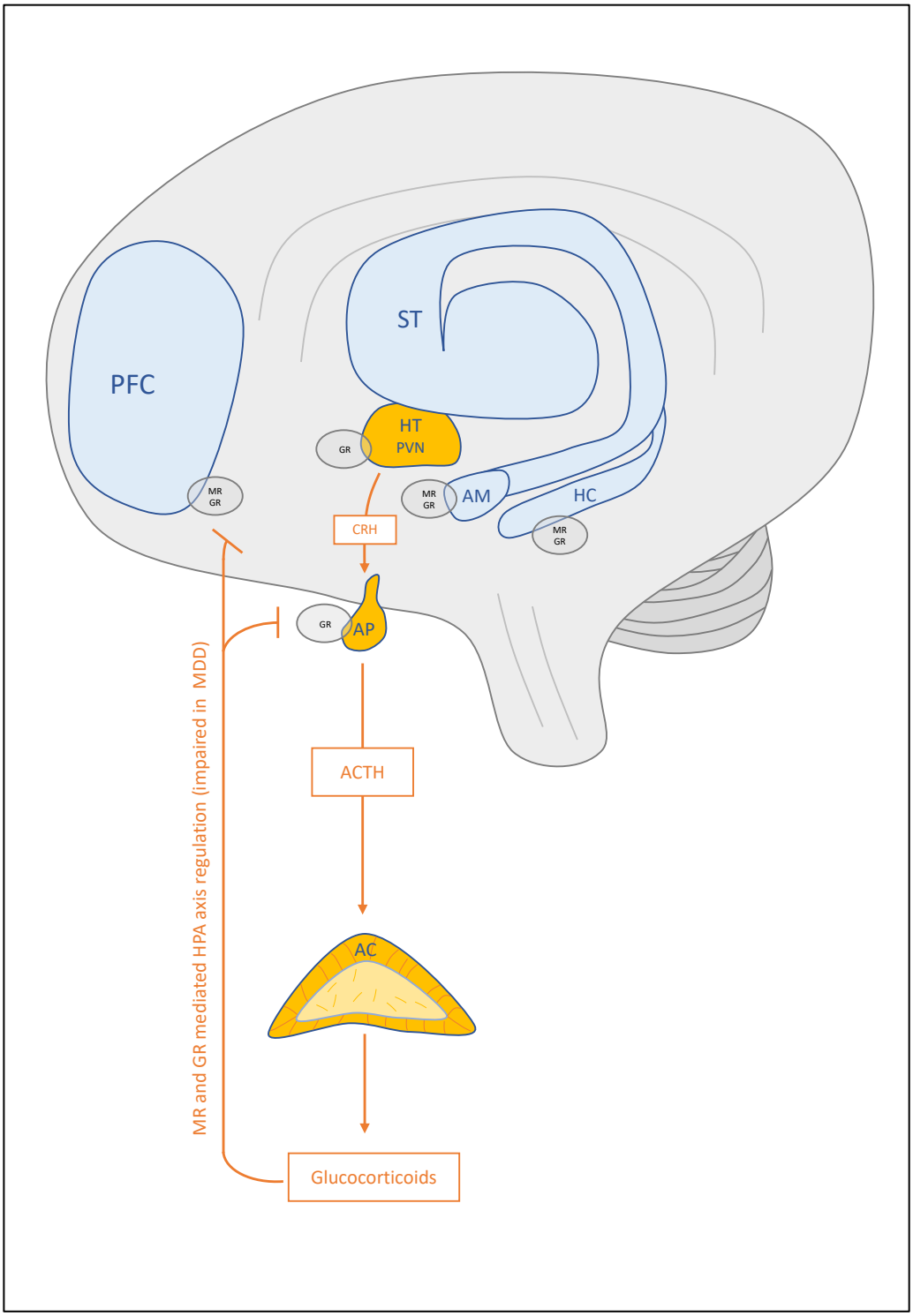

Figure 1. Schematic illustration of selected stress response processes.

Note: The hypothalamic-pituitary-adrenal (HPA) axis is illustrated **in orange**. Upon stress exposure, the paraventricular nucleus (PVN) of the hypothalamus (HT) initiates corticotropin-releasing hormone (CRH) secretion which activates the anterior pituitary (AP) to secrete the adrenocorticotrophic hormone (ACTH). The adrenocorticotrophic hormone, in turn, stimulates the adrenal cortex (AC) to secrete glucocorticoids which regulate via mineralocorticoid receptors (MR) and glucocorticoid receptors (GR) the activity of the HPA axis. Illustration based on existing models (de Kloet & Joëls, 2020; de Kloet et al., 2005; Pariante & Lightman, 2008). The hippocampus (HC), prefrontal cortex (PFC), amygdala (AM), and striatum (ST) are illustrated **in blue**. The brain areas are involved in the stress response by regulating HPA axis activity (HC and PFC) and cognitive processes that take place in reaction to acute stress (HC, PFC, AM, and ST) as will be outlined later (section 1.6). Illustration based on existing models (de Kloet & Joëls, 2020; Vogel et al., 2016).

The regulating effects of glucocorticoids on HPA axis activity can be mimicked by pharmacological MR and GR modulation. Mineralocorticoid receptor blockade by the antagonist spironolactone, for instance, leads to increased concentrations of the glucocorticoid cortisol (Otte et al., 2007). Mineralocorticoid receptor stimulation caused by the agonist fludrocortisone, in contrast, has been shown to decrease cortisol concentrations in healthy individuals and patients with MDD (Otte et al., 2003; Otte et al., 2015a). Thus, while MR antagonists inhibit HPA axis negative feedback control, leading to increased cortisol concentrations, MR agonists induce HPA axis negative feedback control, leading to decreased cortisol concentrations. The current research project used the latter processes, examining the effect of MR stimulation by the agonist fludrocortisone on steroid hormone concentrations and social cognitive processes in healthy individuals and patients with MDD (section 2.2.3).

To summarize this section, stress activates the HPA axis which leads to the secretion of the glucocorticoid cortisol. Glucocorticoids bind upon MR and GR, which leads to HPA axis inhibition and decreased steroid hormone concentrations. This effect can be mimicked by administration of the MR agonist fludrocortisone. The current research project made use of this mechanism to examine the effect of MR stimulation by fludrocortisone on steroid hormone secretion and social cognition in healthy individuals and patients with MDD. Aside from the glucocorticoid cortisol, the neuroendocrine stress response involves several other steroid hormones including *aldosterone* and *sulfated dehydroepiandrosterone* (DHEA-S). Therefore, their role within the human organism will be described in the following.

1.2 The steroid hormones cortisol, aldosterone, and sulfated dehydroepiandrosterone (DHEA-S)

In humans, the adrenal cortex produces several steroid hormones, of which cortisol, aldosterone, and DHEA-S are central to this research project. Within the adrenal cortex, cortisol is produced in the zona fasciculata, aldosterone in the zona glomerulosa and the zona reticularis produces *dehydroepiandrosterone* (DHEA) and its sulfated form DHEA-S (Rainey et al., 2002). One class of steroid hormones referred to as *corticosteroids* can be further divided into glucocorticoids and *mineralocorticoids*. The names derive from their main functions within the human organism: glucocorticoids contribute to gluconeogenesis in the liver and mineralocorticoids act within the kidney to contribute to mineral balance (Joëls et al., 2012). The main corticosteroids are cortisol and

corticosterone, and the main mineralocorticoid is aldosterone. While MR bind cortisol as well as aldosterone with a high affinity, GR appear to bind selectively cortisol (Kubzansky & Adler, 2010). The steroid hormones DHEA and DHEA-S are important precursors for the sex hormones estrogen and testosterone (Sahu et al., 2020) and, in contrast to cortisol and aldosterone, no specific receptor has been identified for DHEA and DHEA-S yet (Kamin & Kertes, 2017). The steroid hormones serve diverse functions within the human body and brain and also play an important role in the neuroendocrine response to stress (Kubzansky & Adler, 2010; Sahu et al., 2020). An overview of the steroid hormones examined in the current research project is presented in Figure 2 and their main functions will be outlined in the following.

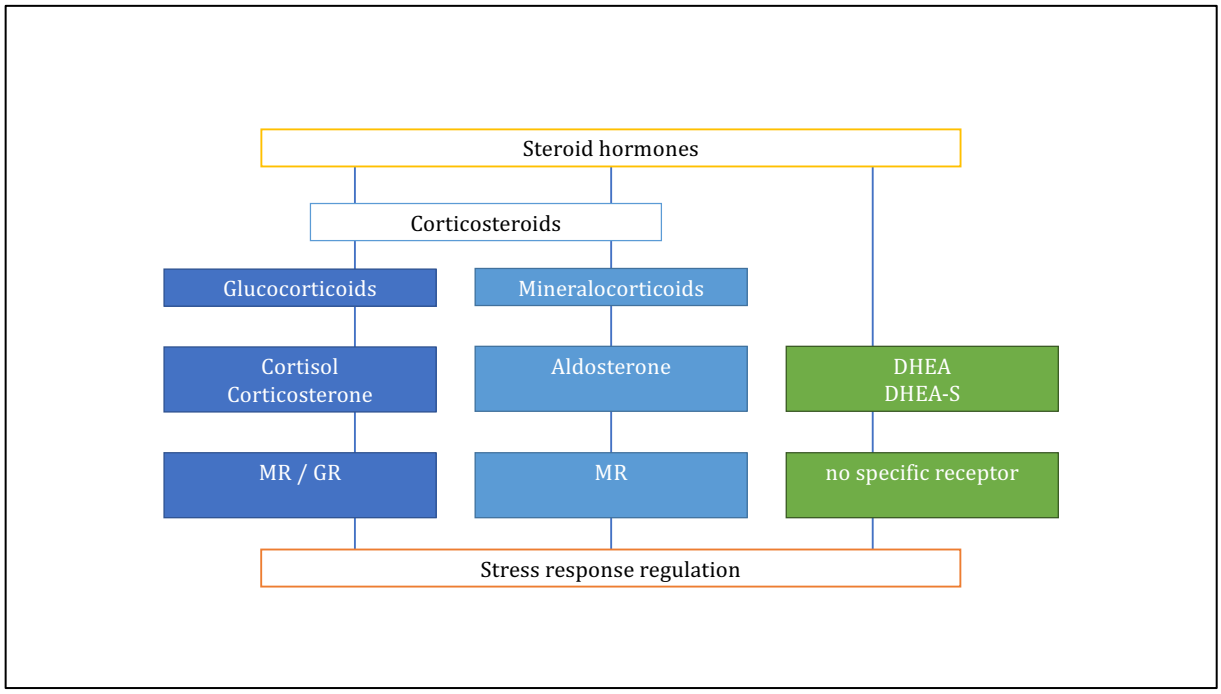

Figure 2. Schematic overview of selected steroid hormones.

Note: Selected steroid hormones involved in the regulation of the stress response. The figure includes the glucocorticoids cortisol and corticosterone and their corresponding mineralocorticoid receptors (MR), as well as glucocorticoid receptors (GR) and the mineralocorticoid aldosterone and its corresponding MR. No specific receptor has been identified for dehydroepiandosterone (DHEA) and dehydroepiandosterone sulphate (DHEA-S) yet. Illustration based on several reviews (Kamin & Kertes, 2017; Kubzansky & Adler, 2010).

Cortisol and corticosterone are glucocorticoids. In rodents, the main glucocorticoid is corticosterone, whereas in humans, cortisol circulates in much higher concentrations (ratio 20:1) compared to corticosterone (de Kloet & Joëls, 2020). Glucocorticoids play an important role for the individual to cope with current stress, to recover from past stress, and to adapt to ongoing/future stress. Through circulation, glucocorticoids are present in every organ and therefore they take diverse actions within the brain as well as peripheral body parts. Glucocorticoids bind upon the MR and GR and their function is determined depending on the tissue where the receptors are expressed in the human body (de Kloet et al., 2019; de Kloet et al., 2005). Consequently, glucocorticoids contribute to the regulation of the stress response in various ways. For instance, as part of the metabolism, glucocorticoids help to provide energy for the individual to cope with stress. Studies show, for instance, that GR in peripheral adipocytes regulate the supply of energy in concert with the brain (de Kloet et al., 2015) and that MR play an important role in adipogenesis (Caprio et al., 2007). Other relevant functions are, among others: immune regulation (Cain & Cidlowski, 2017) and neurogenesis (Fitzsimons et al., 2016). The glucocorticoid cortisol is also involved in the pathophysiology of MDD. For instance, MDD patients show a HPA axis hyperactivity as indicated by increased cortisol concentrations compared with healthy individuals (Stetler & Miller, 2011).

Aldosterone is a mineralocorticoid that binds upon MR and circulating concentrations are between 10-100-times lower than for cortisol and corticosterone (de Kloet et al., 2018; Yongue & Roy, 1987). The corticosteroid increases after HPA axis activation in response to stress, like cortisol, and after activation of the *rennin-angiotensin-aldosterone system*. Within the rennin-angiotensin-aldosterone system, it regulates water-salt-homeostasis in the human body. Accordingly, aldosterone plays an important role in the regulation of blood pressure and electrolytes and is closely related to cardiovascular health (Kubzansky & Adler, 2010). Not surprisingly, alterations of aldosterone concentrations are associated with cardiovascular diseases (Funder & Reincke, 2010). Furthermore, there is increasing evidence for an involvement of aldosterone in MDD (Murck et al., 2019). For instance, there is evidence for increased aldosterone concentrations in patients with MDD compared to healthy individuals (Emanuele et al., 2005).

DHEA is a steroid hormone that is mainly produced by the adrenal cortex, in the zona reticularis, and serves as a prohormone for the female and male sex hormones

estrogen and testosterone. Concentrations of DHEA in humans are age-dependent, with peak levels around 25-30 years of age and continuous decline in the years thereafter. In the human body, DHEA serves as an energy supplier for several body functions and contributes to the metabolism of fat and minerals, as well as the neuroendocrine response to stress (Peixoto et al., 2020; Sahu et al., 2020; Stárka et al., 2015). DHEA is converted into DHEA-S and both hormones show similar physiological actions in the body and brain. Like cortisol and aldosterone, DHEA-S is secreted by the adrenal cortex after activation of the HPA axis (Kamin & Kertes, 2017). Interestingly, there is evidence for opposing actions of cortisol and DHEA-S in stress response regulation. For example, chronic cortisol exposure appears to have neurotoxic effects in the rat hippocampus accompanied with memory deficits (Sebastian et al., 2013), whereas administration of DHEA has been shown to reverse these effects in the hippocampus (Kimonides et al., 1999). Furthermore, DHEA and DHEA-S are closely related to the pathophysiology of MDD. For instance, patients with MDD show decreased concentrations of DHEA-S in comparison to healthy individuals (Kamin & Kertes, 2017).

To summarize this section, cortisol, aldosterone, and DHEA-S play an important role in the human neuroendocrine response to stress and the steroid hormones appear to be involved in the pathophysiology of MDD. While there is evidence that MR stimulation decreases cortisol secretion in healthy individuals and patients with MDD (Otte et al., 2015a), the effects of MR stimulation on aldosterone and DHEA-S remain largely unknown. Therefore, this research project examined the effect of MR stimulation on steroid hormone secretion in MDD. Cortisol and aldosterone bind upon MR, but no specific receptor has been identified for the steroid hormone DHEA-S yet. The actions of the steroid hormones in the human body and brain are largely determined by the receptors they bind upon. Therefore, the following section will provide a detailed description of MR and GR and their role within the human response to stress.

1.3 The glucocorticoid receptors (GR) and mineralocorticoid receptors (MR)

Corticosteroid hormones bind upon GR and MR in the brain and peripheral tissue (de Kloet & Joëls, 2020). Both MR and GR exert *rapid non-genomic* and *slow genomic actions* (de Kloet et al., 2018; de Kloet & Joëls, 2020) and exist in *membrane*-bound or *nuclear*-bound forms (Gray et al., 2017; Popoli et al., 2012). Nuclear GR and MR are encoded from the genes NR3C1 and NR3C2 respectively and can serve as transcription factors that regulate gene transcription (de Kloet & Joëls, 2020).

Glucocorticoid receptors and MR are distributed differently in the brain. Mineralocorticoid receptors are particularly expressed in neurons in limbic brain areas (e.g., hippocampus, lateral septum, and amygdala) and to a lower extent within other parts of the cortex (Ahima et al., 1991; Arriza et al., 1988; de Kloet et al., 2018; de Kloet et al., 2019; de Kloet et al., 2005; Joëls et al., 2018; McEwen et al., 1968; Reul & Kloet, 1985). Glucocorticoid receptors are more widely expressed within the brain, in neurons and glia cells alike, and show especially high expressions in the hypothalamic PVN and in hippocampal regions (de Kloet et al., 2018; Joëls et al., 2018; Reul & Kloet, 1985).

Glucocorticoid receptors and MR have different binding affinities for glucocorticoids. The glucocorticoid cortisol, the main ligand in humans, has a binding affinity that is about ten times lower for GR than MR. Hence, when the individual is at rest and cortisol concentrations are low, MR are predominately occupied. With increasing glucocorticoid concentrations, for example, in reaction to acute stress or during the circadian peak, GR become increasingly occupied (de Kloet et al., 2018; de Kloet et al., 2019; de Kloet et al., 2005; Joëls et al., 2012; Reul & Kloet, 1985). The binding affinity of MR for glucocorticoids differ, in addition, between membrane MR and nuclear MR. Membrane MR bind with a lower affinity for glucocorticoids than nuclear MR. Therefore, nuclear MR are already occupied under rest, whereas membrane MR are less occupied and can respond rapidly to changes in glucocorticoid concentrations (de Kloet & Joëls, 2020; Joëls & de Kloet, 2017). Given the different binding affinities of MR and GR for glucocorticoids and given the constant change in glucocorticoid concentrations, both receptors play different roles in the human stress response. Mineralocorticoid receptors are mainly involved in the basal control of HPA axis activity, for example, circadian and ultradian rhythmicity and the onset of the stress response. Glucocorticoid receptors become occupied with increasing glucocorticoid concentrations and are mainly involved in the termination of the stress response (de Kloet et al., 2018; Herman et al., 2016).

In short, corticosteroids bind upon MR and GR within the human brain and peripheral body parts. Since the binding affinity of corticosteroids is much higher for MR than GR, this has implications for the role of both receptors in the neuroendocrine response to stress. While MR react to rapid changes of corticosteroid concentrations early after stress, GR become increasingly occupied when cortisol concentrations are rising at a later time after stress induction. Thus, the effects of MR and GR mediated actions in the neuroendocrine response to stress appear to be time-dependent. In the following, the role

of both receptors in the psycho-neuro-endocrine stress response will be described, structured chronologically from when the individual was confronted with a stressor.

1.4 Time-dependent MR and GR mediated actions

Depending on the point in time after stress exposure, different behavioral, brain network, neuroendocrine, and cellular processes take place that, together, shape the stress reaction of the individual. In the following, the psycho-neuro-endocrinological stress reaction will be described structured by the time (early and late phase of the stress response) and the process level (behavioral, brain network, neuroendocrine, and cellular level). The early phase of the stress response refers to the timeframe of approximately 0 – 30 minutes and the late phase to the timeframe of approximately 30 minutes to hours post stress exposure. It is important to mention that the transition between the phases is fluent, and that the time designations are approximates, based on recent reviews (de Kloet et al., 2019; de Kloet & Joëls, 2020; Hermans et al., 2014; Joëls et al., 2018; Joëls et al., 2012).

1.4.1 The early phase of the stress response

On the behavioral level, the early phase of the stress response is characterized by attention to and appraisal of the stressor, the initiation of the behavioral response, and the selection of the coping style that is adequate to deal with the stressor. At this stage, the individual is required to take action quickly and these behavioral responses are strongly associated with emotional processes (de Kloet et al., 2019; Joëls et al., 2018).

On the brain network level, the stressor immediately activates the *salience network* which contributes to the direction of attention to potential threats in the environment, to the provision of energy to initiate a behavioral response, and to increased vigilance in order to monitor the situation. Brain areas that are involved in these processes are the amygdala, thalamus, and hypothalamus, among others (Hermans et al., 2014).

On the neuroendocrine level, the early phase of the stress response is characterized by an immediate increase of catecholamines (e.g., dopamine and norepinephrine). After a short delay, HPA axis activation leads to the secretion of cortisol and concentrations peak between 15-30 minutes after stress induction. Then, increasing cortisol starts to bind upon MR and GR receptors to initiate negative feedback control of

the HPA axis, leading to a slow decrease in cortisol concentrations (de Kloet et al., 2019; Hermans et al., 2014).

On the cellular level, the early phase of the stress response is mainly characterized by nongenomic actions starting approximately within seconds to minutes after stress exposure. Glucocorticoids start to increase in this timeframe and, because of higher binding affinities for MR than GR, these rapid nongenomic actions are primarily mediated via MR (de Kloet et al., 2019; de Kloet & Joëls, 2020; Hermans et al., 2014; Joëls et al., 2018). Insight into the cellular actions in response to stress comes from animal studies and will be further outlined at a later time (section 1.5). For instance, in neurons of the hippocampus glucocorticoids bind upon membrane MR, leading to rapid nongenomic glutamatergic signal transmission, as measured by excitatory postsynaptic potentials (Karst et al., 2005). Interestingly, in the basolateral amygdala, these rapid nongenomic actions required about ten times higher doses of corticosteroids than genomic actions (Karst et al., 2010). The observation led to the assumption that membrane MR are *"corticosensors"* which rapidly react when corticosteroids increase in response to acute stress, but are less involved when corticosteroid concentrations are low, when the individual is at rest, for example (de Kloet & Joëls, 2020).

1.4.2 The late phase of the stress response

On the behavioral level, the late phase of the stress response is characterized by recovery from the stressor, rationalizing and contextualizing the information involved in the stressful situation, and saving information about the stressful situation in memory to prepare for future stressful events. The individual processes the stressful situation and, therefore, this phase is associated with higher-order cognitive processes (de Kloet et al., 2019; de Kloet & Joëls, 2020; Joëls et al., 2018).

On the brain network level, the stressor activates the *executive control network* with a delay of about one hour which regulates higher-order cognitive processes such as executive functioning, working memory, and decision-making. This activation is accompanied with a shift of neuronal resources from the salient network described earlier to the executive control network. With this shift, neuronal processes rely increasingly on brain areas such as dorsolateral prefrontal cortex, dorsomedial prefrontal cortex, and dorsal posterior parietal cortex, among others (Hermans et al., 2014).

On the neuroendocrine level, the rise in glucocorticoids leads to increased occupation of GR in addition to MR. Due to HPA axis negative feedback control,

glucocorticoid concentrations decrease over time before they reach basal levels as under non-stressful conditions, from approximately 90 minutes post stress exposure (Edo R de Kloet et al., 2019; Hermans et al., 2014).

On the cellular level, nongenomic actions proceed into the late phase of the stress response, but delayed genomic actions become increasingly important starting approximately one hour after stress exposure (de Kloet et al., 2019; Hermans et al., 2014; Joëls et al., 2018). The genomic actions encompass several complex stages that will be summarized since a detailed description is beyond the scope of this research project. In brief: delayed genomic actions are mediated via nuclear MR and GR and, indirectly, via membrane MR and GR which initiate second messenger processes. Free circulating glucocorticoids in the blood can cross the blood-brain barrier after leaving corticosteroid binding globulin. Glucocorticoids that enter the cell and bind upon MR and GR can initiate genomic actions by releasing heat shock proteins. Mineralocorticoid receptors and GR then bind upon glucocorticoid response elements in the deoxyribonucleic acid (DNA), thereby initiating transcription of target genes (de Kloet & Joëls, 2020; Joëls et al., 2012; Popoli et al., 2012).

To summarize this section, the psycho-neuro-endocrinological stress reaction is characterized by an early and late phase which encompass several behavioral, brain network, neuroendocrine, and cellular processes. These processes interact with each other to enable the individual to cope with the stressor. The early phase is characterized by immediate attention to, and appraisal of the stressful information, primarily associated with the salience network, emotional processes, and non-genomic MR and GR actions. The late phase of the stress response is characterized by processing the stressful information in the long-term, primarily associated with the executive control network, higher-order cognitive processes, and genomic MR and GR actions. Important information for this research project is the observation that the glucocorticoid and glutamate system are closely related, and that MR play a crucial role in this context. This research project examined the interplay between both systems within the psycho-neuro-endocrinological stress response, by observing the effects of MR and NMDA-R stimulation on steroid hormone secretion and social cognitive processes. Therefore, the following section provides information about the interplay between the glucocorticoid system and glutamate system.

1.5 Glucocorticoids and the glutamate system

Glutamate is the primary excitatory neurotransmitter in the central nervous system. The neurotransmitter binds upon several receptors including metabotropic (i.e. mGluR) and ionotropic (i.e. NMDA-R, AMPA) glutamate receptors (Murrough et al., 2017). This research project will focus on the ionotropic NMDA-R and will touch upon the interplay of the glucocorticoid and glutamate system. For detailed information, there are several reviews on the role of the glutamate system in the stress system (Gray et al., 2017; Popoli et al., 2012) and in MDD (Murrough et al., 2017).

Glutamate is released from synapses and when the neurotransmitter, together with glycine, binds upon already depolarized ionotropic glutamate receptors, the NMDA-R becomes activated. The activation of NMDA-R initiates an influx of cations (Na^+ and Ca^{2+}, among others) which lead to depolarization of the post-synaptic membrane. This way, the release of glutamate from synapses contributes to synaptic signal transmission in the short-term, by inducing postsynaptic excitability through activation of NMDA-R. In the long-term, depending on the magnitude and time of glutamate receptor activation, glutamatergic signal transmission can modulate neuronal plasticity and, in case of excessive activation, to excitotoxicity (Murrough et al., 2017).

Converging lines of evidence indicate that stress or glucocorticoid exposure can affect glutamate transmission rapidly via membrane MR and GR and can have delayed effects mediated via nuclear GR. There are various effects of glucocorticoids on the glutamate system, including increased glutamate release and accompanying NMDA-R activation as well as long-lasting effects such as changes in neuronal plasticity (Gray et al., 2017; Popoli et al., 2012). In the following, selected exemplary studies are presented to provide information relevant for this research project. Studies are structured by rapid and delayed MR and GR mediated effects of glucocorticoids on glutamate transmission.

1.5.1 Rapid and delayed effects of glucocorticoids on glutamate transmission

Research indicates that MR and GR mediated nongenomic actions are involved in rapid glutamatergic transmission. There is evidence from animal research that corticosterone rapidly increases glutamate transmission in the hippocampus of mice via nongenomic MR (Karst et al., 2005) and GR mediated actions (Wang & Wang, 2009). Similar effects have been observed in the prefrontal cortex and frontal cortex of mice, where corticosterone increased the releasable pool of glutamate vesicles via MR and GR mediated nongenomic actions (Treccani et al., 2014).

Rapid effects of glucocorticoids mediated via membrane MR and delayed effects mediated via nuclear GR shape glutamate transmission. In an important animal experiment, Karst et al. (2010) demonstrated that corticosterone rapidly activates glutamate transmission via MR in the amygdala of mice, similar to what was previously observed in the hippocampus (Karst et al., 2005). The rapid MR mediated effects on glutamate transmission started within approximately ten minutes. In the amygdala, however, the effects lasted for several hours and the authors concluded that genomic GR mediated actions are important for maintaining the long-lasting effects of glucocorticoids on glutamate transmission (Karst et al., 2010).

N-methyl-D-aspartate receptors play an important role in the delayed long-lasting effects of glucocorticoids on glutamate transmission. Yuen et al. (2011) showed that behavioral stress and corticosterone exposure increased glutamate transmission for several hours via intracellular GR in the prefrontal cortex. Importantly, these long-term changes were accompanied by an increased number and increased activity of NMDA-R at the synaptic membrane (Yuen et al., 2011). Thus, stress or corticosterone exposure appears to induce rapid effects on glutamate transmission within several minutes via membrane MR and GR. Delayed and long-lasting changes in glutamate transmission that last for several hours seem to be mediated via intracellular GR and accompanied by changes in NMDA-R activity.

One important aspect for the delayed long-lasting effects of glucocorticoids on glutamate transmission is the magnitude of (stress) corticosterone exposure. In line with earlier studies (e.g. Karst et al., 2010), Karst and Joëls (2016) showed that corticosterone exposure modified glutamatergic transmission in neurons of the basolateral amygdala for several hours. The long-term effects, however, varied depending on the magnitude of corticosterone exposure: low corticosterone doses (moderate stress) initially enhanced and later diminished glutamatergic transmission, whereas high corticosterone doses (severe stress) had reverse effects. The authors speculate that this mechanism might underlie the frequent observation of enhanced encoding of emotional information in individuals who were exposed to severe stress after traumatic experiences (Karst & Joëls, 2016) for example.

Chronic stress or corticosteroid exposure affects NMDA-R mediated glutamate transmission contributing to long-term changes in neuronal plasticity in several brain areas. Animal research has shown, for instance, that chronic stress or corticosteroid exposure has adverse effects on neuronal plasticity and that blocking the NMDA-R

reverses these adverse effects in the prefrontal cortex (Martin & Wellman, 2011) and hippocampus (Christian et al., 2011). Just recently, similar effects have been observed in humans. Blockade of the NMDA-R by memantine reversed the adverse effects of chronic corticosteroids exposure on neuronal plasticity in the hippocampus of humans (Brown et al., 2019).

To summarize this section (see Figure 3), glucocorticoids have rapid effects on glutamate transmission that appear to be mediated via membrane MR and GR and that contribute to increased glutamate release and glutamatergic NMDA-R activation among others. Glucocorticoids can also have delayed effects on NMDA-R mediated glutamate transmission, that can last for several hours and that appear to be mediated via intracellular GR. Chronic stress or glucocorticoid exposure, in addition, can have long-lasting effects on NMDA-R mediated glutamate transmission, contributing to changes in neuronal plasticity. It becomes apparent that the interplay between MR and GR is important for the neuroendocrine stress response and related glutamatergic signaling. Thus far, the role of MR and NMDA-R in the psycho-neuro-endocrinological stress response has been outlined. To this point, the focus has mainly been on to the neuro-endocrinological part of the stress response, while less attention has been given to the psychological aspects. Therefore, the following section will focus on the role of MR and NMDA-R in (social) cognitive processes.

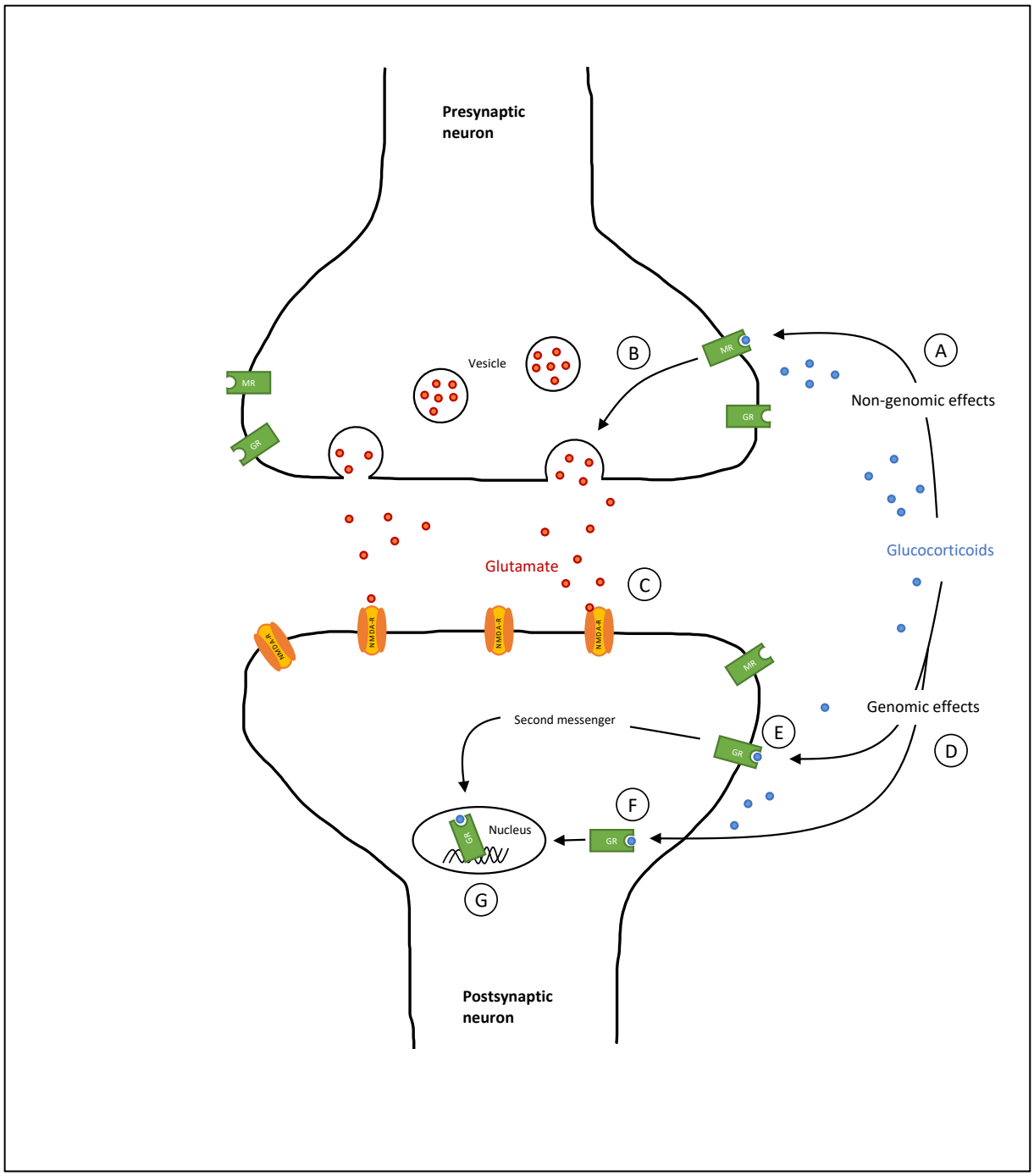

Figure 3. Schematic illustration of glucocorticoid effects on glutamate transmission.

Note: Glucocorticoids exert (A) rapid non-genomic effects by binding upon membrane mineralocorticoid receptors (MR) and glucocorticoid receptors (GR) that lead to (B) increased release of glutamate which (C) binds upon N-methyl-D-aspartate receptors (NMDA-R). Glucocorticoids also exert (D) delayed genomic effects by binding upon (E) membrane MR and GR, which initiate second messenger processes and by binding upon (F) nuclear MR and GR. The genomic effects contribute to (G) gene transcription in the nucleus. Illustration based on existing models (Popoli et al., 2012).

1.6 The role of MR and N-methyl-D-aspartate receptors (NMDA-R) in (social) cognition

As described previously (section 1.1 to 1.5), the human stress response encompasses various psycho-neuro-endocrinological processes that, all together, determine how the individual deals with a stressor. In reaction to acute stress, HPA axis activation leads to secretion of glucocorticoids which bind upon MR and GR. Both receptors play an important role in regulating psycho-neuro-endocrinological processes within the human stress response and their actions are closely related to the actions of glutamatergic NMDA-R (de Kloet & Joëls, 2020; Popoli et al., 2012). So far, the focus of research has mainly been on the role of MR and NMDA-R in the neuro-endocrinological part of the stress response. This section will focus upon the psychological part of the human response to stress. The role of MR and NMDA-R in cognitive and social cognitive processes will be examined in more detail. *Social cognition* describes the cognitive processes of identification, perception, and interpretation of socially salient information in the environment (Weightman et al., 2019). The current research project examined empathy, recognition of facial emotion expressions, and selective attention to facial emotional stimuli that can be classified as processes of social cognition. Social cognition tasks activate specific brain areas referred to as the *social brain* in humans. Among these social brain areas are the amygdala, hippocampus, and prefrontal cortex, which are especially important for this research project because they are also highly involved in the processing of stress (Sandi & Haller, 2015). Overall, research emphasizes that the human response to stress is involved in social cognitive processes. However, there is a great heterogeneity in the observations, which might be explained by the variety of stress manipulations, as well as measures of social cognition that are used in the field (von Dawans et al., 2020). For example, the human stress response can be manipulated by the induction of psychosocial stress through the *Trier Social Stress Test* (TSST; Kirschbaum et al., 1993), by the induction of psychophysiological stress through the *Socially Evaluated Cold-Pressor Test* (SECPT; Schwabe et al., 2008), or by the pharmacological modulation of MR and/or GR as well as NMDA-R. The following sections will focus upon studies that are most relevant for this research project. For a detailed overview of studies exploring the relationship between the human stress response and social cognition, as well as methods of stress manipulations and measures of social cognition, see the following review (von Dawans et al., 2020).

1.6.1 The MR in (social) cognition

Evidence for MR involvement in cognitive processes is found in animal and human research. One early study showed that manipulation of MR in rats influenced the behavioral reactivity to novel cues in the environment (Oitzl et al., 1994). Such observations motivated research of the role of MR in cognition and, with increasing research, the picture evolved that stimulation of MR has cognitive-enhancing effects, while blockade of the receptors has impairing effects on cognition. For instance, loss of forebrain MR in mice impaired learning and memory (Arp et al., 2014; Berger et al., 2006; Brinks et al., 2009). Overexpression of forebrain MR, in contrast, improved memory (Ferguson & Sapolsky, 2008; Lai et al., 2007).

Similar effects of MR modulation on cognition have been observed in humans. In healthy humans, MR stimulation has been shown to improve learning (Groch et al., 2013), several memory processes (visuospatial, short-term, working) (Hinkelmann et al., 2015), and spatial memory retrieval (Piber et al., 2016). Blockade of MR, in contrast, showed impaired effects on memory (Rimmele, Besedovsky, Lange, & Born, 2013; Schwabe, Tegenthoff, Höffken, & Wolf, 2013), selective attention (Cornelisse, Joëls, & Smeets, 2011; Otte et al., 2007), and executive functioning (Schwabe, Höffken, Tegenthoff, & Wolf, 2013). Overall, both animal and human research emphasize the important role of MR in cognitive processes. While strong evidence exists for cognitive-enhancing effects of MR stimulation on memory and learning, much less is known about the role of MR in social cognition processes.

Some evidence for MR involvement in social cognition comes from animal research. For example, one study found that the genetic deletion of limbic-hippocampal MR impaired social discrimination in mice (Ter Horst et al., 2014). Animal research emphasizes that high stress exposure during the lifespan contributes to an altered glucocorticoid stress response which, in turn, contributes to disturbances in social information processing and social behavior. Thus, stress appears to have a great impact on social behavior in animals (Sandi & Haller, 2015). It is unclear, whether these observations can be translated to humans because the relationship between the human stress response and social cognitive processes is far from understood.

Several studies in healthy individuals emphasize an involvement of the human stress response in social cognition. For example, psychosocial stress induced through the TSST increased *emotional empathy* (the ability to empathize with another person) but not *cognitive empathy* (the ability to understand another person's emotions) in healthy men.

Thus, acute stress appears to influence social cognition by enhancing the magnitude a person feels with another person, which could promote prosocial behavior in stressful situations (Wolf et al., 2015). Brain imaging studies promote the important role of the amygdala here. For example, acute stress induced by aversive movie clips enhanced the sensitivity and lowered the specificity of processing emotional facial expressions, which was linked to amygdala activity (van Marle et al., 2009). Interestingly, MR appear to play a crucial role in this context. Vogel et al. (2015) induced psychophysiological stress with the SECPT while blocking MR with spironolactone. Acute stress enhanced emotional vigilance processing, which was accompanied by a heightened connectivity between the amygdala and striatum. Interestingly, the shift in emotional processing was prevented after MR blockade (Vogel et al., 2015). Considered together, the studies emphasize that within the stress response, MR play a central role in regulating the processing of emotional salient information and that the receptor might be relevant for allocating brain resources to support these social cognitive processes.

Further support for the importance of MR in social cognition is found in pharmacological studies. Mineralocorticoid receptor stimulation by fludrocortisone has been shown to enhance selective attention to negative emotional stimuli, yet it had no effect on emotion recognition (Schultebraucks et al., 2016). Several other studies administered the exogenous glucocorticoid hydrocortisone, which, depending on the administered dose, serves as an MR or GR agonist. Because glucocorticoids have a higher binding affinity for MR compared to GR (section 1.3), it is mainly MR which are occupied when hydrocortisone is administered in low doses. After administration of high-dose hydrocortisone, GR are also occupied (von Dawans et al., 2020). Interestingly, the effects of hydrocortisone on social cognition appear to be dose dependent. Low-dose hydrocortisone has been shown to increase the inhibition of processing angry faces, while high doses had no effect on the processing of emotional salient information, when compared to a placebo (Taylor et al., 2011). The findings emphasize that MR, rather than GR, may be crucial for processing emotional information within the human response to stress. However, not all studies found evidence for MR involvement in social cognition. For example, in one study, administration of low-dose hydrocortisone showed no effect on cognitive empathy, emotional empathy, or the recognition of facial emotion expressions (Duesenberg et al., 2016).

The picture is complemented by research showing time-dependent effects of low-dose hydrocortisone administration on social cognition. For example, Henckens et al.

(2010) found a reduction in overall amygdala reactivity to emotional stimuli soon (75min) after hydrocortisone administration, when rapid non-genomic effects take place. Late (258min) after hydrocortisone administration, when delayed genomic effects take place, the reduction in processing emotional information was abolished for negative emotional stimuli associated with increased connectivity between amygdala and prefrontal cortex. It appears that rapid non-genomic MR mediated actions contribute to heightened sensitivity of the amygdala to process emotional information, delayed genomic MR mediated actions normalize amygdala sensitivity controlled by the prefrontal cortex (Henckens et al., 2010).

To summarize, there is evidence that MR are involved in (social) cognitive processes. While MR stimulation appears to have cognitive-enhancing effects on learning and memory, research on MR involvement in social cognition is inconclusive. Within the psycho-neuro-endocrinological stress response, MR appear to play a crucial role in processing social cognitive processes. One could speculate that in the early phase of the stress response, MR mediated non-genomic actions enhance sensitivity to process emotional salient information linked to the amygdala; while in the late phase of the stress response, MR mediated genomic actions contribute to normalize emotional processing controlled by the prefrontal cortex. Clearly, further research is required to examine whether the beneficial effects of MR stimulation on learning and memory are extendable to social cognition, this was one aim of the current research project (section 2.1). In the stress system, the effects of MR stimulation and NMDA-R mediated glutamate transmission are closely related (section 1.5). Therefore, the role of NMDA-R in (social) cognition in animals and healthy individuals is worth examining and research on this topic will be described in the following section.

1.6.2 The NMDA-R in (social) cognition

Research in animals and humans suggest an important role of NMDA-R in cognitive processes. Early studies in mice showed that NMDA-R blockade impaired amygdala-associated learning (Miserendino et al., 1990), while stimulation of NMDA-R by D-cycloserine (DCS) enhanced learning (Monahan et al., 1989), memory (Flood et al., 1992; Thompson et al., 1992), and amygdala-associated extinction of conditioned fear (Walker et al., 2002). Thus, NMDA-R stimulation in animals appears to enhance cognitive processes and NMDA-R blockade appears to have reverse effects. Since these early observations, an increasing number of studies found evidence for NMDA-R playing an

important role in learning and memory in animals. Several studies have translated these observations to humans (Davis et al., 2006; Otto et al., 2016).

For instance, in healthy humans, NMDA-R stimulation by DCS has been shown to have beneficial effects on memory (Feld et al., 2013), decision making (Scholl et al., 2014), and hippocampus-associated learning (Onur et al., 2010), although not all studies concur (Otto et al., 2009). The important role of NMDA-R in learning and memory seems to be closely related to the role of NMDA-R in synaptic plasticity. Several studies in mice indicate that blocking NMDA-R impairs synaptic transmission in the hippocampus and that this is accompanied by impairments in learning and memory (Lee & Silva, 2009). Overall, these studies emphasize an important role of NMDA-R in cognition, especially in memory and learning, both in animals and in humans. Far less is understood about the role of NMDA-R in social cognition.

One recent study is particularly important for this research project because it examined the role of NMDA-R in social cognition healthy individuals in a similar experimental setting (Chen et al., 2020). Chen et al. (2020) examined social cognition with similar experimental tasks to our study (e.g., autobiographical memory task, facial expression recognition task, and facial dot-probe task) three hours after administration of the NMDA-R partial agonist DCS with the same dosage (250 mg) as in this research project. NMDA-R stimulation enhanced specificity of autobiographical memory and increased positive emotional memory. Interestingly, DCS administration had no effect on facial expression recognition or selective attention to facial emotional stimuli. The results confirm cognitive-enhancing effects of NMDA-R stimulation by DCS on memory. However, the study found no evidence for NMDA-R involvement in social cognition when measured through facial emotion recognition and selective attention to emotional faces (Chen et al., 2020). One might speculate that the specific beneficial effects of NMDA-R stimulation on memory are attributable to the crucial role of NMDA-R in synaptic plasticity (Lee & Silva, 2009). Yet, it is far from understood whether the beneficial effects of NMDA-R modulation are specific to learning and memory or extendable to processes of social cognition.

Another study in patients suffering from NMDA-R encephalitis complements the overall picture. Compared to healthy individuals, the patients suffered from impairments in general cognitive functions (verbal memory, visual memory, working memory, attention, processing speed, and executive functioning) and some aspects of social cognition (judging social behavior violations and understanding social situations based on mental states). Interestingly, emotion recognition was not affected in these patients.

However, due to the small sample size (n = 7) these results need to be interpreted with caution (McKeon et al., 2016), before any firm conclusions on the role of NMDA-R in social cognition can be drawn. More stable evidence for NMDA-R involvement in social cognition comes from studies that used the NMDA-R antagonist ketamine. For example, administration of ketamine impaired facial emotion recognition (Ebert et al., 2012) and there is evidence that this is accompanied by reduced neuronal activity in the amygdala and other limbic regions (Abel et al., 2003).

Overall, there is first evidence for NMDA-R involvement in social cognition in healthy individuals. Most evidence focusses on the cognitive-enhancing effects of NMDA-R stimulation on learning and memory, far less is known about the role of NMDA-R in social cognition and the few studies available show heterogenous results. One explanation might lie in the central role NMDA-R play in synaptic plasticity which is closely related to processes of learning and memory. Clearly, there is need for further research which examines whether NMDA-R stimulation has beneficial effects on social cognition, which was one aim of this research project (section 2.1).

To summarize this section, research emphasizes that MR and NDMA-R are involved in (social) cognition. While studies indicate that MR and NMDA-R stimulation has beneficial effects on cognitive processes such as learning and memory, research on the role of both receptors in social cognition is sparse. Thus far, the focus has been on the relationship between the human stress response and (social) cognitive processes in healthy individuals. This research project examined the effects of MR and NMDA-R stimulation on steroid hormone secretion and social cognition in healthy individuals and patients with MDD. Therefore, the following sections will focus upon the psychiatric disorder MDD.

1.7 Major depressive disorder (MDD)

The central aim of the current research project was to examine the effect of MR and NMDA-R stimulation on steroid hormone secretion and social cognition in healthy individuals and patients with MDD. So far, the focus has mainly been upon healthy individuals and, therefore, the following sections will provide information on the psychiatric disorder MDD. The section starts with a description of the clinical picture (section 1.7.1), the pathophysiology of MDD (section 1.7.2), and will then focus upon the role of MR and NMDA-R in (social) cognition in MDD and other psychiatric disorders

(section 1.7.3). This section focusses on information that is most relevant for the current research project. Detailed information on the clinical picture of MDD and the biological systems involved in the pathophysiology of MDD are available in the following reviews (Murrough et al., 2017; Otte et al., 2016).

1.7.1 The clinical picture of MDD

Major depressive disorder is a psychiatric disorder with a high burden of disease (James et al., 2018). Estimates of MDD prevalence (proportion of people with MDD in 2015) vary depending on the country, for instance, 4.4. % for the global population and 5.2 % for the population in Germany. Importantly, MDD prevalence is higher in women (5.1%) than in men (3.6%) (World Health Organization, 2017).

According to the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5), a diagnosis of MDD is indicated if the individual suffers from depressed mood and/or diminished pleasure or interest in almost all activities nearly every day and for a period of minimum two weeks. In addition, at least four (three, if both symptoms above are present) other symptoms must be present nearly every day during this period: weight gain/loss, insomnia/hypersomnia, diminished ability to think or concentrate, and recurrent thoughts of death, among others. For diagnosis, the symptoms must cause distress or impairment in social, occupational, and other areas of functioning (American Psychiatric Association, 2014).

1.7.2 The pathophysiology of MDD

The pathophysiology of MDD is complex and involves several biological systems that interact with each other and shape the clinical picture of MDD. Biological systems that appear to play an important role are the autonomic nervous system, the immune system, the HPA axis, and the glutamate system (Otte et al., 2016). Since this research project focused on the HPA axis and the glutamate system (section 1.1 & section 1.5), the role of these biological systems in the pathophysiology of MDD will be outlined in more detail in the following sections.

The HPA axis, as well as MR and GR, is closely related to the pathophysiology of MDD. Although several biological systems are involved in MDD, impairments in HPA axis functioning are among the most consistently described in the literature findings (Otte et al., 2016). Overall, patients with MDD show HPA axis hyperactivity, which is accompanied

by increased cortisol concentrations in comparison with healthy individuals (Stetler & Miller, 2011). However, studies on cortisol concentrations in MDD show heterogenous results (Knorr et al., 2010). One explanation is that the pathophysiology of MDD varies between different patient populations. For instance, older MDD patients show stronger HPA axis dysregulations than younger MDD patients (Murri et al., 2014), hypercortisolemia is more pronounced in psychotic MDD compared to non-psychotic MDD or healthy individuals (Keller et al., 2006), and in melancholic MDD compared to atypical MDD (Juruena et al., 2018). Furthermore, impairments in HPA axis functioning in MDD are closely related to alterations in MR and GR functioning. Studies indicate that GR stimulation has no inhibiting effect on cortisol secretion in MDD patients and this impairment has been termed *glucocorticoid resistance* (Pariante, 2017; Pariante & Lightman, 2008). Moreover, studies indicate that patients with psychotic MDD show diminished MR mediated negative feedback inhibition of the HPA axis (Lembke et al., 2013; Schatzberg, 2015). However, HPA axis regulation via MR appears to be intact in younger patients with MDD. Another study in a relatively young sample (M = 26 years) showed that MR stimulation decreased cortisol concentrations equally in MDD patients and healthy individuals (Otte et al., 2015a). In line with these observations which suggest that MR and GR functioning are related to the pathophysiology of MDD, post-mortem studies indicate that patients with MDD suffer from decreased MR expression in the hippocampus and prefrontal cortex (Klok et al., 2011; Medina et al., 2013). Furthermore, altered MR gene expression has been found in the hypothalamus (Wang et al., 2008), the anterior cingulate cortex and the dorsolateral prefrontal cortex (Qi et al., 2013). The importance of MR for MDD pathophysiology is further emphasized by the observation that MR stimulation by fludrocortisone has beneficial effects on antidepressant treatment of patients with MDD (Otte et al., 2010). Considered together, the observations suggest that the HPA axis and MR are involved in the pathophysiology of MDD, and that stimulation of MR might improve symptomatology of patients with MDD.

The glutamate system and the NMDA-R play an important role in the pathophysiology of MDD. Converging lines of evidence suggest that there are dysregulations of the glutamate system and NMDA-R functioning in MDD (Murrough et al., 2017; Sanacora et al., 2008). For instance, glutamate concentrations are increased in MDD patients (Altamura et al., 1993; Sanacora et al., 2004) and have been shown to be related to depression severity (Mitani et al., 2006). Furthermore, alterations in NMDA-R functioning have been observed in the prefrontal cortex of MDD patients (Feyissa et al.,

2009) and frontal cortex of suicide victims (Nowak et al., 1995). In recent years, particular focus has been on NMDA-R since several studies have shown antidepressant effects for the antagonist ketamine (Krystal et al., 2019). Interestingly, there is evidence that the partial NMDA-R agonist DCS has antidepressant effects (Heresco-Levy et al., 2013; Schade & Paulus, 2016). Furthermore, administration of DCS has been shown to have beneficial effects on cognitive processes in several psychiatric disorders (Peyrovian et al., 2019). All things considered, the observations emphasize that the glutamate system and NMDA-R are involved in the pathophysiology of MDD and there is evidence that NMDA-R stimulation might improve symptomatology of patients with MDD.

1.7.3 The MR and NMDA-R in (social) cognition in MDD

As aforementioned, MR and NMDA-R are involved in (social) cognitive processes in healthy individuals (section 1.6.1), there is also evidence that both receptors play a crucial role in the pathophysiology of MDD (section 1.7.2). So far, there are few clinical studies that examined the role of MR and NMDA-R in (social) cognitive processes in MDD samples. The following section will provide information on the role of MR and NMDA-R in (social) cognitive processes in MDD and other psychiatric disorders which are relevant for this research project. A detailed overview of the role of MR and NMDA-R in the symptomatology of psychiatric disorders can be found in wider literature (Peyrovian et al., 2019; Wingenfeld & Otte, 2019).

Mineralocorticoid receptors appear to play an important role in cognitive processes in psychiatric disorders. For instance, one study found that variations in the MR gene (NR3C2) which is associated with an increased risk for MDD, was also associated with a negative memory bias (Vogel et al., 2014). Furthermore, some studies emphasize that MR modulation might have cognitive-enhancing effects in clinical samples. For the current research project, studies in MDD samples are most important. One study could show that MR stimulation improved memory and executive functioning both in MDD patients and healthy individuals (Otte et al., 2015a). Another study that examined social cognition found that MR blockade reduced *cognitive empathy* in MDD patients (Wingenfeld et al., 2016). This is in line with the observation that MR stimulation enhanced *emotional empathy* both in patients with borderline personality disorder and healthy individuals (Wingenfeld et al., 2014). Thus, MR stimulation appears to have (social) cognitive-enhancing effects in MDD patients and other psychiatric populations, hence it may serve as a potential treatment target to improve cognitive deficits in MDD.

However, not all studies concur. For instance, MR stimulation showed no effect on autobiographical memory retrieval in patients with borderline personality disorder, patients with MDD, nor healthy individuals (Fleischer et al., 2015).

N-methyl-D-aspartate receptors' role in cognition has rarely been studied in psychiatric samples and most evidence comes from studies in anxiety-related disorders. One meta-analysis found that administration of DCS showed a small improvement in cognitive behavioral exposure therapy for anxiety-related disorders (Mataix-Cols et al., 2017), suggesting beneficial therapeutic effects of NMDA-R modulation in psychiatric populations. Several clinical studies confirm cognitive-enhancing effects of NMDA-R stimulation by DCS in psychiatric disorders (e.g., anxiety disorders, Alzheimer's disease, and schizophrenia) (Peyrovian et al., 2019). However, research on the effect of NMDA-R stimulation in MDD is sparse. There is evidence for anti-depressant effects of NMDA-R modulation after high (1000mg/d) DCS administration (Heresco-Levy et al., 2013) but not in low doses (250mg/d) (Heresco-Levy et al., 2006). The most promising evidence for beneficial antidepressant effects of NMDA-R modulation comes from research with the NMDA-R antagonist ketamine. Several studies emphasize the rapid antidepressant effects of ketamine, these observations underline that glutamate signaling plays an important role in the pathophysiology of MDD (Murrough et al., 2017). Interestingly, there is new evidence that suggests DCS might promote the antidepressant effects of ketamine. One study found that DCS administration failed to maintain treatment effects of ketamine in patients with MDD, but showed an enhancement of ketamine's long-term anti-suicidal effects (Chen et al., 2019). In sum, NMDA-R modulation appears to have beneficial antidepressant effects in MDD patients, and the receptor might thus serve as a treatment target to improve (social) cognition in MDD. However, there is a lack of research on the role of NMDA-R in (social) cognition in MDD. This research project aimed to fill this gap.

To summarize this section, MDD is a severe mental disorder that is very common in the global population. The HPA axis and MR, as well as the glutamate system and NMDA-R, play an important role in the pathophysiology of MDD. Importantly, MR and NMDA-R modulation has been shown to have beneficial antidepressant effects in patients with MDD. Accordingly, the receptors might serve as potential treatment targets to improve the symptomatology of patients with MDD. While there is some evidence for cognitive-enhancing effects of MR modulation in MDD, the role of NMDA-R in the symptomatology of MDD has rarely been studied. Therefore, this research project

examined the role of MR and NMDA-R in social cognitive processes in healthy individuals and patients with MDD.

1.8 Theoretical and empirical rationale in brief

In individuals who are confronted with stress, perception of the stressor activates the HPA axis leading to the secretion of glucocorticoids, such as cortisol and other steroid hormones such as aldosterone and DHEA-S (section 1.1 & section 1.2). Glucocorticoids bind upon MR and GR, and exert different effects in the psycho-neuro-endocrinological stress response, depending on the time post stress exposure (early or late phase of the stress response) and on the receptor type (genomic or non-genomic MR and GR), among other factors (section 1.3 & section 1.4). Furthermore, the glucocorticoid system is closely related to the glutamate system. By binding upon MR and GR, glucocorticoids contribute to glutamatergic signal transmission via NMDA-R (section 1.5 and Figure 3). Interestingly, disturbances in the glucocorticoid and glutamate system contribute to the pathophysiology of MDD. Patients with MDD show HPA axis hyperactivity accompanied by increased cortisol concentrations and, additionally, alterations in the glutamate system, expressed in heightened glutamate concentrations and NMDA-R dysfunctions among others (section 1.7). Therefore, it appears to be particularly important that there is some evidence that confirms: MR stimulation decreases cortisol secretion in healthy individuals and patients with MDD (section 1.1), separate MR and NMDA-R stimulation improves (social) cognition in healthy individuals (section 1.6.1) as well as several psychiatric disorders (section 1.7), and separate MR and NMDA-R stimulation has beneficial effects on the symptomatology of patients with MDD (section 1.7). Consequently, there is need for research that examines whether MR and NMDA-R might serve as potential treatment targets to influence steroid hormone secretion and to improve social cognition in healthy individuals and patients with MDD. Therefore, this research project examined the following research question:

What is the effect of MR and NMDA-R stimulation on steroid hormone secretion and social cognition in healthy individuals and patients with MDD?

The aforementioned empirical background (section 1.1 to section 1.7) led to the assumptions of this research project: that MR stimulation by fludrocortisone could influence steroid hormone secretion and also have beneficial effects on social cognition in

healthy individuals and patients with MDD. In addition, it was assumed that the beneficial effects of MR stimulation on social cognition might be enhanced by simultaneous MR and NMDA-R stimulation. The latter assumption was based on the observation that MR stimulation by glucocorticoids increases glutamatergic signal transmission via NMDA-R (section 1.5 and Figure 3). Accordingly, the theoretical model of the current research project was that MR stimulation by fludrocortisone might increase glutamate transmission via NMDA-R. In addition, simultaneous MR stimulation by fludrocortisone, and NMDA-R stimulation by DCS, might contribute to synergistic effects expressed by additionally enhanced glutamatergic signal transmission. The theoretical model is summarized in the following (Figure 4), the aims as well as the design of this research project will be described in detail thereafter (section 2).

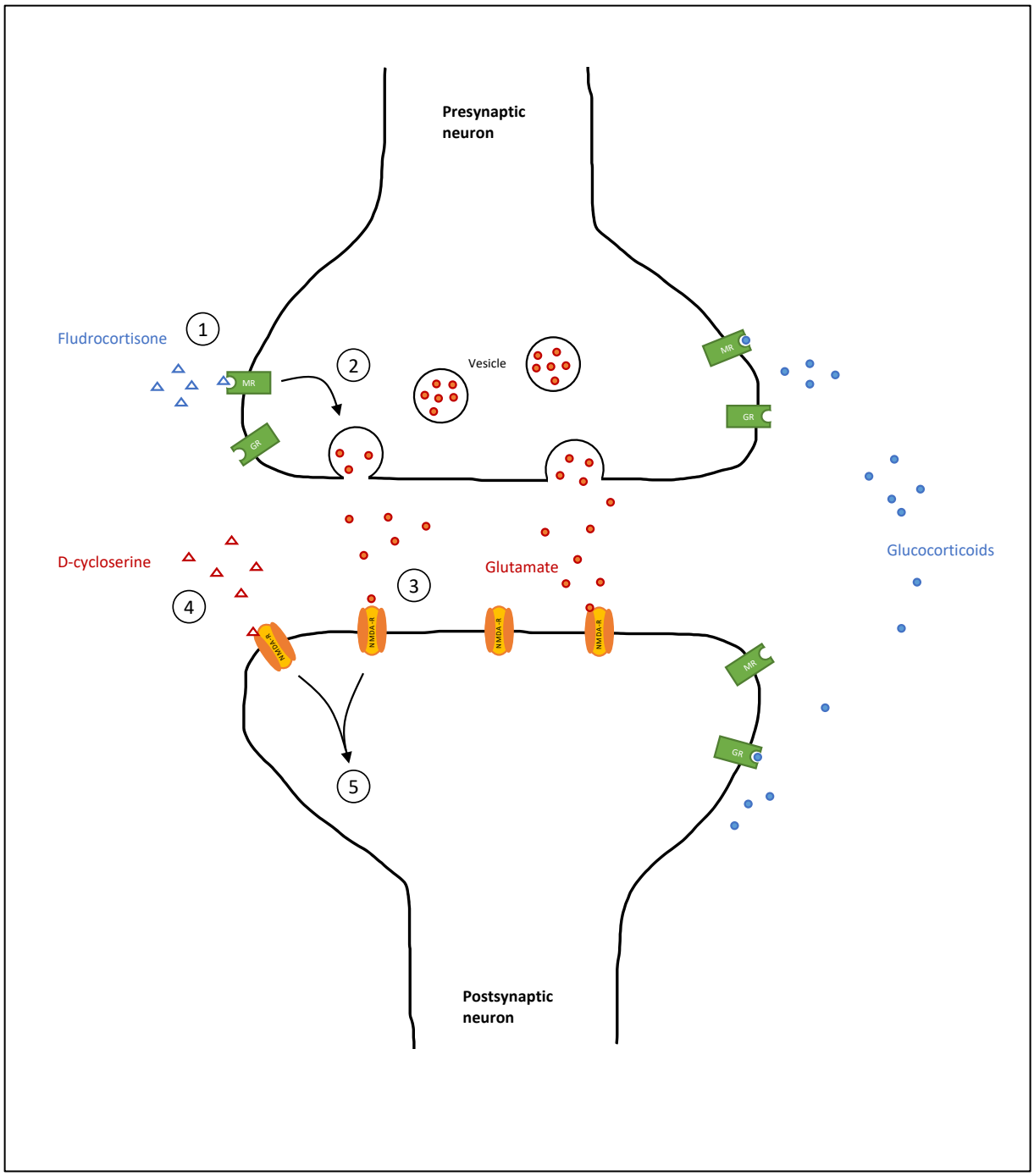

Figure 4. Schematic illustration of the theoretical model.

Note: The theoretical model assumes that glucocorticoid effects on glutamate transmission can be mimicked by pharmacological receptor modulation. Administration of (1) the MR agonist fludrocortisone may lead to (2) increased glutamate transmission via (3) NMDA-R activation. Simultaneous administration of fludrocortisone and (4) the partial NMDA-R agonist D-cycloserine may lead to synergistic effects being expressed by (5) enhanced glutamatergic signal transmission. Illustration based on existing models (Popoli et al., 2012).

2 Aims and Design of the Research Project

The aim of this research project was to examine the role of MR and NMDA-R in steroid hormone secretion and social cognitive processes in healthy individuals and patients with MDD. The three studies of the research project were based on the same sample of healthy individuals and patients with MDD. In the following sections, information refers to all three studies, if not otherwise specified. The first study aimed to examine the effect of MR and NMDA-R stimulation on steroid hormone secretion in healthy individuals and patients with MDD (Nowacki et al., 2020b). The aim of the second and third study was to examine whether the beneficial effect of MR stimulation can be extended to social cognitive processes and whether simultaneous stimulation of NMDA-R has additional beneficial effects (Nowacki et al., 2020a; Nowacki et al., 2021). The following sections will outline the research questions and hypotheses as well as the rationale of the studies.

2.1 Research questions and hypotheses

2.1.1 First study: Effects of MR and NMDA-R stimulation on steroid hormones

The aim of the first study was to examine the effect of MR and NMDA-R stimulation on steroid hormone secretion in healthy individuals and patients with MDD (Nowacki et al., 2020b). In short, the rationale of the first study was based on the following observations: MR stimulation inhibits the HPA axis which leads to decreased secretion of cortisol in healthy individuals and patients with MDD (section 1.1 & section 1.7). The steroid hormones cortisol, aldosterone, and DHEA-S are involved in the human stress response and in the pathophysiology of MDD (section 1.1, section 1.2, & section 1.7). Furthermore, there is evidence that the glucocorticoid and glutamate system are closely related. Glucocorticoids induce MR stimulation which contributes to increased glutamate transmission via NMDA-R (section 1.5). Despite the close relationship, the effect of separate MR and simultaneous MR and NMDA-R stimulation on steroid hormone secretion remains largely unknown. Therefore, the first study of this research project examined the effects of MR and NMDA-R stimulation on cortisol, aldosterone, and DHEA-S in in healthy individuals and patients with MDD. The following research question and hypothesis were raised.

Research question: What is the effect of MR and NMDA-R stimulation on steroid hormone secretion in healthy individuals and patients with MDD?

Hypothesis: MR stimulation by fludrocortisone leads to decreased cortisol concentrations in healthy individuals and patients with MDD in comparison to no stimulation (placebo).

2.1.2 Second study: Effects of MR and NMDA-R stimulation on social cognition (cognitive and emotional empathy)

The aim of the second study was to examine the effect of MR and NMDA-R stimulation on social cognition (cognitive and emotional empathy) in healthy individuals and patients with MDD (Nowacki et al., 2020a). In short, the rationale of the study was based on the following observations: MR stimulation has beneficial effects on cognitive processes of memory and executive functioning in healthy individuals and patients with MDD (section 1.6.1 & 1.7.3) and contributes to glutamatergic NMDA-R activation (section 1.5). Furthermore, NMDA-R stimulation has enhancing effects on (social) cognition in health and disease (section 1.6.2 & 1.7.3). Whether the beneficial effects of MR stimulation can be extended to social cognition and whether simultaneous MR and NMDA-R stimulation has additional beneficial effects remains largely unknown. Therefore, the second study examined the effect of MR and NMDA-R stimulation on social cognition (cognitive and emotional empathy) in healthy individuals and patients with MDD. The following research question and hypotheses were raised.

Research question: What is the effect of MR and NMDA-R stimulation on social cognition (cognitive and emotional empathy) in healthy individuals and patients with MDD?

Hypothesis (A): MR stimulation by fludrocortisone leads to higher scores in cognitive and emotional empathy in healthy individuals and patients with MDD in comparison to no stimulation (placebo).

Hypothesis (B): Simultaneous MR and NMDA-R stimulation by fludrocortisone and DCS leads to higher scores in cognitive and emotional empathy in healthy individuals and patients with MDD in comparison to separate MR stimulation and to no stimulation (placebo).

2.1.3 Third study: Effects of MR and NMDA-R stimulation on social cognition (emotion recognition and selective attention to emotional stimuli)

The aim of the third study was to examine the effect of MR and NMDA-R stimulation on social cognition (facial emotion recognition and selective attention to emotional stimuli) in healthy individuals and patients with MDD (Nowacki et al., 2021). The rationale of the third study mirrors the second study as previously mentioned (section 2.1.2). The following research question and hypotheses were raised.

Research question: What is the effect of MR and NMDA-R stimulation on social cognition (facial emotion recognition and selective attention to emotional stimuli) in healthy individuals and patients with MDD?

Hypothesis (A): MR stimulation by fludrocortisone leads to higher scores in facial emotion recognition and reduced selective attention to emotional stimuli in healthy individuals and patients with MDD in comparison to no stimulation (placebo).

Hypothesis (B): Simultaneous MR and NMDA-R stimulation by fludrocortisone and DCS leads to higher scores in facial emotion recognition and reduces selective attention to emotional stimuli in healthy individuals and patients with MDD in comparison to separate MR stimulation and to no stimulation (placebo).

2.2 Realization of the studies

The studies were conducted at Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Hindenburgdamm 30, 12203 Berlin, Germany. The studies were approved by the local ethics committee (Landesamt für Gesundheit und Soziales Berlin, 16-0031- EK 11) and conducted in accordance with the latest version of the Declaration of Helsinki. All participants provided written informed consent.

The following sections will describe the realization of the studies. The section starts with a description of the recruitment of the participants (section 2.2.1) and randomization and blinding procedure (section 2.2.2), before the section ends with a description of the treatments (section 2.2.3) and measurements (section 2.2.4) used in the studies. The following sections provide summarized information on the realization of

the studies. For further information on study realization, please see corresponding publications (Nowacki et al., 2020a; Nowacki et al., 2020b; Nowacki et al., 2021).

2.2.1 Participant recruitment

In total, 232 healthy individuals (*n* = 116) and patients with MDD (*n* = 116) were recruited to take part in the studies. Major depressive disorder patients were recruited via the in- and out-patient unit of the department of psychiatry and psychotherapy of the Charité – Universitätsmedizin Berlin, via the internet, and through flyers distributed in psychiatric and psychotherapeutic practices. Healthy individuals were recruited via the internet and through flyers distributed in universities and other public buildings. Major depressive disorder patients and healthy individuals were matched in age, sex, and education years. All participants received an expense allowance for participation. Detailed information on the inclusion and exclusion criteria, procedure, and assessments are described in the publications (Nowacki et al., 2020a; Nowacki et al., 2020b; Nowacki et al., 2021).

2.2.2 Randomization and blinding

Randomization and blinding were conducted by the pharmacy of the Charité – Universitätsmedizin Berlin. Randomization was conducted with a parallel-group four-block design (4, 3, 1, 2; 3, 4, 2, 1; and so forth), to ensure an equal number of healthy individuals (*n* = 29) and MDD patients (*n* = 29) in each of the four treatment conditions. To ensure double-blinding, the pharmacy provided two identical-looking capsules that contained either 0.4 mg fludrocortisone, 250 mg D-cycloserine (DCS), and/or placebo. Participants were randomized to the following treatment conditions: (A) placebo + placebo, (B) fludrocortisone + placebo, (C) placebo + DCS, or (D) fludrocortisone + DCS. Further information on randomization and blinding are described in the publications (Nowacki et al., 2020a; Nowacki et al., 2020b; Nowacki et al., 2021).

2.2.3 Treatments

MR stimulation: In order to stimulate MR, 0.4 mg fludrocortisone (tradename: Astonin H) was used. Drug and dosage choices were based on several earlier studies of the research group in which fludrocortisone effectively modulated cortisol secretion and demonstrated effects on cognitive processes in health and disease (Otte et al., 2015a; Schultebraucks et al., 2016; Wingenfeld et al., 2014). Fludrocortisone, also referred to as

9α-fluorocortisol, binds with a high affinity to MR and has some GR potency. Compared to cortisol and aldosterone, fludrocortisone has a much higher potency for both receptors (Agarwal et al., 1977; Grossmann et al., 2004). In blood, fludrocortisone is measurable 20 minutes after oral intake, peak concentrations are reached between 90-120 minutes, and the mean half-time is 4.9 hours (Quinkler et al., 2015). Clinically, fludrocortisone is used to treat adrenal deficiency and postural hypotension (de Kloet, 2014). Several recent studies emphasize that fludrocortisone has antidepressant effects in MDD patients (Otte et al., 2010) and cognitive-enhancing effects in health and disease (Wingenfeld & Otte, 2019).

NMDA-R stimulation: In order to stimulate NMDA-R, 250 mg D-cycloserine (DCS; tradename: Cycloserine) was used. Drug and dosage choices were based on earlier studies in which cognitive-enhancing effects were observed in healthy individuals (Onur et al., 2010; Scholl et al., 2014). D-cycloserine binds upon the NMDA-R glycine site as a partial agonist in low doses and serves as an antagonist in higher doses. Clinically, the antibiotic is primarily used to treat tuberculosis (Peyrovian et al., 2019). Furthermore, some studies emphasize antidepressant effects in MDD patients (Heresco-Levy et al., 2013; Schade & Paulus, 2016) and recent research provides evidence for cognitive-enhancing effects in several psychiatric disorders (Peyrovian et al., 2019).

2.2.4 Measurements

First study: Salivary steroid hormone concentrations (cortisol, aldosterone, and DHEA-S), blood pressure, and heart rate were measured before and after drug administration at several measurement time points on the testing day. Measurements took place at the same time for all participants due to circadian rhythmicity of cortisol secretion (Joëls et al., 2012). Cardiovascular risk parameters were measured in blood samples collected at the in-person-visit on a separate day. For further information on the measurements, please see publication (Nowacki et al., 2020b).

Second study: In order to measure empathy, a modified version (Deuter et al., 2018) of the *Multifaceted Empathy Test* (MET) (Dziobek et al., 2008) was used. The MET measures cognitive empathy (the ability to understand another person's emotions) and emotional empathy (the ability to empathize with another person). Participants were asked to rate pictures of individuals in an emotional state with regard to cognitive and emotional empathy. For further information on the measurements, please see publication (Nowacki et al., 2020a).

Third study: The *Facial Emotion Recognition Task* was used to measure recognition of facial emotion expression, as in an earlier study of the research group (Duesenberg et al., 2016). In the task, participants were asked to recognize the emotion of neutral, sad, or angry facial expressions presented in 40% and 80% intensities on a computer screen. Selective attention to emotional stimuli was measured with the *Emotional Dot-Probe paradigm* (MacLeod et al., 1986) in a modified version (Schultebraucks et al., 2016). The task measures selective attention to sad, happy, or neutral facial expressions by asking participants to indicate the position of a vertical bar that appeared subsequent to an emotional facial expression on a computer screen. For further information on the measurements, please see publication (Nowacki et al., 2021).

To summarize this section, this research project examined the effect of MR and NMDA-R stimulation on steroid hormone secretion and social cognition in healthy individuals and patients with MDD in three experimental clinical studies (Nowacki et al., 2020a; Nowacki et al., 2020b; Nowacki et al., 2021). The following sections will provide detailed information on the first study (section 3: effects of MR and NMDA-R stimulation on steroid hormones), second study (section 4: effects of MR and NMDA-R stimulation on empathy), and third study (section 5: effects of MR and NMDA-R stimulation on emotion recognition and selective attention to emotional stimuli).

3 Effects of MR and NMDA-R Stimulation on Steroid Hormones (First Study)

This chapter has been published as: Nowacki, J., Wingenfeld, K., Kaczmarczyk, M., Chae, W. R., Salchow, P., Abu-Tir, I., Piber, D., Hellmann-Regen, J., & Otte, C. (2020). Steroid hormone secretion after stimulation of mineralocorticoid and NMDA receptors and cardiovascular risk in patients with depression. *Translational Psychiatry*, 10(1), 109.

DOI: 10.1038/s41398-020-0789-7
https://doi.org/10.1038/s41398-020-0789-7

Nowacki et al. *Translational Psychiatry* (2020)10:109
https://doi.org/10.1038/s41398-020-0789-7

Translational Psychiatry

ARTICLE Open Access

Steroid hormone secretion after stimulation of mineralocorticoid and NMDA receptors and cardiovascular risk in patients with depression

Jan Nowacki[1], Katja Wingenfeld[1], Michael Kaczmarczyk[1], Woo Ri Chae[1], Paula Salchow[1], Ikram Abu-Tir[1], Dominique Piber[1], Julian Hellmann-Regen[1] and Christian Otte[1]

Abstract

Major depressive disorder (MDD) is associated with altered mineralocorticoid receptor (MR) and glucocorticoid receptor function, and disturbed glutamatergic signaling. Both systems are closely intertwined and likely contribute not only to the pathophysiology of MDD, but also to the increased cardiovascular risk in MDD patients. Less is known about other steroid hormones, such as aldosterone and DHEA-S, and how they affect the glutamatergic system and cardiovascular disease risk in MDD. We examined salivary cortisol, aldosterone, and DHEA-S secretion after stimulation of MR and glutamatergic NMDA receptors in 116 unmedicated depressed patients, and 116 age- and sex-matched healthy controls. Patients (mean age = 34.7 years, SD = ±13.3; 78% women) and controls were randomized to four conditions: (a) control condition (placebo), (b) MR stimulation (0.4 mg fludrocortisone), (c) NMDA stimulation (250 mg D-cycloserine (DCS)), and (d) combined MR/NMDA stimulation (fludrocortisone + DCS). We additionally determined the cardiovascular risk profile in both groups. DCS had no effect on steroid hormone secretion, while cortisol secretion decreased in both fludrocortisone conditions across groups. Independent of condition, MDD patients showed (1) increased cortisol, increased aldosterone, and decreased DHEA-S concentrations, and (2) increased glucose levels and decreased high-density lipoprotein cholesterol levels compared with controls. Depressed patients show profound alterations in several steroid hormone systems that are associated both with MDD pathophysiology and increased cardiovascular risk. Prospective studies should examine whether modulating steroid hormone levels might reduce psychopathology and cardiovascular risk in depressed patients.

Introduction

Stress is a risk factor for the development of major depressive disorder (MDD)[1] and cardiovascular disease (CVD)[2]. Furthermore, stress activates the hypothalamus–pituitary–adrenal (HPA) axis leading to the release of the steroid hormone cortisol and consecutive enhanced secretion of the neurotransmitter glutamate[3]. Both systems are closely intertwined[3,4], and altered secretion of cortisol and glutamate is not only involved in the pathogenesis of MDD[5,6], but may also contribute to the increased cardiovascular risk of depressed patients[1,7–9]. However, to our knowledge, steroid hormone secretion after separate or combined stimulation of the HPA axis, and glutamatergic system in depressed patients and healthy controls has not been studied so far.

Cortisol acts upon glucocorticoid receptors (GR) and mineralocorticoid receptors (MR) in the central nervous system. While GR are widely distributed in the brain, MR are predominantly expressed in the hippocampus and prefrontal cortex. MR are predominantly occupied during basal cortisol secretion, whereas GR are increasingly occupied as cortisol levels rise, for example, after stress. Cortisol binding to GR and MR inhibits HPA axis activity[1,10,11]. In MDD, this negative feedback is impaired and

Correspondence: Jan Nowacki (jan.nowacki@charite.de)
[1]Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Hindenburgdamm 30, 12203 Berlin, Germany

cortisol levels increase[12], possibly because of impaired MR function[13–15].

Elevated cortisol alters glutamate signaling in the hippocampus and prefrontal cortex[3]. Not surprisingly, MDD is associated with disturbed glutamatergic signaling. For example, decreased levels of glutamatergic metabolites have been reported in the medial frontal cortex of patients with MDD[6,16]. Glutamate acts on metabotropic and ionotropic receptors[3], including the N-methyl-D-aspartate (NMDA) receptor, which has been closely implicated in the pathogenesis of MDD[17]. In fact, the U.S. Food and Drug Administration[18] recently approved the rapid-acting NMDA receptor antagonist ketamine as a treatment for treatment-resistant depression after its efficacy was shown in several trials[19]. Importantly, ketamine strongly elevates cortisol levels[20,21]. However, the glutamate system is extremely complex and there is evidence that D-cycloserine (DCS), a partial agonist at the glycine binding site of the NMDA receptor, exhibits antidepressant effects[22,23], and increases glutamate and GABA in the brain to the same extent as ketamine[24].

As well as contributing to the pathogenesis of MDD, alterations in HPA activity and glutamate signaling may also contribute to the elevated risk of CVD in depressed patients[7,25,26]. Other steroid hormones, such as increased aldosterone[27,28] and decreased DHEA-S levels[29], are also closely linked to CVD. Importantly, increased aldosterone levels[30–32] and decreased DHEA-S concentrations[33] have been found in depressed patients, and both hormones interact with the glutamate system[34–36].

Taken together, the HPA axis and the glutamatergic system play an important role in the pathogenesis of depression and might represent an important link to CVD. However, little is known about the interplay of both systems in MDD. To address this, we examined (a) salivary cortisol, aldosterone, and DHEA-S secretion after stimulation of MR and glutamatergic NMDA receptors, and (b) the cardiovascular risk profile in 116 unmedicated depressed patients and 116 age- and sex-matched healthy controls.

Materials and methods

Participants

In total, we examined 116 MDD patients and 116 healthy controls. We recruited patients from our in- and outpatient clinics for affective disorders (Department of Psychiatry and Psychotherapy of the Charité – Universitätsmedizin Berlin), via our website, and through flyers distributed in outpatient psychiatric practices and psychotherapy institutes. Healthy participants were recruited via our website and through flyers distributed in universities and other public spaces.

We matched depressed patients with healthy controls based on sex, age, and education duration. For every enrolled depressed patient, we recruited a control subject who was matched on these characteristics. Inclusion criteria were 18–65 years of age, a diagnosis of MDD according to the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5)[37], and a score of 18 or more on the Hamilton rating scale for depression (HAMD)[38].

Exclusion criteria were intake of psychotropic medication during the last 5 days (except antidepressants as sleep medication and benzodiazepines as needed), substance abuse or dependency within the last 6 months, any current episode or history of schizophrenia, schizoaffective, or bipolar disorder (for healthy individuals, the presence of any psychiatric disorders), neuroendocrine disorders, current or past organic brain disease, acute suicidality, endocrine disorders or intake of medication with neuroendocrine effects, pregnancy or lactation, unstable cardiovascular conditions, known intolerance of study medication, or significantly abnormal laboratory values.

All participants provided written informed consent and received an expense allowance. The study was conducted in accordance with the latest version of the Declaration of Helsinki and was approved by the local ethics committee (Landesamt für Gesundheit und Soziales Berlin, 16-0031-EK 11).

Experimental design

We used a randomized double-blind placebo-controlled parallel group design. The pharmacy of the Charité – Universitätsmedizin Berlin conducted the block randomization and blinded the medication. To stimulate MR, we used 0.4 mg fludrocortisone. To stimulate NMDA receptors, we used 250 mg DCS. In the control condition, we administered placebo. Participants were randomly assigned to one of the following four conditions: (a) control condition (placebo + placebo), (b) MR stimulation only (fludrocortisone + placebo), (c) NMDA stimulation only (placebo + DCS), and (d) combined MR and NMDA stimulation (fludrocortisone + DCS). Twenty-nine depressed patients and 29 healthy controls took part in each condition.

Procedure

Participants were assessed for eligibility by telephone interview, and eligible participants were invited for the formal screening visit. An experienced clinician (physician or psychologist) from our team interviewed participants to acquire demographic information and to diagnose or exclude MDD according to DSM-5 criteria[37]. The HAMD interview[38] was also conducted and participants were asked to complete the Beck Depression Inventory[39] before undergoing an electrocardiogram. To assess the cardiovascular risk profile, we measured blood pressure and heart rate, and took blood samples for laboratory analyses.

The experiment (separate or combined stimulation of MR and NMDA receptors) took place at least 24 h and not >7 days after the screening visit.

All experiments started at the same time (11:30 h) to control for influences of the circadian rhythm on cortisol secretion[40]. After arriving at the laboratory, participants rested for 30 min before the first blood pressure and heart rate measurements were taken. Two baseline saliva samples were taken at 11:55 h and 12:00 h. From 12:00 h on, we measured blood pressure and heart rate and took saliva samples every hour until 18:00 h. Participants received the first medication at 12:05 h and the second medication at 13:05 h (Supplementary Fig. 1).

At three time points (prior to medication 11:50 h, during the experiment 13:50 h, and at the end of the experiment 17:50 h), we assessed the current mood state of all participants with a visual analogue mood scale (VAMS). We asked all participants to answer the question "how are you currently feeling?" by making a cross on the VAMS, which ranged from 0 (very bad) to 100 (very good).

Between measurements, participants were allowed to walk around, read, or watch a movie. Participants were allowed to drink water, but did not eat during the experiment (11:30 h until 18:00 h). Ten minutes before every measurement, participants were asked to rest, sit on a chair, and stop drinking water. An experimenter was present during the whole testing period. Participants conducted cognitive tasks on a computer between 16:00 h and 17:00 h for ~45 min.

Cardiovascular risk assessment

The cardiovascular risk assessment took place at the screening visit. We measured systolic and diastolic blood pressure (mmHg), and heart rate (bpm) using the Boso Medicus Uno (Bosch + Sohn, Germany) apparatus as a hemodynamometer. Blood samples were analyzed by the Labor Berlin (Charité – Universitätsmedizin Berlin). We measured total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, C-reactive protein (CRP), and glucose (all measured in mg/l or mg/dl respectively).

Steroid hormone measurement

We collected saliva samples with Code Blue Salivettes® (Sarstedt, Germany) on the day of the experiment. Steroid levels were analyzed in the neurobiological laboratory at the Department of Psychiatry and Psychotherapy of the Charité – Universitätsmedizin Berlin. For all salivary analyses, we used enzyme-linked immunosorbent assays (ELISA; IBL International GmbH, Germany). For cortisol analyses (measured in nmol/L), an ELISA kit with a detection limit of 0.08 nmol/L was used. For aldosterone analyses (measured in pg/mL), we used an ELISA kit with a detection limit of 12 pg/mL. DHEA-S levels (measured in ng/mL) were measured using an ELISA kit optimized for saliva with a detection limit of 0.05 ng/mL. The intra-assay coefficients of variation were <8% and the inter-assay coefficients of variation were <10% for all analyses. To improve comparability, we converted all steroid hormone measurement units into pg/mL for all figures.

Statistical analyses

Statistical analyses were conducted with IBM SPSS Statistics (version 25). Greenhouse–Geisser corrections or Welch tests were applied if assumptions of sphericity or homogeneity of variances were violated. Post hoc analyses were conducted with Bonferroni tests or contrasts if applicable.

To analyze demographic variables, we used chi-squared tests for categorical data and independent *t*-tests for continuous data. If the assumptions of the chi-squared test were violated, we used Fisher's exact test. For cardiovascular risk assessment (blood pressure, heart rate, cholesterol, HDL cholesterol, LDL cholesterol, CRP, and glucose), we used independent *t*-tests for group comparisons.

Steroid hormone concentrations (cortisol, aldosterone, and DHEA-S) were analyzed with mixed ANOVAs with within-subject factor time (measurement time points), between-subject factors group (depressed patients and healthy controls), and condition (placebo, fludrocortisone, DCS, and fludrocortisone + DCS).

All non-normally distributed data were log transformed. Missing values for single cortisol and DHEA-S measurement time points in four participants were replaced by mean imputation (mean value of the preceding and subsequent measurement time points) to avoid loss of data.

The current mood state was analyzed with mixed ANOVAs with within-subject factor time (measurement time points), between-subject factors group (depressed patients and healthy controls), and condition (placebo, fludrocortisone, DCS, and fludrocortisone + DCS).

We calculated correlations between the cardiovascular risk assessment (blood pressure, heart rate, cholesterol, HDL cholesterol, LDL cholesterol, CRP, and glucose) measured at the screening visit and steroid hormone secretion during the experiment. For the steroid hormones cortisol, aldosterone, and DHEA-S, we calculated area under the curve values with respect to the ground.

Sample size was calculated with G*Power[41]. Effect sizes for condition effects were based on the fludrocortisone effects on cortisol ($\eta^2 = 0.12$) reported in our earlier study[42]. The effect size ($\eta^2 = 0.10$) for group (depressed patients versus controls) were based on a meta-analysis on differences in the cortisol response to stress between depressed patients and controls[43]. Using mixed ANOVAs with $\eta^2 = 0.10$, $\alpha = 0.05$, and $1 - \beta = 0.95$, we calculated a

total sample size of $n = 120$. To be able to find smaller effects and considering possible dropouts, we conservatively recruited a larger sample of $n = 232$ participants: $n = 116$ per group, and $n = 58$ per condition.

Results

Sample characteristics

Depressed patients and healthy controls did not differ in age, sex, education duration, or intake of hormonal contraceptives. There were more smokers among depressed patients than among healthy controls (Table 1). Therefore, we repeated the analyses in nonsmokers to examine a possible confounding effect of smoking status. Additional analyses on sample and depression characteristics with respect to condition are presented in the supplement (Supplementary Tables 1 and 2).

Depressed patients took the following medication: benzodiazepines as needed ($n = 13$), low-dose antidepressants as sleep medication ($n = 5$), cetirizine ($n = 1$), pantoprazole ($n = 1$), ramipril ($n = 3$), lercanidipine ($n = 1$), simvastatin ($n = 2$), rosuvastatin ($n = 1$), L-thyroxine ($n = 11$), propylthiouracil ($n = 1$), dorzolamide ($n = 1$), actaea racemosa ($n = 1$), sumatriptan ($n = 1$), amlodipine ($n = 2$), indapamide ($n = 1$), valsartan ($n = 1$), and zopiclone ($n = 2$). Healthy controls took the following medication: salbutamole ($n = 1$), L-thyroxine ($n = 10$), tapentadol ($n = 1$), mesalazine ($n = 1$), ramipril ($n = 1$), metoprolol ($n = 1$), and estradiol ($n = 1$).

Steroid hormone response to separate or combined MR and NMDA receptor stimulation

Cortisol

We found a main effect of group on cortisol levels ($F(1,222) = 4.0$, $p < 0.05$, $\eta^2 = 0.02$), indicating that depressed patients had higher cortisol concentrations compared with healthy controls independent of condition and time (Fig. 1a). In addition, we found a main effect of condition ($F(3,222) = 4.8$, $p < 0.01$, $\eta^2 = 0.06$) and time ($F(3,696) = 266.2$, $p < 0.001$, $\eta^2 = 0.55$) on cortisol concentrations, and an interaction between condition × time ($F(9,696) = 11.0$, $p < 0.001$, $\eta^2 = 0.13$). One-way ANOVAs with Bonferroni post hoc tests revealed decreased cortisol secretion in both fludrocortisone conditions compared with the placebo and DCS-only conditions (all $p < 0.05$; Fig. 1b, c). Analyses in nonsmokers confirmed the results.

Aldosterone

We found a main effect of group on aldosterone levels ($F(1,222) = 10.2$, $p < 0.01$, $\eta^2 = 0.04$), indicating that depressed patients had higher aldosterone concentrations compared with healthy controls independent of condition and time (Fig. 2a). We found no main effect of condition ($p > 0.05$; Fig. 2b, c) but a main effect of time ($F(2,660) = 33.7$, $p < 0.001$, $\eta^2 = 0.13$) on aldosterone levels, indicating a decrease in aldosterone concentrations. Analyses in nonsmokers confirmed the results.

DHEA-S

There was a main effect of group on DHEA-S concentrations ($F(1,222) = 8.3$, $p < 0.01$, $\eta^2 = 0.04$), indicating that depressed patients had lower DHEA-S concentrations compared with healthy controls independent of condition and time (Fig. 3a). We found no main effect of condition ($p > 0.05$; Fig. 3b, c) but a main effect of time ($F(3,704) = 12.3$, $p < 0.001$, $\eta^2 = 0.05$) on DHEA-S concentrations. Analyses in nonsmokers revealed a slightly reduced effect size for the main effect of group ($F(1,179) = 3.7$, $p = 0.056$, $\eta^2 = 0.02$) and confirmed the time effect ($p < 0.001$).

Table 1 Sample characteristics.

	Healthy controls	Depressed patients	Statistics
n	116	116	
Age, mean (*SD*)	34.9 (13.2)	34.7 (13.3)	$t(230) = 0.1$, $p = 0.90$
Women, *n* (%)	91 (78%)	91 (78%)	
Education years	12.1 (1.3)	11.8 (1.3)	$t(230) = 1.6$, $p = 0.12$
BMI	23.5 (3.4)	24.0 (4.3)	$t(230) = -0.9$, $p = 0.36$
Smoker	14 (12%)	30 (26%)	$\chi^2(1) = 7.2$, $p < 0.01$
Hormonal contraception	19 (21%)	19 (21%)	
HAMD	1.6 (1.3)	21.5 (3.4)	$t(149) = -58.7$, $p < 0.001$
BDI	1.4 (1.8)	25.7 (8.3)	$t(125) = -30.8$, $p < 0.001$

Values represent mean (SD) or *n* (%).
BMI body mass index, *HAMD* Hamilton ratings scale for depression, *BDI* Beck Depression Inventory.

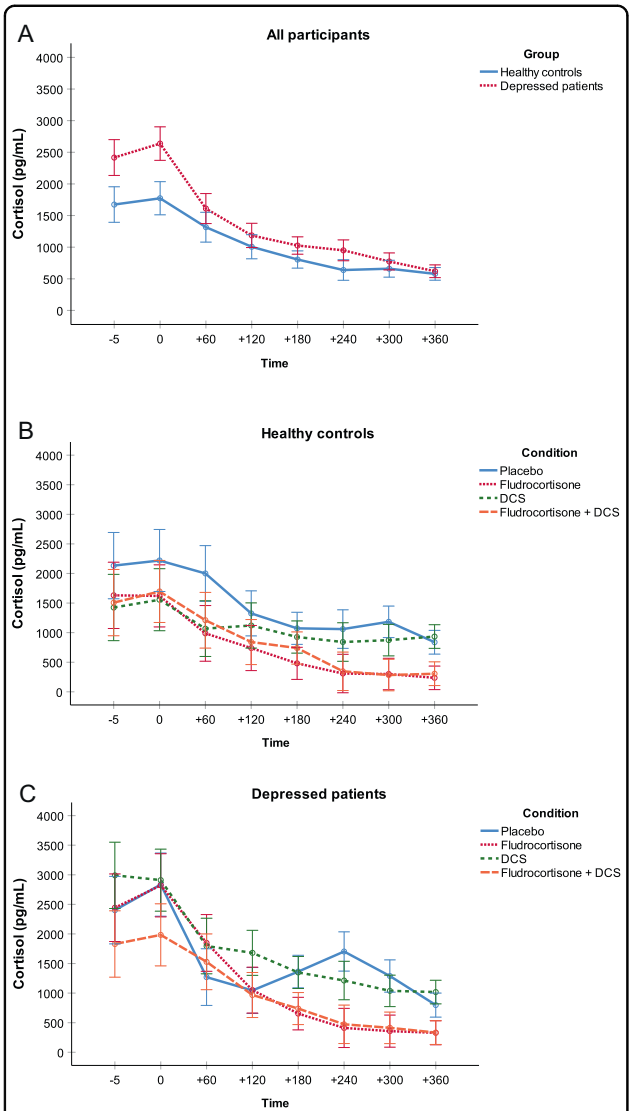

Fig. 1 Cortisol secretion. Mean cortisol secretion in pg/mL (SE) for **a** both groups across conditions, **b** healthy controls for each condition, and **c** depressed patients for each condition.

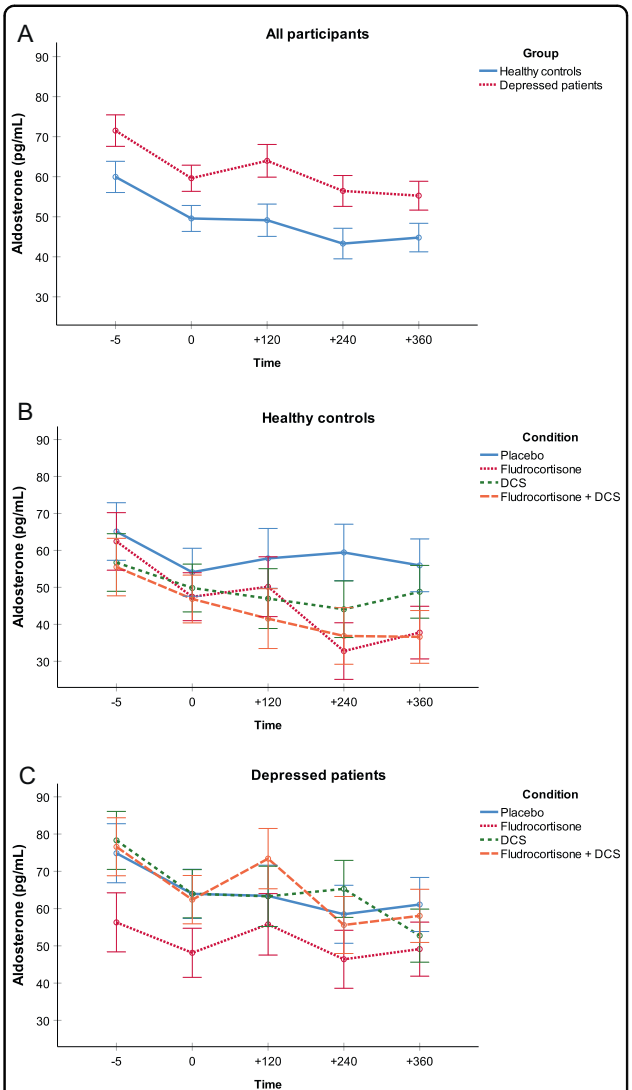

Fig. 2 Aldosterone secretion. Mean aldosterone secretion in pg/mL (SE) for **a** both groups across conditions, **b** healthy controls for each condition, and **c** depressed patients for each condition.

Blood pressure and heart rate

For systolic blood pressure, we found a main effect of time ($F(5,1150) = 43.6$, $p < 0.001$, $\eta^2 = 0.16$) and a condition × time interaction ($F(15,1150) = 2.4$, $p < 0.01$, $\eta^2 = 0.31$), indicating an increase in systolic blood pressure within each condition over time (Supplementary Fig. 2). There was a main effect of time on diastolic blood pressure ($F(5,1205) = 44.6$, $p < 0.001$, $\eta^2 = 0.17$) and a condition × time interaction ($F(16,1205) = 2.9$, $p < 0.001$, $\eta^2 = 0.37$), indicating an overall increase in diastolic blood pressure within each condition over time (Supplementary Fig. 3).

We found a main effect of group on heart rate ($F(1,224) = 15.4$, $p < 0.001$, $\eta^2 = 0.06$) and a main effect of time on heart rate ($F(4,1025) = 188.5$, $p < 0.001$, $\eta^2 = 0.46$). In addition, we found a group × condition interaction ($F(3,224) = 2.9$, $p < 0.05$, $\eta^2 = 0.04$), indicating increased heart rate in depressed patients compared with healthy controls within the fludrocortisone-only condition (Supplementary Fig. 4). Analyses in nonsmokers confirmed these results.

Mood assessment

We found a main effect of group on current mood state ($F(1,222) = 200.0$, $p < 0.01$, $\eta^2 = 0.47$), indicating that depressed patients were in a worse mood compared with healthy controls (Supplementary Fig. 5). However, there was neither a main effect of condition or time nor a condition × group interaction. We found an interaction

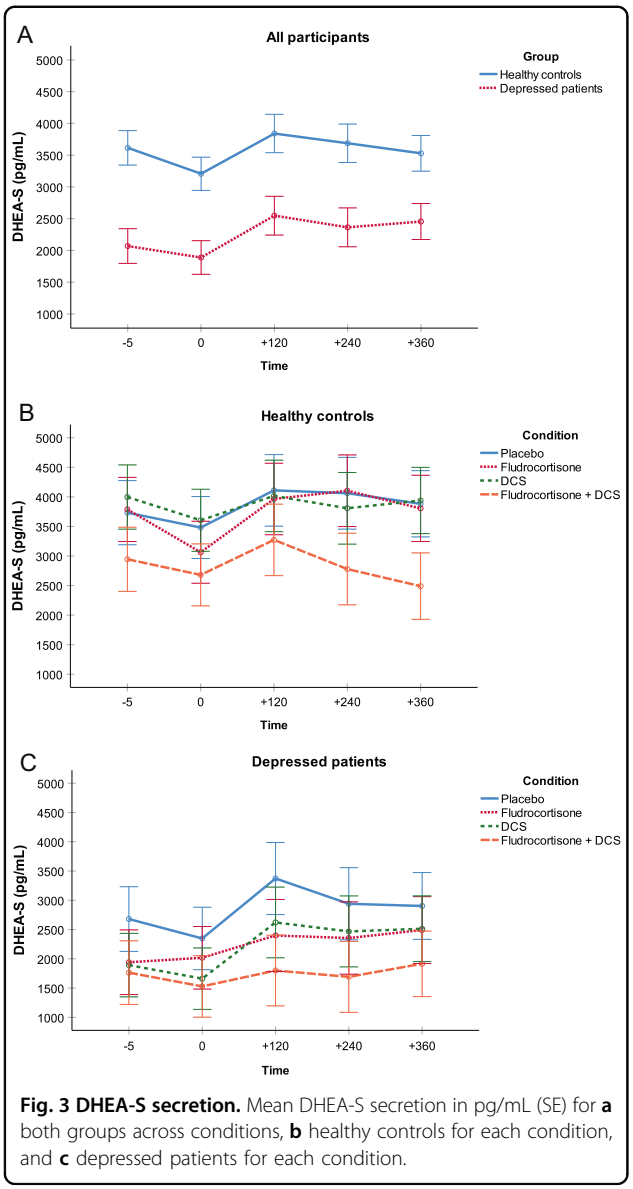

Fig. 3 DHEA-S secretion. Mean DHEA-S secretion in pg/mL (SE) for **a** both groups across conditions, **b** healthy controls for each condition, and **c** depressed patients for each condition.

between group × time ($F(1,378) = 10.5$, $p < 0.001$, $\eta^2 = 0.05$), indicating that subjective mood ratings of depressed patients slightly increased during the experiment, while healthy individuals exhibited a slight decrease in subjective mood (Supplementary Fig. 5).

Cardiovascular risk

Depressed patients and healthy controls did not differ in blood pressure, heart rate, total cholesterol, LDL cholesterol, and CRP. However, we found lower HDL cholesterol and higher glucose levels in depressed patients than in healthy controls (Table 2). Analyses in the group of nonsmokers confirmed the results and also showed higher diastolic blood pressure in depressed patients ($M = 80.6$, SD = 7.3) than in healthy controls ($M = 77.9$, SD = 8.7; $p < 0.05$).

Correlational analyses

We calculated correlations between cardiovascular risk variables and steroid hormone values. To control for multiple testing, we performed Bonferroni corrections ($p = 0.0018$, after 0.05/27). We found a correlation between cortisol and aldosterone ($r = 0.34$, $p < 0.001$), but no other correlations were significant.

Discussion

The aim of this study was to examine salivary cortisol, aldosterone, and DHEA-S secretion in depressed patients and healthy controls after stimulation of MR with fludrocortisone and glutamatergic NMDA receptors with DCS. We also examined CVD risk in these participants. We report four main results: (1) NMDA receptor stimulation with DCS did not affect steroid hormone secretion in depressed patients and healthy individuals, (2) MR stimulation with fludrocortisone inhibited cortisol secretion across groups, (3) depressed patients showed higher cortisol and aldosterone values, but lower DHEA-S concentrations compared with healthy individuals independent of condition, and (4) depressed patients had higher glucose levels and lower HDL cholesterol values than healthy controls.

There is evidence for a bidirectional association between glutamate signaling and the HPA axis. For example, stress and elevated cortisol levels increase glutamate signaling in the hippocampus and prefrontal cortex[3]. Conversely, the NMDA receptor antagonist ketamine activates the HPA axis and elevates cortisol levels[20,21,44,45]. However, in this study, the partial NMDA receptor agonist DCS did not affect cortisol secretion, which is in line with earlier studies[46–48]. Interestingly, a previous study showed that ketamine but not memantine (another NMDA receptor antagonist) increases cortisol secretion[44]. These findings suggest that ketamine increases cortisol secretion independently of the NMDA receptor. DCS has attracted tremendous interest in neuroscience research because of its role in learning, neuroplasticity, memory, and as a potential antidepressant[22,49,50]. Our results suggest that these effects are independent of HPA activity.

NMDA receptor stimulation did not affect hormone secretion, but the MR agonist fludrocortisone inhibited cortisol (but not aldosterone or DHEA-S) across groups. It is well known that MR stimulation inhibits HPA activity[51,52]. In accordance with our previous findings, the cortisol responses to fludrocortisone did not differ between young, unmedicated depressed patients and healthy individuals in the current study[53]. This suggests that MR function is intact in these patients. However, Lembke et al.[14] found attenuated MR-mediated inhibition

Table 2 Cardiovascular risk in depressed patients and healthy controls.

	Healthy controls	Depressed patients	Statistics
Systolic blood pressure	119.4 (13.2)	119.1 (12.1)	$t(230) = 0.2$, $p = 0.87$
Diastolic blood pressure	78.4 (8.5)	79.7 (7.2)	$t(223) = -1.2$, $p = 0.22$
Heart rate	71.6 (10.8)	73.7 (10.0)	$t(230) = -1.5$, $p = 0.12$
Total cholesterol	181.2 (35.6)	184.6 (39.1)	$t(229) = -0.7$, $p = 0.49$
HDL cholesterol	69.4 (19.6)	64.1 (17.4)	$t(229) = 2.1$, $p = 0.03$*
LDL cholesterol	108.0 (33.2)	112.4 (33.3)	$t(229) = -1.0$, $p = 0.32$
CRP	1.6 (3.6)	1.8 (2.8)	$t(229) = -0.5$, $p = 0.59$
Glucose	84.2 (14.8)	88.3 (13.1)	$t(230) = -2.2$, $p = 0.03$*

Values represent mean (SD) and significant differences are marked (*).
HDL cholesterol high-density lipoprotein cholesterol, *LDL cholesterol* low-density lipoprotein cholesterol, *CRP* C-reactive protein.

of cortisol secretion in patients with psychotic depression. Furthermore, Juruena et al.[13] observed diminished MR function in patients with treatment-resistant depression. Given the well-established glucocorticoid resistance in depressed patients[54], the authors speculated that patients with treatment-resistant depression (and potentially patients with psychotic depression) are not able to compensate for GR resistance by increasing MR function. However, our findings suggest that this might be possible in less severely depressed patients.

The third main result was that depressed patients had higher cortisol and aldosterone values, but lower DHEA-S concentrations than healthy individuals. To our knowledge, this is the first study to demonstrate alterations of three important steroid hormones in the same patients. Importantly, all of these hormones are stress responsive[27,55] and have been associated with depression[5,30,33,56,57]. While increased cortisol secretion in depressed patients is well established[5,12], only few studies have examined aldosterone secretion in MDD. However, these studies have consistently found increased aldosterone levels in depressed patients compared with healthy individuals[30,57]. Furthermore, salivary aldosterone was associated with depression severity[58] and predicted treatment response to standard antidepressants[59,60]. In turn, patients with primary aldosteronism exhibit more depressive symptoms compared with the general population[61,62]. Finally, animal studies demonstrated that aldosterone increases earlier than corticosterone after induction of depressive behavior[63] and that subchronic treatment with aldosterone induces depression-like behaviors in rats[64]. In sum, these studies suggest that aldosterone is closely involved in the pathophysiology of MDD.

With our cross-sectional design, we cannot determine causality between depression and altered steroid hormone secretion. However, there is strong evidence that alterations in steroid hormones contribute to the development of depression. For example, childhood adversity is associated with altered cortisol and DHEA secretion[55], which in turn increases the risk of depression[65,66]. Furthermore, aldosterone induces depressive symptoms in animals[64,67]. On the other hand, depression itself can alter steroid hormone concentrations. For example, the lifestyle of depressed patients (such as poor diet, reduced sleep, and less physical activity) affects steroid hormone secretion[68]. Therefore, the association between depression and altered steroid secretion is likely bidirectional, leading to a vicious circle of more severe depression and more profound disturbances in steroid hormone signaling.

These alterations in steroid hormone secretion likely contribute to the increased cardiovascular risk in depressed patients. Indeed, we show in the present study that depressed patients had higher glucose levels and lower HDL cholesterol values than healthy controls. Increased aldosterone is an established risk factor for mortality in CVD, and blocking MR has beneficial effects on many CVD endpoints, including mortality[69,70]. Several prospective studies in different populations have shown that higher cortisol values are associated with cardiovascular mortality[71–73]. In addition, a meta-analysis of 25 studies showed an association between low DHEA-S levels and increased mortality in patients with CVD[29]. Therefore, our findings have strong clinical implications because they suggest that these endocrine alterations in depressed patients contribute to their increased CVD risk and their increased mortality. In our cross-sectional study, however, there was no correlation between any steroid hormone and any CVD risk factor. One explanation might be that we examined a relatively young population of unmedicated depressed patients and healthy controls, who did not suffer (yet) from severe metabolic and/or cardiovascular conditions. Indeed, our participants were younger and less physically impaired compared with participants of studies that found an association between

steroid hormones and CVD[74], or an association between steroid hormones and cardiovascular mortality[71–73]. Future longitudinal studies should examine whether modulating these endocrine systems can improve CVD risk and psychopathology in depressed patients. Randomized controlled trials have revealed encouraging evidence that DHEA both decreases CVD risk and improves depressive symptoms[75,76].

Our study had several limitations. First, in our sample there were much more women than men (78% women). Thus, our results cannot be generalized to men. Furthermore, we studied a comparably young population of depressed patients with few medical comorbidities, so our results cannot be generalized to older people and patient groups with severe medical conditions. However, the homogeneous nature of our group of depressed patients is also a strength as it increases internal validity. Second, there was no specific time of day when we collected the plasma samples and, therefore, our blood samples were not restricted to fasting glucose or fasting lipids, but included non-fasting values as well. However, according to the consensus statement from the European Atherosclerosis Society and European Federation of Clinical Chemistry and Laboratory Medicine there is no clinically relevant difference between fasting and non-fasting lipid measurements[77]. Furthermore, there is also evidence that non-fasting glucose values are associated with incident CVD[78,79]. Overall, the clinical significance of non-fasting glucose and non-fasting lipid values seems to be established. Third, we did not measure HbA1c as a long-term marker of glucose concentrations. Fourth, due to the limited quantity of saliva, we were restricted to three steroids. Therefore, we chose those steroids for which several earlier studies had been shown an association with MDD[5,30,33,56,57]. The plasma concentrations of the sulfated form (DHEA-S) is between 250 and 500 times higher (women and men, respectively) than the concentrations of DHEA[80]. In addition, both steroid hormones are correlated[81,82]. Therefore, we believe that measuring DHEA-S also provides a reliable assessment of DHEA values. Fifth, we used 250 mg of DCS, which is considered a moderate dosage that can lead to partial agonism of the NMDA receptor. However, it has been suggested that DCS acts as an NMDA receptor antagonist at high doses in the range of 750–1000 mg (ref. [17]). The NMDA receptor antagonist ketamine increases cortisol secretion[20,21]. However, the NMDA receptor antagonist memantine does not[44]. We cannot exclude that a higher dosage of DCS that acts as an NMDA receptor antagonist would have affected steroid hormone secretion and further studies should examine this question. Sixth, while the MR affinity of fludrocortisone is ~150 times higher than its GR affinity[83], fludrocortisone has some glucocorticoid potency. The extent of its glucocorticoid potency ranges from negligible to rather moderate depending on the source of the literature and variable being examined[84,85]. Thus, remaining GR activity could have contributed to the effects of fludrocortisone in our study. Finally, even though we recruited a relatively large sample ($n = 232$), we still might have lacked power to find an association between NMDA receptor stimulation and steroid secretion even though the effect sizes were small and presumably clinically irrelevant.

Strengths of the study include the lack of antidepressive treatment during the study, careful matching of healthy individuals to depressed patients based on age, sex, and years of education, and strongly controlled experimental conditions during saliva collection with almost no missing data. In addition, the demographic characteristics did not differ between participants across the four conditions, except for fewer male participants in the fludrocortisone + DCS condition compared with other conditions. However, our main findings were independent of condition—MDD patients showed increased cortisol, increased aldosterone, and decreased DHEA-S concentrations, and increased glucose levels and decreased HDL cholesterol levels compared with controls. Therefore, the differences in sex distribution across the four conditions likely did not affect the main results.

In conclusion, we found that steroid hormone alterations and cardiovascular risk are higher in patients with depression than in healthy individuals. Future research should prospectively examine whether manipulating these steroid systems can improve the symptoms and cardiovascular risk of patients with depression.

Acknowledgements

This study was funded by a grant from the German Research Foundation (Deutsche Forschungsgemeinschaft, DFG; OT 209/7-3) to C.O. and K.W. M.K. is participant in the BIH-Charité Clinician Scientist Program funded by the Charité – Universitätsmedizin Berlin and the Berlin Institute of Health. The data have been presented at the congress of the "German College of Neuropsychopharmacology" (AGNP) 2019 and at the congress of the "European College of Neuropsychopharmacology" (ECNP) 2019 in abstract and poster form. We acknowledge support from the German Research Foundation (DFG) and the Open Access Publication Funds of Charité – Universitätsmedizin Berlin.

Conflict of interest

C.O. has received honoraria for lectures and/or scientific advice from Allergan, Ferring, Fortbildungskolleg, Limes Klinikgruppe, Lundbeck, MedOnline, Medical Tribune, Neuraxpharm, SAGE Therapeutics, and Stillachhaus. All other authors declare that they have no conflict of interest.

Supplementary Information accompanies this paper at (https://doi.org/10.1038/s41398-020-0789-7).

Received: 8 November 2019 Revised: 12 March 2020 Accepted: 25 March 2020

Published online: 20 April 2020

References

1. Otte, C. et al. Major depressive disorder. *Nat. Rev. Dis. Prim.* **2**, 16065 (2016).
2. Brotman, D. J., Golden, S. H. & Wittstein, I. S. The cardiovascular toll of stress. *Lancet* **370**, 1089–1100 (2007).
3. Popoli, M., Yan, Z., McEwen, B. S. & Sanacora, G. The stressed synapse: the impact of stress and glucocorticoids on glutamate transmission. *Nat. Rev. Neurosci.* **13**, 22 (2012).
4. Treccani, G. et al. Stress and corticosterone increase the readily releasable pool of glutamate vesicles in synaptic terminals of prefrontal and frontal cortex. *Mol. Psychiatry* **19**, 433 (2014).
5. Stetler, C. & Miller, G. E. Depression and hypothalamic-pituitary-adrenal activation: a quantitative summary of four decades of research. *Psychosom. Med.* **73**, 114–126 (2011).
6. Murrough, J. W., Abdallah, C. G. & Mathew, S. J. Targeting glutamate signalling in depression: progress and prospects. *Nat. Rev. Drug Discov.* **16**, 472–486 (2017).
7. Penninx, B. W. J. H. Depression and cardiovascular disease: epidemiological evidence on their linking mechanisms. *Neurosci. Biobehav. Rev.* **74**, 277–286 (2017).
8. Nemeroff, C. B. & Goldschmidt-Clermont, P. J. Heartache and heartbreak—the link between depression and cardiovascular disease. *Nat. Rev. Cardiol.* **9**, 526 (2012).
9. Carney, R. M. & Freedland, K. E. Depression and coronary heart disease. *Nat. Rev. Cardiol.* **14**, 145 (2016).
10. ter Heegde, F., De Rijk, R. H. & Vinkers, C. H. The brain mineralocorticoid receptor and stress resilience. *Psychoneuroendocrinology* **52**, 92–110 (2015).
11. de Kloet, E., Meijer, O., de Nicola, A., de Rijk, R. & Joëls, M. Importance of the brain corticosteroid receptor balance in metaplasticity, cognitive performance and neuro-inflammation. *Front. Neuroendocrinol.* **49**, 124–145 (2018).
12. Pariante, C. M. & Lightman, S. L. The HPA axis in major depression: classical theories and new developments. *Trends Neurosci.* **31**, 464–468 (2019).
13. Juruena, M. F. et al. The role of mineralocorticoid receptor function in treatment-resistant depression. *J. Psychopharmacol.* **27**, 1169–1179 (2013).
14. Lembke, A. et al. The mineralocorticoid receptor agonist, fludrocortisone, differentially inhibits pituitary–adrenal activity in humans with psychotic major depression. *Psychoneuroendocrinology* **38**, 115–121 (2013).
15. Hinkelmann, K. et al. Mineralocorticoid receptor function in depressed patients and healthy individuals. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* **71**, 183–188 (2016).
16. Moriguchi, S. et al. Glutamatergic neurometabolite levels in major depressive disorder: a systematic review and meta-analysis of proton magnetic resonance spectroscopy studies. *Mol. Psychiatry* **24**, 952–964 (2019).
17. Chan, S. Y., Matthews, E. & Burnet, P. W. ON or OFF?: modulating the N-methyl-D-aspartate receptor in major depression. *Front. Mol. Neurosci.* **9**, 169 (2017).
18. Food and Drug Administration. *FDA Approves New Nasal Spray Medication for Treatment-resistant Depression; Available Only at a Certified Doctor's Office or Clinic*. Retrieved June 11 (2019) from.
19. Krystal, J. H., Abdallah, C. G., Sanacora, G., Charney, D. S. & Duman, R. S. Ketamine: a paradigm shift for depression research and treatment. *Neuron* **101**, 774–778 (2019).
20. Khalili-Mahani, N., Martini, C. H., Olofsen, E., Dahan, A. & Niesters, M. Effect of subanaesthetic ketamine on plasma and saliva cortisol secretion. *Br. J. Anaesth.* **115**, 68–75 (2015).
21. Khalili-Mahani, N. et al. Ketamine interactions with biomarkers of stress: a randomized placebo-controlled repeated measures resting-state fMRI and PCASL pilot study in healthy men. *NeuroImage* **108**, 396–409 (2015).
22. Heresco-Levy, U. et al. A randomized add-on trial of high-dose D-cycloserine for treatment-resistant depression. *Int. J. Neuropsychopharmacol.* **16**, 501–506 (2013).
23. Schade, S. & Paulus, W. D-Cycloserine in neuropsychiatric diseases: a systematic review. *Int. J. Neuropsychopharmacol* **19**, 1–7 (2016).
24. Kantrowitz, J. T., Milak, M. S., Mao, X., Shungu, D. C. & Mann, J. Jd-Cycloserine an NMDA glutamate receptor glycine site partial agonist, induces acute increases in brain glutamate plus glutamine and gaba comparable to ketamine. *Am. J. Psychiatry* **173**, 1241–1242 (2016).
25. Whooley, M. A. & Wong, J. M. Depression and cardiovascular disorders. *Annu. Rev. Clin. Psychol.* **9**, 327–354 (2013).
26. Zheng, Y. et al. Metabolites of glutamate metabolism are associated with incident cardiovascular events in the PREDIMED PREvencion con Dieta MEDiterranea (PREDIMED) Trial. *J. Am. Heart Assoc.* **5**, e003755 (2016).
27. Kubzansky, L. D. & Adler, G. K. Aldosterone: A forgotten mediator of the relationship between psychological stress and heart disease. *Neurosci. Biobehav. Rev.* **34**, 80–86 (2010).
28. Dahal, K. et al. Aldosterone antagonist therapy and mortality in patients with ST-segment elevation myocardial infarction without heart failure: a systematic review and meta-analysis. *JAMA Intern. Med.* **178**, 913–920 (2018).
29. Wu, T. T. et al. Prognostic value of dehydroepiandrosterone sulfate for patients with cardiovascular disease: a systematic review and meta-analysis. *J. Am. Heart Assoc.* **6**, e004896 (2017).
30. Emanuele, E., Geroldi, D., Minoretti, P., Coen, E. & Politi, P. Increased plasma aldosterone in patients with clinical depression. *Arch. Med. Res.* **36**, 544–548 (2005).
31. Murck, H., Büttner, M., Kircher, T. & Konrad, C. Genetic, molecular and clinical determinants for the involvement of aldosterone and its receptors in major depression. *Nephron. Physiol.* **128**, 17–25 (2014).
32. Häfner, S. et al. To live alone and to be depressed, an alarming combination for the renin–angiotensin–aldosterone-system (RAAS). *Psychoneuroendocrinology* **37**, 230–237 (2012).
33. Hu, Q. et al. Clinical significance of decreased protein expression of dehydroepiandrosterone sulfate in the development of depression: a meta-analysis. *J. Affect. Disord.* **174**, 416–423 (2015).
34. Gabor, A. & Leenen, F. H. Central mineralocorticoid receptors and the role of angiotensin II and glutamate in the paraventricular nucleus of rats with angiotensin II–induced hypertension. *Hypertension* **61**, 1083–1090 (2013).
35. Zoupa, E., Gravanis, A. & Pitsikas, N. The novel dehydroepiandrosterone (DHEA) derivative BNN27 counteracts behavioural deficits induced by the NMDA receptor antagonist ketamine in rats. *Neuropharmacology* **151**, 74–83 (2019).
36. Zaric, M. et al. Regional-specific effects of cerebral ischemia/reperfusion and dehydroepiandrosterone on synaptic NMDAR/PSD-95 complex in male Wistar rats. *Brain Res.* **1688**, 73–80 (2018).
37. Association A. P. *Diagnostic and statistical manual of mental disorders (DSM-5®)* (American Psychiatric Pub, 2013).
38. Hamilton M. *The Hamilton rating scale for depression. Assessment of depression* 143–152 (Springer, 1986).
39. Beck, A. T., Ward, C., Mendelson, M., Mock, J. & Erbaugh, J. Beck depression inventory (BDI). *Arch. Gen. Psychiatry* **4**, 561–571 (1961).
40. Edwards, S., Clow, A., Evans, P. & Hucklebridge, F. Exploration of the awakening cortisol response in relation to diurnal cortisol secretory activity. *Life Sci.* **68**, 2093–2103 (2001).
41. Faul, F., Erdfelder, E., Lang, A.-G. & Buchner, A. G* Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav. Res. methods* **39**, 175–191 (2007).
42. Schultebraucks, K. et al. Selective attention to emotional cues and emotion recognition in healthy subjects: the role of mineralocorticoid receptor stimulation. *Psychopharmacology* **233**, 3405–3415 (2016).
43. Burke, H. M., Davis, M. C., Otte, C. & Mohr, D. C. Depression and cortisol responses to psychological stress: a meta-analysis. *Psychoneuroendocrinology* **30**, 846–856 (2005).
44. Hergovich, N. et al. Comparison of the effects of ketamine and memantine on prolactin and cortisol release in men: a randomized, double-blind, placebo-controlled trial. *Neuropsychopharmacology* **24**, 590 (2001).
45. Krystal, J. H. et al. Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans: psychotomimetic, perceptual, cognitive, and neuroendocrine responses. *Arch. Gen. Psychiatry* **51**, 199–214 (1994).
46. van Berckel, B. N. et al. The partial NMDA agonist D-cycloserine stimulates LH secretion in healthy volunteers. *Psychopharmacology* **138**, 190–197 (1998).
47. van Berckel, B. N. et al. Behavioral and neuroendocrine effects of the partial NMDA agonist D-cycloserine in healthy subjects. *Neuropsychopharmacology* **16**, 317 (1997).
48. Feld, G. B., Lange, T., Gais, S. & Born, J. Sleep-dependent declarative memory consolidation—unaffected after blocking NMDA or AMPA receptors but enhanced by NMDA coagonist D-cycloserine. *Neuropsychopharmacology* **38**, 2688 (2013).
49. Otto, M. W. et al. Enhancement of psychosocial treatment with d-cycloserine: models, moderators, and future directions. *Biol. Psychiatry* **80**, 274–283 (2016).
50. Peyrovian, B. et al. The glycine site of NMDA receptors: a target for cognitive enhancement in psychiatric disorders. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* **92**, 387–404 (2019).

51. Wingenfeld, K. & Otte, C. Mineralocorticoid receptor function and cognition in health and disease. *Psychoneuroendocrinology* **105**, 25–35 (2019).
52. de Kloet, E. R., de Kloet, S. F., de Kloet, C. S. & de Kloet, A. D. Top-down and bottom-up control of stress-coping. *J. Neuroendocrinol.* **31**, e12675 (2019).
53. Otte, C. et al. Mineralocorticoid receptor stimulation improves cognitive function and decreases cortisol secretion in depressed patients and healthy individuals. *Neuropsychopharmacology* **40**, 386–393 (2015).
54. Pariante, C. M. Why are depressed patients inflamed? A reflection on 20 years of research on depression, glucocorticoid resistance and inflammation. *Eur. Neuropsychopharmacol.* **27**, 554–559 (2017).
55. Kamin, H. S. & Kertes, D. A. Cortisol and DHEA in development and psychopathology. *Hormones Behav.* **89**, 69–85 (2017).
56. Knorr, U., Vinberg, M., Kessing, L. V. & Wetterslev, J. Salivary cortisol in depressed patients versus control persons: a systematic review and meta-analysis. *Psychoneuroendocrinology* **35**, 1275–1286 (2010).
57. Murck, H. et al. The renin-angiotensin-aldosterone system in patients with depression compared to controls–a sleep endocrine study. *BMC Psychiatry* **3**, 15 (2003).
58. Segeda, V., Izakova, L., Hlavacova, N., Bednarova, A. & Jezova, D. Aldosterone concentrations in saliva reflect the duration and severity of depressive episode in a sex dependent manner. *J. Psychiatr. Res.* **91**, 164–168 (2017).
59. Büttner, M. et al. Target-based biomarker selection–mineralocorticoid receptor-related biomarkers and treatment outcome in major depression. *J. Psychiatr. Res.* **66**, 24–37 (2015).
60. Murck, H., Braunisch, M. C., Konrad, C., Jezova, D. & Kircher, T. Markers of mineralocorticoid receptor function: changes over time and relationship to response in patients with major depression. *Int. Clin. Psychopharmacol.* **34**, 18–26 (2019).
61. Velema, M. S. et al. Health-related quality of life and mental health in primary aldosteronism: a systematic review. *Horm. Metab. Res.* **49**, 943–950 (2017).
62. Künzel, H. E. Psychopathological symptoms in patients with primary hyperaldosteronism – possible pathways. *Horm. Metab. Res.* **44**, 202–207 (2012).
63. Franklin, M., Bermudez, I., Murck, H., Singewald, N. & Gaburro, S. Sub-chronic dietary tryptophan depletion – an animal model of depression with improved face and good construct validity. *J. Psychiatr. Res.* **46**, 239–247 (2012).
64. Hlavacova, N. et al. Subchronic treatment with aldosterone induces depression-like behaviours and gene expression changes relevant to major depressive disorder. *Int. J. Neuropsychopharmacol.* **15**, 247–265 (2012).
65. Harris, T. et al. Morning cortisol as a risk factor for subsequent major depressive disorder in adult women. *Br. J. Psychiatry* **177**, 505–510 (2000).
66. Goodyer, I. M., Herbert, J., Tamplin, A. & Altham, P. Recent life events, cortisol, dehydroepiandrosterone and the onset of major depression in high-risk adolescents. *Br. J. Psychiatry* **177**, 499–504 (2000).
67. Franklin, M. et al. Aldosterone signals the onset of depressive behaviour in a female rat model of depression along with SSRI treatment resistance. *Neuroendocrinology* **102**, 274–287 (2015).
68. Lopresti, A. L., Hood, S. D. & Drummond, P. D. A review of lifestyle factors that contribute to important pathways associated with major depression: diet, sleep and exercise. *J. Affect. Disord.* **148**, 12–27 (2013).
69. Gomez-Sanchez, E. Third generation mineralocorticoid receptor antagonists; why we need a fourth. *J. Cardiovascular Pharmacol.* **67**, 26 (2016).
70. DuPont, J. J. & Jaffe, I. Z. 30 YEARS OF THE MINERALOCORTICOID RECEPTOR: the role of the mineralocorticoid receptor in the vasculature. *J. Endocrinol.* **234**, T67–T82 (2017).
71. Kumari, M., Shipley, M., Stafford, M. & Kivimaki, M. Association of diurnal patterns in salivary cortisol with all-cause and cardiovascular mortality: findings from the Whitehall II study. *J. Clin. Endocrinol. Metab.* **96**, 1478–1485 (2011).
72. Vogelzangs, N. et al. Urinary cortisol and six-year risk of all-cause and cardiovascular mortality. *J. Clin. Endocrinol. Metab.* **95**, 4959–4964 (2010).
73. Hammer, F. et al. High evening salivary cortisol is an independent predictor of increased mortality risk in patients with systolic heart failure. *Int. J. Cardiol.* **203**, 69–73 (2016).
74. Buglioni, A. et al. Circulating aldosterone and natriuretic peptides in the general community. *Hypertension* **65**, 45–53 (2015).
75. Schmidt, P. J. et al. Dehydroepiandrosterone monotherapy in midlife-onset major and minor depression. *Arch. Gen. Psychiatry* **62**, 154–162 (2005).
76. Weiss, E. P., Villareal, D. T., Fontana, L., Han, D.-H. & Holloszy, J. O. Dehydroepiandrosterone (DHEA) replacement decreases insulin resistance and lowers inflammatory cytokines in aging humans. *Aging (Albany NY)* **3**, 533 (2011).
77. Nordestgaard, B. G. et al. Fasting is not routinely required for determination of a lipid profile: clinical and laboratory implications including flagging at desirable concentration cut-points-a joint consensus statement from the European Atherosclerosis Society and European Federation of Clinical Chemistry and Laboratory Medicine. *Eur. Heart J.* **37**, 1944–1958 (2016).
78. Benn, M. et al. Nonfasting glucose, ischemic heart disease, and myocardial infarction: a Mendelian randomization study. *J. Am. Coll. Cardiol.* **59**, 2356–2365 (2012).
79. Imano, H. et al. Non-fasting blood glucose and risk of incident coronary heart disease in middle-aged general population: The Circulatory Risk in Communities Study (CIRCS). *Preventive Med.* **55**, 603–607 (2012).
80. Webb, S. J., Geoghegan, T. E., Prough, R. A. & Michael Miller, K. K. The biological actions of dehydroepiandrosterone involves multiple receptors. *Drug Metab. Rev.* **38**, 89–116 (2006).
81. Straub, R. H. et al. Serum dehydroepiandrosterone (DHEA) and DHEA sulfate are negatively correlated with serum interleukin-6 (IL-6), and DHEA inhibits IL-6 secretion from mononuclear cells in man in vitro: possible link between endocrinosenescence and immunosenescence. *J. Clin. Endocrinol. Metab.* **83**, 2012–2017 (1998).
82. Folan, M. M. et al. Dehydroepiandrosterone, dehydroepiandrosterone-sulfate, and cortisol concentrations in intensive care unit patients. *Crit. Care Med.* **29**, 965–970 (2001).
83. Agarwal, M., Coupry, F. & Philippe, M. Physiological activity and receptor binding of 9α fluorohydrocortisone. *Biochem. Biophys. Res. Commun.* **78**, 747–753 (1977).
84. Grossmann, C. et al. Transactivation via the human glucocorticoid and mineralocorticoid receptor by therapeutically used steroids in CV-1 cells: a comparison of their glucocorticoid and mineralocorticoid properties. *Eur. J. Endocrinol.* **151**, 397–406 (2004).
85. Miller D. Adrenocorticoids. in *Foye's Principles of Medicinal Chemistry*, 6th edn, (eds Lemke, T. L. & Williams, D. A.) 890–891 (Lippincott Williams & Wilkins, a Wolters Kluwer business: Baltimore, MD, 2008).

4 Effects of MR and NMDA-R Stimulation on Empathy (Second Study)

This chapter has been published as: Nowacki, J., Wingenfeld, K., Kaczmarczyk, M., Chae, W. R., Abu-Tir, I., Deuter, C. E., Piber, D., Hellmann-Regen, J., & Otte, C. (2020). Cognitive and emotional empathy after stimulation of brain mineralocorticoid and NMDA receptors in patients with major depression and healthy controls. *Neuropsychopharmacology*, 45(13), 2155-2161.

DOI: 10.1038/s41386-020-0777-x

https://doi.org/10.1038/s41386-020-0777-x

Neuropsychopharmacology www.nature.com/npp

ARTICLE OPEN

Cognitive and emotional empathy after stimulation of brain mineralocorticoid and NMDA receptors in patients with major depression and healthy controls

Jan Nowacki[1], Katja Wingenfeld[1], Michael Kaczmarczyk[1], Woo Ri Chae[1], Ikram Abu-Tir[1], Christian Eric Deuter[1], Dominique Piber[1], Julian Hellmann-Regen[1] and Christian Otte[1]

Mineralocorticoid receptors (MR) are predominantly expressed in the hippocampus and prefrontal cortex. Both brain areas are associated with social cognition, which includes cognitive empathy (ability to understand others' emotions) and emotional empathy (ability to empathize with another person). MR stimulation improves memory and executive functioning in patients with major depressive disorder (MDD) and healthy controls, and leads to glutamate-mediated N-methyl-D-aspartate receptor (NMDA-R) signaling. We examined whether the beneficial effects of MR stimulation can be extended to social cognition (empathy), and whether DCS would have additional beneficial effects. In this double-blind placebo-controlled single-dose study, we randomized 116 unmedicated MDD patients (mean age 34 years, 78% women) and 116 age-, sex-, and education years-matched healthy controls to four conditions: MR stimulation (fludrocortisone (0.4 mg) + placebo), NMDA-R stimulation (placebo + D-cycloserine (250 mg)), MR and NMDA-R stimulation (both drugs), or placebo. Cognitive and emotional empathy were assessed by the Multifaceted Empathy Test. The study was registered on clinicaltrials.gov (NCT03062150). MR stimulation increased cognitive empathy across groups, whereas NMDA-R stimulation decreased cognitive empathy in MDD patients only. Independent of receptor stimulation, cognitive empathy did not differ between groups. Emotional empathy was not affected by MR or NMDA-R stimulation. However, MDD patients showed decreased emotional empathy compared with controls but, according to exploratory analyses, only for positive emotions. We conclude that MR stimulation has beneficial effects on cognitive empathy in MDD patients and healthy controls, whereas NMDA-R stimulation decreased cognitive empathy in MDD patients. It appears that MR rather than NMDA-R are potential treatment targets to modulate cognitive empathy in MDD.

Neuropsychopharmacology (2020) 45:2155–2161; https://doi.org/10.1038/s41386-020-0777-x

INTRODUCTION

Patients with major depressive disorder (MDD) exhibit cognitive deficits in executive functioning, memory, and attention that may persist even after remission [1, 2]. Interestingly, there is evidence that MDD patients show altered concentrations of the steroid hormone cortisol that, in turn, is associated with deficits in these cognitive domains [3–5]. Yet, little is known about the role of cortisol in MDD patients in other cognitive domains that are also clinically relevant to MDD, such as social cognition—the process of identification, perception, and interpretation of social information [6].

Cortisol is released in response to stress and acts via glucocorticoid receptors (GR) and mineralocorticoid receptors (MR). GR are expressed across the brain, while MR are predominantly expressed in the hippocampus and prefrontal cortex [7–9]. Importantly, these brain areas are closely associated with processes of social cognition [10].

There is increasing evidence for an important role of the MR in cognitive processes in MDD patients and healthy individuals [5, 11]. In healthy humans, MR blockade impaired memory and executive functioning [12, 13], whereas MR stimulation by fludrocortisone improved memory processes [14, 15]. Because MR stimulation appears to improve cognitive processes in healthy individuals, it might serve as a potential treatment target to improve cognitive deficits in MDD patients. Indeed, we found in our own group that MR stimulation improved verbal memory, and executive functioning in MDD patients and healthy controls [16]. However, it remains open to question whether these beneficial effects of MR stimulation on cognition in MDD patients can be extended to social cognition.

Depressed patients often exhibit deficits in social cognition that contribute to impaired social functioning and quality of life [6]. One important aspect of social cognition is empathy that consists of a cognitive and an emotional component. While the former describes the ability to understand others' emotions, the latter refers to the ability to feel with another person [17]. Overall, it appears, that several aspects of empathy are impaired in MDD patients [18]. However, the results are heterogeneous with studies showing decreased emotional empathy for positive emotions [19], increased emotional empathy for negative emotions [20], or no empathy differences compared with healthy controls (e.g., ref. [21]).

We previously found that modulating the MR affects social cognition (empathy) in health and disease. For instance, blockade

[1]Department of Psychiatry and Psychotherapy, Campus Benjamin Franklin, Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Hindenburgdamm 30, 12203 Berlin, Germany
Correspondence: Christian Otte (christian.otte@charite.de)

Received: 8 April 2020 Revised: 19 June 2020 Accepted: 10 July 2020
Published online: 28 July 2020

of the MR reduced cognitive empathy in MDD patients [19]. Furthermore, MR stimulation enhanced emotional empathy in patients with borderline personality disorder and healthy controls [22]. Thus, MR stimulation appears to enhance empathy and these beneficial effects might be applicable to MDD patients also. Although our studies provide first evidence for beneficial effects of MR stimulation, the mechanisms of action by which fludrocortisone enhances social cognition (empathy) remain to be examined.

In reaction to stress, glucocorticoids activate the release of glutamate via the MR in the hippocampus and prefrontal cortex. Glutamate, in turn, binds upon the N-methyl-D-aspartate receptor (NMDA-R) [8, 23, 24], which is involved in several cognitive processes. For instance, knockout of the NMDA-R in the forebrain is associated with impaired social cognition in mice [25] and stimulation of the receptor by the agonist D-cycloserine (DCS) has beneficial effects on decision making, memory, and learning in healthy individuals [26, 27] and in a range of psychiatric populations [28]. Accordingly, the beneficial effects of MR stimulation on social cognition (empathy) might be enhanced by modulation of NMDA-R signaling.

In the current study, we examined whether the beneficial effects of MR stimulation by fludrocortisone can be extended to social cognition (empathy) in MDD patients. In addition, we examined whether these potential beneficial effects can be enhanced by coadministration of the partial NMDA-R agonist DCS. We hypothesized that (1) MR stimulation by fludrocortisone enhances cognitive and emotional empathy in MDD patients, and that (2) simultaneous NMDA-R stimulation by DCS additionally enhances the effects of MR stimulation on cognitive and emotional empathy.

MATERIALS AND METHODS

Participants

We examined 116 patients with MDD and 116 healthy controls. We recruited MDD patients from the Department of Psychiatry of the Charité—Universitätsmedizin Berlin (in- and outpatients), via our website and by means of flyers distributed in outpatient psychiatric and psychotherapy practices. We recruited healthy controls via our website, and by means of flyers that we distributed in universities and other public buildings. MDD patients and healthy controls were matched for age, sex, and years of education. All participants gave their written informed consent and received an expense allowance for participation. The study was approved by the local ethics committee (Landesamt für Gesundheit und Soziales Berlin, 16-0031-EK 11) and was conducted according to the Declaration of Helsinki. The study was registered on clinicaltrials.gov (NCT03062150).

We included participants in the age between 18–65 years. For MDD patients, additional inclusion criteria were the diagnosis of MDD according to the Diagnostic and Statistical Manual of Mental Disorders [DSM-5; ref. [29]] and a minimum score of 18 on the 17-item Hamilton rating scale for depression (HAMD) [30].

Exclusion criteria for all participants were: intake of any psychotropic medication within the past 5 days (except for antidepressant sleep medication and benzodiazepines on demand), substance abuse or dependency within the last half-year, diagnosis of schizoaffective disorder, bipolar disorder, or schizophrenia (healthy controls were free of any current, or past psychiatric disorder and psychotropic medication), pregnancy or lactation period, study medication intolerance, neuroendocrine disorders, organic brain disease (current or past), acute suicidality, endocrine disorders, neuroendocrine medication intake, abnormal cardiovascular conditions, or abnormal clinical laboratory.

Procedure

All participants were pre-assessed for eligibility during a short telephone interview, except for inpatient MDD patients who were pre-assessed based on their medical record. Thereafter, participants were invited for an in-person visit for further diagnostic evaluation. During the in-person visit, participants gave their written informed consent, followed by a diagnostic clinical interview conducted by a trained and experienced physician or psychologist. The aim of the interview was to check inclusion and exclusion criteria, to diagnose or preclude any psychiatric disorder and to assess the general medical condition of the participants. For the diagnosis of psychiatric disorders, we conducted the Structured Clinical Interview for DSM-5 [31]. To measure the severity of the MDD, we used the HAMD [30] and the Beck Depression Inventory [BDI; ref. [32]]. To evaluate the general medical condition, we measured blood pressure and heart rate, we took blood samples for a safety laboratory, and we conducted an electrocardiography. All participants who were eligible for participation were invited for the testing day. The testing day was scheduled between 24 h and 1 week after the in-person visit.

Eligible participants were randomly assigned to one of four single-dose treatment conditions: (A) placebo + placebo, (B) fludrocortisone + placebo, (C) placebo + DCS, or (D) fludrocortisone + DCS. The Charité—Universitätsmedizin Berlin pharmacy conducted the randomization. We used a parallel group design with simple randomization: every four participants were randomized to one out of the four treatment conditions. Randomization was conducted separately for each group (stratified randomization) to ensure that 29 MDD patients and 29 healthy controls were randomized to each treatment condition.

The Charité—Universitätsmedizin Berlin pharmacy provided the medication to ensure blinding of participants and examiners. Participants received two identical-looking capsules that contained either fludrocortisone, DCS, or placebo. We used 0.4 mg fludrocortisone to stimulate the MR and 250 mg DCS to stimulate the NMDA-R. The dosage was based on studies that found cognitive enhancing effects in humans for one-time drug administration. For fludrocortisone, this was based on our own studies [15, 16, 33] and for DCS it was based on studies of other research groups [27, 34].

The testing day started for all participants at 1130 h. After a resting period of 30 min, participants received the first medication at 1200 h and the second medication at 1300 h. The Multifaceted Empathy Test (MET) was conducted between 1600 and 1700 h. This procedure was identical for all participants, in order to control for circadian rhythm of cortisol secretion [35]. Furthermore, by the afternoon cortisol concentrations have already much declined compared to peak levels after awakening [36] allowing agonistic effects at MR, which are largely but not fully occupied when cortisol levels are low [37].

We measured steroid hormone concentrations (cortisol, aldosterone, and DHEA-S), as well as blood pressure and heart rate every hour from 1200 to 1800 h. The effect of the treatment conditions on the cortisol response can be summarized as follows: cortisol concentrations decreased in both fludrocortisone conditions (fludrocortisone and fludrocortisone + DCS) across groups, whereas DCS had no effect on steroid hormone concentrations as described elsewhere [38].

Multifaceted Empathy Test

We used a modified version [39] of the MET [40] to asses cognitive and emotional empathy. The computerized task consists of 30 pictures of people in emotional situations that are presented on a black screen. Pictures were presented in blocks of ten. In alternating order, participants were asked to rate ten pictures for cognitive empathy, and then ten pictures for emotional empathy. All blocks were presented twice, once for cognitive and once for emotional empathy. The order was pseudo-randomized, which implied that subsequent blocks presented different pictures. To measure cognitive empathy, participants were asked to indicate the emotion the person feels

Table 1. Demographics characteristics.

	MDD	HC	Statistics
Age years	34.7 ± 13.3	34.9 ± 13.2	t (230) = 0.1, p = 0.90
Sex (women)	91 (78%)	91 (78%)	
Education duration	11.8 ± 1.3	12.1 ± 1.3	t (230) = 1.6, p = 0.12
In relationship (yes)	50 (43%)	67 (58%)	$\chi^2(1) = 5.0$, $p < 0.05$
Hormonal contraception	19 (21%)	19 (21%)	
HAMD	21.5 (3.4)	1.6 (1.3)	t (149) = −58.7, $p < 0.001$
BDI	25.7 (8.3)	1.4 (1.8)	t (125) = −30.8, $p < 0.001$

Demographic characteristics for depressed patients (MDD) and HC. Values represent mean ± SD or *n* (%).
HAMD Hamilton Rating Scale for Depression, *BDI* Beck Depression Inventory, *HC* healthy controls, *MDD* major depressive disorder.

on the picture by choosing one out of four suggested emotions presented on the screen. The sum of all correct answers was calculated, leading to a minimum score of 0 and a maximum score of 30. To measure emotional empathy, participants were asked to indicate how much they empathize with the person on the picture on a Likert scale ranging from 1 (not at all) to 9 (very much). The sum score was calculated, leading to a minimum score for emotional empathy of 30 and a maximum score of 270. In addition, the mean score for positive and the mean score for negative emotions was calculated for cognitive and emotional empathy.

Statistical analysis

For all statistical analyses, we used IBM SPSS Statistics (version 25). The analyses of the demographic information were conducted with *t*-tests for continuous data and chi-squared tests for categorical data.

The analyses of cognitive and emotional empathy were conducted with separate ANOVAs with the factors group (MDD patients vs. healthy controls), MR stimulation (fludrocortisone conditions vs. non-fludrocortisone conditions), and NMDA-R stimulation (DCS conditions vs. non-DCS conditions). Post hoc tests were Bonferroni corrected for multiple testing, and we used independent *t*-tests or paired sample *t*-tests, respectively.

We conducted several exploratory analyses: first, we analyzed whether cognitive and emotional empathy differed for positive and negative emotions. We used mixed ANOVAs with the between-subject factors group, MR stimulation, and NMDA-R stimulation and the within-subject factor valence (positive and negative emotions).

Second, within the group of female participants, we analyzed the effect of hormonal contraceptives with separate ANOVAs for cognitive and emotional empathy, and the factors hormonal contraception (intake vs. no intake of hormonal contraceptives), MR stimulation (fludrocortisone conditions vs. non-fludrocortisone conditions), and NMDA-R stimulation (DCS conditions vs. non-DCS conditions).

Third, we calculated change scores (delta) for cortisol, DHEA-S, and aldosterone by subtracting the mean of the two baseline values from the minimum post drug administration value for each hormone. Correlations between these delta values, and cognitive and emotional empathy for each treatment condition were calculated. Bonferroni corrections were applied to control for multiple testing.

Fourth, we calculated correlations between cognitive and emotional empathy, and depression severity (HAMD and BDI scores) within the group of MDD patients. Bonferroni corrections were applied to control for multiple testing.

The current study was powered based on the findings by Schultebraucks et al. [33], where we found an effect size of d = 0.70 for fludrocortisone vs. placebo on emotional dot probe in healthy young participants. Thus, we chose the emotional dot probe paradigm as primary outcome variable (unpublished data), and three other cognitive paradigms as secondary endpoints. The MET is one of the latter.

RESULTS

Demographic information

MDD patients (n = 116) and healthy controls (n = 116) did not differ in age, sex, years of education, and in the proportion of hormonal contraception users among female participants. Fewer MDD patients were in a relationship than healthy controls. As expected, MDD patients had higher mean HAMD scores and higher mean BDI scores, as compared with healthy controls (see Table 1).

Cognitive empathy

We found no main effect of group ($p > 0.05$), but a main effect of MR stimulation ($F(1,224) = 9.4$, $p < .01$, $\eta^2 = 0.04$), indicating enhanced cognitive empathy after fludrocortisone administration (Fig. 1a). We also found a main effect of NMDA-R stimulation ($F(1,224) = 4.5$, $p < 0.05$, $\eta^2 = 0.02$) and an interaction of group × NMDA-R stimulation ($F(1,224) = 4.8$, $p < 0.05$, $\eta^2 = 0.02$). Post hoc tests indicated less cognitive empathy after DCS administration within the group of MDD patients only ($t(114) = 2.8$, $p < 0.01$; Fig. 1b). We found no interaction of MR stimulation × NMDA-R stimulation ($F(1,224) = 0.5$, $p = 0.48$, $\eta^2 = 0.002$). Thus, combined stimulation of MR and NMDA-R had no effect on cognitive empathy across groups.

In exploratory analyses, we additionally found a main effect of valence ($F(1,224) = 151.3$, $p < 0.001$, $\eta^2 = 0.40$), indicating higher scores of cognitive empathy for positive emotions compared with negative emotions across groups.

Emotional empathy

We found a main effect of group ($F(1,224) = 6.8$, $p < 0.05$, $\eta^2 = 0.03$), indicating less emotional empathy in MDD patients compared with healthy controls. We found no main effect of MR stimulation or NMDA-R stimulation (all $ps > 0.05$), and we found no interaction of MR stimulation × NMDA-R stimulation ($F(1,224) = 0.2$, $p = 0.63$, $\eta^2 = 0.001$) or any other interaction (all $ps > 0.05$). Thus, separate and combined stimulation of MR and NMDA-R had no effect on emotional empathy across groups (Fig. 2).

In exploratory analyses, we additionally found a main effect of valence ($F(1,224) = 4.1$, $p < 0.05$, $\eta^2 = 0.02$) and an interaction of group × valence ($F(1,224) = 39.7$, $p < 0.001$, $\eta^2 = 0.15$). Post hoc tests revealed that MDD patients showed less emotional empathy compared with healthy controls for positive emotions only ($t(224) = 5.5$, $p < 0.001$; Fig. 3).

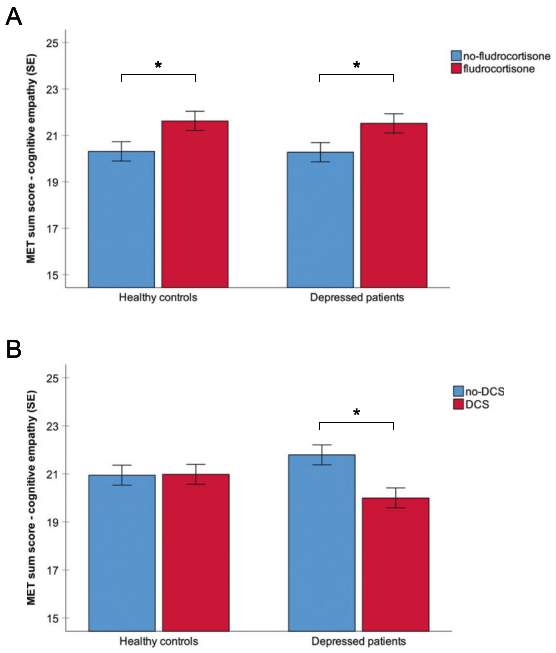

Fig. 1 Cognitive empathy after MR and NMDA-R stimulation in patients with MDD and healthy controls. a Cognitive empathy scores were higher across groups after MR stimulation and **b** lower after NMDA-R stimulation in MDD patients. MET Multifaceted Empathy Test, error bars show standard error (SE), and significant differences are marked (*).

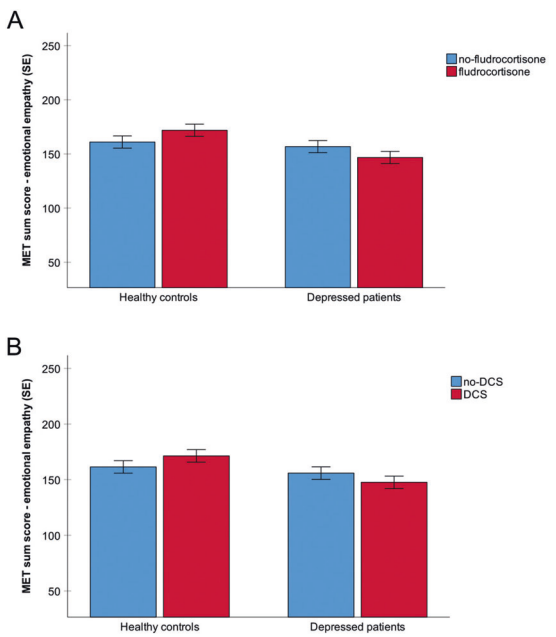

Fig. 2 Emotional empathy after MR and NMDA-R stimulation in patients with MDD and healthy controls. MR stimulation (**a**) and NMDA-R stimulation (**b**) had no effect on emotional empathy scores in MDD patients and healthy controls. MET Multifaceted Empathy Test, error bars show standard error (SE).

Hormonal contraception

Exploratory analyses in women revealed no main effect of hormonal contraception, and no interaction between hormonal contraception × MR or × NMDA-R stimulation for cognitive and emotional empathy (all *ps* > 0.05). Thus, hormonal contraceptives had no effect, and did not influence the effect of MR or NMDA-R stimulation, on cognitive and emotional empathy in female participants.

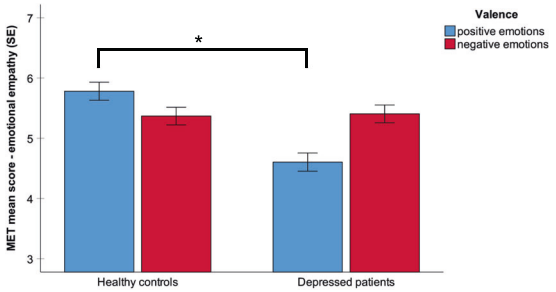

Fig. 3 Emotional empathy for positive and negative emotions in patients with MDD and healthy controls. Emotional empathy scores were lower in MDD patients compared with healthy controls for positive emotions. MET Multifaceted Empathy Test, error bars show standard error (SE), and significant differences are marked (*).

Correlational analyses

In exploratory analyses, we found no significant correlations between cognitive and emotional empathy and cortisol, DHEA-S, and aldosterone secretion within each treatment condition. Only 1 out of 24 correlations reached conventional significance ($p < 0.05$), and this result did not survive Bonferroni correction ($p = 0.002$, after 0.05/24).

In addition, we found no significant correlations between cognitive and emotional empathy, and depression severity in MDD patients. None of the four correlations survived Bonferroni corrections ($p = 0.013$, after 0.05/4) or reached conventional significance ($p < 0.05$).

DISCUSSION

We examined the separate and combined effect of MR and NMDA-R stimulation on cognitive and emotional empathy in MDD patients and healthy controls. Our main results are: (1) MR stimulation by fludrocortisone enhanced cognitive empathy in MDD patients and healthy controls, (2) NMDA-R stimulation by DCS decreased cognitive empathy in MDD patients, (3) cognitive empathy did not differ between MDD patients and healthy controls, and (4) emotional empathy was lower in MDD patients compared with healthy controls, but according to exploratory analyses, only for positive emotions.

Our results partly confirm our first hypothesis that MR stimulation by fludrocortisone enhances cognitive and emotional empathy in MDD patients. We found that MR stimulation enhanced cognitive, but not emotional empathy in MDD patients and healthy controls. This is partly in line with prior research of our group [19, 22]. We previously showed that MR blockade decreased cognitive empathy in MDD patients to the level of healthy controls [19], and that MR stimulation enhanced emotional empathy in patients with borderline personality disorder and healthy controls [22]. Together, our studies provide strong evidence for the involvement of the MR in social cognition (empathy) in health and disease. The current research shows, in addition, that the beneficial effects of MR stimulation on executive functioning and memory in MDD patients [16] can be extended to social cognition (empathy), another clinically relevant cognitive domain in MDD [6].

We could not confirm our second hypothesis that simultaneous MR and NMDA-R stimulation enhances the effects of single MR stimulation on cognitive and emotional empathy in MDD patients. In contrast, stimulation of the NMDA-R decreased cognitive empathy in MDD patients, but not in healthy controls and the combined stimulation of both receptors showed no (additive) effect. We assumed synergistic effects of the MR agonist

fludrocortisone and the partial NMDA-R agonist DCS on empathy, based on findings that (1) MR stimulation leads to glutamatergic NMDA-R activation [8, 41], and (2) that MR agonism with fludrocortisone [15, 16, 33] and partial NMDA-R agonism with DCS [27, 34] have cognitive enhancing effects. However, the use of the partial NMDA-R agonist DCS does not allow examination of whether potential beneficial effects of MR stimulation depend on the NMDA-R. This would rather require NMDA-R blockage with an antagonist parallel to MR stimulation. Interestingly, the NMDA-R antagonist memantine improved memory by inducing neurogenesis in mice [42], and reversed the adverse effects of long-term glucocorticoid administration on hippocampus volume in humans over a period of several months [43].

The following should be considered when interpreting our results. First, the MR agonist fludrocortisone inhibits the hypothalamic–pituitary–adrenal axis leading to decreased secretion of cortisol [44, 45]. Given the higher binding affinity of cortisol for MR than GR, the decrease in cortisol concentrations is accompanied by lower GR occupation relative to MR occupation [46]. Thus, we examined the interplay between MR and GR-mediated effects rather than isolated effects of MR stimulation on empathy. Second, in the current study, we examined empathy 4 h after MR stimulation by fludrocortisone. Within this timeframe, late genomic MR and GR-mediated effects rather than early non-genomic actions occur [7]. Animal studies have shown rapid effects of glucocorticoids on glutamate transmission in the hippocampus, which were mediated by MR [8, 41]. It is thus possible that in humans, MR stimulation by fludrocortisone exerts early effects on glutamate transmission. Future studies should examine this question.

In terms of mechanisms, studies have shown that cognitive empathy processes are associated with the prefrontal cortex and emotional empathy processes are linked to the hippocampus, amygdala, and hypothalamus [47]. Interestingly, in MDD patients decreased MR expression has been found in the prefrontal cortex and hippocampus [48–50], and for the NMDA-R studies showed receptor downregulations in the prefrontal cortex and upregulations in the amygdala [51]. These findings fit very well with our finding that MR and NMDA-R stimulation changes cognitive empathy in MDD patients. Several other lines of evidence suggest an important role of the MR and NMDA-R in MDD. For instance, MR stimulation by fludrocortisone improved antidepressant treatment [52], and NMDA-R stimulation by DCS showed antidepressant effects [53]. Our research adds to the literature, showing that both receptors shape processes of social cognition (empathy) in MDD patients.

Our analyses of emotional valence indicated that independent of receptor stimulation, MDD patients showed less emotional empathy for positive emotions than healthy controls. The finding fits very well with studies showing that MDD patients suffer from impaired processing of positive emotions (e.g., refs. [54–56]). Furthermore, we replicated and extended an earlier study by our group [19] in a larger and younger sample of MDD patients. However, we did not replicate our earlier finding of increased cognitive empathy in MDD patients compared with healthy controls. Several other studies found no difference in cognitive empathy, but a bias toward negative emotional stimuli in MDD patients compared with healthy controls [20, 57]. Overall, the results suggest that MDD patients suffer from a mood congruent bias in emotional empathy processes that Beck [58] described in the cognitive model of depression (see also refs. [10, 54, 59]).

Our study had several strengths. First, we examined a comparatively large sample of unmedicated MDD patients. Rütgen et al. [60] argued that most research in the field had examined medicated patients, which restricts generalizability and that might have contributed to the heterogeneity of results in the field [18]. Importantly, one small longitudinal study showed that antidepressants influenced empathy in MDD patients [60]. Second, we carefully matched MDD patients and healthy controls according to age, sex, and education years. Third, there was an equal number of women using oral contraceptives in both groups. Although oral contraceptives have been shown to impact on cognitive empathy, affective responsiveness, and perception of emotional valence [61–63], in the current study we did not find an effect of oral contraceptives on cognitive and emotional empathy.

We would also like to acknowledge some limitations. Our study used the MET as a single empathy measurement instrument, which restricts generalizability of our findings. Several studies that used multiple empathy measurement instruments in the same sample found group differences for some, but not all empathy paradigms [19, 64, 65]. A related limitation is that the MET only assess state empathy. Other measurement instruments assess trait empathy, such as the Interpersonal Reactivity Index [66]. Recently, Banzhaf et al. [57] showed that empathy alterations in MDD patients are different for state and trait empathy. Future studies should therefore use several empathy measurements to ensure a widespread assessment of the concept of empathy. However, this needs to be weighed against the problem of multiple testing associated with several outcome variables.

Overall, our research shows that the beneficial effects of MR stimulation by fludrocortisone on several cognitive domains can be extended to aspects of social cognition, i.e., cognitive empathy in MDD patients and healthy controls. It appears that MR rather than NMDA-R are potential treatment targets to modulate cognitive empathy in MDD.

FUNDING AND DISCLOSURES

This study was funded by a grant from the German Research Foundation (Deutsche Forschungsgemeinschaft, DFG; OT 209/7–3) to C.O. and K.W. M.K. is a participant in the BIH-Charité Clinician Scientist Program funded by the Charité—Universitätsmedizin Berlin and the Berlin Institute of Health. C.O. has received honoraria for lectures and/or scientific advice from Allergan, Ferring, Fortbildungskolleg, Limes Klinikgruppe, Lundbeck, MedOnline, Medical Tribune, Neuraxpharm, SAGE Therapeutics, and Stillachhaus. All other authors reported no potential conflicts of interest. The study was conducted in cooperation with NeuroCure – Cluster of Excellence at Charité – Universitätsmedizin Berlin. Open access funding provided by Projekt DEAL.

ACKNOWLEDGEMENTS

We would like to thank all participants for their participation in the current study.

AUTHOR CONTRIBUTIONS

C.O., K.W., and J.N. developed the conception and design of the study. J.N., M.K., W.R.C., and D.P. contributed to the data acquisition. J.N. and C.E.D. contributed to the analysis and interpretation of the data of the study under supervision of C.O. and K.W. J.H.-R. analyzed the salivary and blood data. All authors contributed to the draft and revision of the work and all authors gave final approval of the version to be published.

ADDITIONAL INFORMATION

Supplementary Information accompanies this paper at (https://doi.org/10.1038/s41386-020-0777-x).

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

REFERENCES

1. Rock P, Roiser J, Riedel W, Blackwell A. Cognitive impairment in depression: a systematic review and meta-analysis. Psychological Med. 2014;44:2029–40.
2. Semkovska M, Quinlivan L, O'Grady T, Johnson R, Collins A, O'Connor J, et al. Cognitive function following a major depressive episode: a systematic review and meta-analysis. Lancet Psychiatry. 2019;6:851–61.

3. Hinkelmann K, Moritz S, Botzenhardt J, Riedesel K, Wiedemann K, Kellner M, et al. Cognitive impairment in major depression: association with salivary cortisol. Biol Psychiatry. 2009;66:879–85.
4. Keller J, Gomez R, Williams G, Lembke A, Lazzeroni L, Murphy GM Jr, et al. HPA axis in major depression: cortisol, clinical symptomatology and genetic variation predict cognition. Mol Psychiatry. 2017;22:527.
5. Wingenfeld K, Otte C. Mineralocorticoid receptor function and cognition in health and disease. Psychoneuroendocrinology. 2019;105:25–35.
6. Weightman MJ, Knight MJ, Baune BT. A systematic review of the impact of social cognitive deficits on psychosocial functioning in major depressive disorder and opportunities for therapeutic intervention. Psychiatry Res. 2019;274:195–212.
7. de Kloet ER, de Kloet SF, de Kloet CS, de Kloet AD. Top-down and bottom-up control of stress-coping. J Neuroendocrinol. 2019;31:e12675.
8. Joëls M, Karst H, Sarabdjitsingh RA. The stressed brain of humans and rodents. Acta Physiol. 2018;223:e13066.
9. de Kloet E, Meijer O, de Nicola A, de Rijk R, Joëls M. Importance of the brain corticosteroid receptor balance in metaplasticity, cognitive performance and neuro-inflammation. Front Neuroendocrinol. 2018; 49:124–45.
10. Disner SG, Beevers CG, Haigh EA, Beck AT. Neural mechanisms of the cognitive model of depression. Nat Rev Neurosci. 2011;12:467.
11. Vogel S, Fernández G, Joëls M, Schwabe L. Cognitive adaptation under stress: a case for the mineralocorticoid receptor. Trends Cogn Sci. 2016;20:192–203.
12. Otte C, Moritz S, Yassouridis A, Koop M, Madrischewski AM, Wiedemann K, et al. Blockade of the mineralocorticoid receptor in healthy men: effects on experimentally induced panic symptoms, stress hormones, and cognition. Neuropsychopharmacology. 2007;32:232–8.
13. Rimmele U, Besedovsky L, Lange T, Born J. Blocking mineralocorticoid receptors impairs, blocking glucocorticoid receptors enhances memory retrieval in humans. Neuropsychopharmacology. 2013;38:884–94.
14. Hinkelmann K, Wingenfeld K, Kuehl LK, Fleischer J, Heuser I, Wiedemann K, et al. Stimulation of the mineralocorticoid receptor improves memory in young and elderly healthy individuals. Neurobiol Aging. 2015;36:919–24.
15. Piber D, Schultebraucks K, Mueller SC, Deuter CE, Wingenfeld K, Otte C. Mineralocorticoid receptor stimulation effects on spatial memory in healthy young adults: a study using the virtual Morris Water Maze task. Neurobiol Learn Mem.2016;136:139–46.
16. Otte C, Wingenfeld K, Kuehl LK, Kaczmarczyk M, Richter S, Quante A, et al. Mineralocorticoid receptor stimulation improves cognitive function and decreases cortisol secretion in depressed patients and healthy individuals. Neuropsychopharmacology. 2015;40:386–93.
17. Walter H. Social cognitive neuroscience of empathy: concepts, circuits, and genes. Emot Rev. 2012;4:9–17.
18. Schreiter S, Pijnenborg G, Aan Het, Rot M. Empathy in adults with clinical or subclinical depressive symptoms. J Affect Disord. 2013;150:1–16.
19. Wingenfeld K, Kuehl LK, Dziobek I, Roepke S, Otte C, Hinkelmann K. Effects of mineralocorticoid receptor blockade on empathy in patients with major depressive disorder. Cogn Affect Behav Neurosci. 2016;16:902–10.
20. Merkl A, Neumann W-J, Huebl J, Aust S, Horn A, Krauss JK, et al. Modulation of beta-band activity in the subgenual anterior cingulate cortex during emotional empathy in treatment-resistant depression. Cereb Cortex. 2015;26:2626–38.
21. Thoma P, Zalewski I, von Reventlow HG, Norra C, Juckel G, Daum I. Cognitive and affective empathy in depression linked to executive control. Psychiatry Res. 2011;189:373–8.
22. Wingenfeld K, Kuehl LK, Janke K, Hinkelmann K, Dziobek I, Fleischer J, et al. Enhanced emotional empathy after mineralocorticoid receptor stimulation in women with borderline personality disorder and healthy women. Neuropsychopharmacology. 2014;39:1799–804.
23. Popoli M, Yan Z, McEwen BS, Sanacora G. The stressed synapse: the impact of stress and glucocorticoids on glutamate transmission. Nat Rev Neurosci. 2012;13:22.
24. Mikasova L, Xiong H, Kerkhofs A, Bouchet D, Krugers HJ, Groc L. Stress hormone rapidly tunes synaptic NMDA receptor through membrane dynamics and mineralocorticoid signalling. Sci Rep. 2017;7:1–12.
25. Jacobs S, Tsien JZ. Adult forebrain NMDA receptors gate social motivation and social memory. Neurobiol Learn Mem. 2017;138:164–72.
26. Feld GB, Lange T, Gais S, Born J. Sleep-dependent declarative memory consolidation—unaffected after blocking NMDA or AMPA receptors but enhanced by NMDA coagonist D-cycloserine. Neuropsychopharmacology. 2013;38:2688.
27. Onur OA, Schlaepfer TE, Kukolja J, Bauer A, Jeung H, Patin A, et al. The N-methyl-D-aspartate receptor co-agonist D-cycloserine facilitates declarative learning and hippocampal activity in humans. Biol Psychiatry. 2010;67:1205–11.
28. Peyrovian B, Rosenblat JD, Pan Z, Iacobucci M, Brietzke E, McIntyre RS. The glycine site of NMDA receptors: A target for cognitive enhancement in psychiatric disorders. Prog Neuropsychopharmacol Biol Psychiatry. 2019;92:387–404.
29. American Psychiatric Association. Diagnostic and statistical manual of mental disorders (5th ed.). Washington, DC; American Psychiatric Association Publishing. 2013.
30. Hamilton M. The Hamilton rating scale for depression. Assessment of depression. Berlin, Heidelberg. 1986. p. 143–52.
31. First M, Williams J, Karg R, Spitzer R. Structured clinical interview for DSM-5—research version (SCID-5 for DSM-5, research version; SCID-5-RV). Arlington, VA: American Psychiatric Association; 2015. p. 1–94.
32. Beck AT, Ward C, Mendelson M, Mock J, Erbaugh J. Beck depression inventory (BDI). Arch Gen Psychiatry. 1961;4:561–71.
33. Schultebraucks K, Deuter CE, Duesenberg M, Schulze L, Hellmann-Regen J, Domke A, et al. Selective attention to emotional cues and emotion recognition in healthy subjects: the role of mineralocorticoid receptor stimulation. Psychopharmacology. 2016;233:3405–15.
34. Scholl J, Günthner J, Kolling N, Favaron E, Rushworth MF, Harmer CJ, et al. A role beyond learning for NMDA receptors in reward-based decision-making—a pharmacological study using d-cycloserine. Neuropsychopharmacology. 2014;39:2900.
35. Joëls M, Sarabdjitsingh RA, Karst H. Unraveling the time domains of corticosteroid hormone influences on brain activity: rapid, slow, and chronic modes. Pharmacol Rev. 2012;64:901–38.
36. Fitzsimons CP, Herbert J, Schouten M, Meijer OC, Lucassen PJ, Lightman S. Circadian and ultradian glucocorticoid rhythmicity: Implications for the effects of glucocorticoids on neural stem cells and adult hippocampal neurogenesis. Front Neuroendocrinol. 2016;41:44–58.
37. Kalman BA, Spencer RL. Rapid corticosteroid-dependent regulation of mineralocorticoid receptor protein expression in rat brain. Endocrinology 2002;143:4184–95.
38. Nowacki J, Wingenfeld K, Kaczmarczyk M, Chae WR, Salchow P, Abu-Tir I, et al. Steroid hormone secretion after stimulation of mineralocorticoid and NMDA receptors and cardiovascular risk in patients with depression. Transl Psychiatry. 2020;10:109.
39. Deuter CE, Nowacki J, Wingenfeld K, Kuehl LK, Finke JB, Dziobek I, et al. The role of physiological arousal for self-reported emotional empathy. Autonomic Neurosci. 2018;214:9–14.
40. Dziobek I, Rogers K, Fleck S, Bahnemann M, Heekeren HR, Wolf OT, et al. Dissociation of cognitive and emotional empathy in adults with Asperger syndrome using the Multifaceted Empathy Test (MET). J Autism Developmental Disord. 2008;38:464–73.
41. Karst H, Berger S, Turiault M, Tronche F, Schütz G, Joëls M. Mineralocorticoid receptors are indispensable for nongenomic modulation of hippocampal glutamate transmission by corticosterone. Proc Natl Acad Sci USA. 2005;102:19204–7.
42. Amin SN, El-Aidi AA, Ali MM, Attia YM, Rashed LA. Modification of hippocampal markers of synaptic plasticity by memantine in animal models of acute and repeated restraint stress: implications for memory and behavior. Neuromolecular Med. 2015;17:121–36.
43. Brown ES, Kulikova A, Van Enkevort E, et al. A randomized trial of an NMDA receptor antagonist for reversing corticosteroid effects on the human hippocampus. Neuropsychopharmacology. 2019;44:2263–7.
44. Otte C, Jahn H, Yassouridis A, Arlt J, Stober N, Maass P, et al. The mineralocorticoid receptor agonist, fludrocortisone, inhibits pituitary-adrenal activity in humans after pre-treatment with metyrapone. Life Sci. 2003;73:1835–45.
45. Buckley TM, Mullen BC, Schatzberg AF. The acute effects of a mineralocorticoid receptor (MR) agonist on nocturnal hypothalamic–adrenal–pituitary (HPA) axis activity in healthy controls. Psychoneuroendocrinology. 2007;32:859–64.
46. de Kloet ER, Joëls M. Mineralocorticoid receptors and glucocorticoid receptors in HPA stress responses during coping and adaptation. Oxford Research Encyclopedia of Neuroscience; 2020.
47. Tone EB, Tully EC. Empathy as a "risky strength": a multilevel examination of empathy and risk for internalizing disorders. Dev Psychopathol. 2014;26:1547–65.
48. Klok MD, Alt SR, Lafitte AJI, Turner JD, Lakke EA, Huitinga I, et al. Decreased expression of mineralocorticoid receptor mRNA and its splice variants in postmortem brain regions of patients with major depressive disorder. J Psychiatr Res. 2011;45:871–8.
49. Medina A, Seasholtz AF, Sharma V, Burke S, Bunney W Jr, Myers RM, et al. Glucocorticoid and mineralocorticoid receptor expression in the human hippocampus in major depressive disorder. J Psychiatr Res. 2013;47:307–14.
50. Qi X-R, Kamphuis W, Wang S, Wang Q, Lucassen PJ, Zhou J-N, et al. Aberrant stress hormone receptor balance in the human prefrontal cortex and hypothalamic paraventricular nucleus of depressed patients. Psychoneuroendocrinology. 2013;38:863–70.
51. Murrough JW, Abdallah CG, Mathew SJ. Targeting glutamate signalling in depression: progress and prospects. Nat Rev Drug Discov. 2017;16:472.
52. Otte C, Hinkelmann K, Moritz S, Yassouridis A, Jahn H, Wiedemann K, et al. Modulation of the mineralocorticoid receptor as add-on treatment in depression: a randomized, double-blind, placebo-controlled proof-of-concept study. J Psychiatr Res. 2010;44:339–46.

53. Heresco-Levy U, Gelfin G, Bloch B, Levin R, Edelman S, Javitt DC, et al. A randomized add-on trial of high-dose D-cycloserine for treatment-resistant depression. Int J Neuropsychopharmacol. 2013;16:501–6.
54. Leppänen JM. Emotional information processing in mood disorders: a review of behavioral and neuroimaging findings. Curr Opin Psychiatry. 2006;19:34–9.
55. Kellough JL, Beevers CG, Ellis AJ, Wells TT. Time course of selective attention in clinically depressed young adults: An eye tracking study. Behav Res Ther. 2008;46:1238–43.
56. Gollan JK, Hoxha D, Hunnicutt-Ferguson K, Norris CJ, Rosebrock L, Sankin L, et al. Twice the negativity bias and half the positivity offset: Evaluative responses to emotional information in depression. J Behav Ther Exp Psychiatry. 2016;52:166–70.
57. Banzhaf C, Hoffmann F, Kanske P, Fan Y, Walter H, Spengler S, et al. Interacting and dissociable effects of alexithymia and depression on empathy. Psychiatry Res. 2018;270:631–8.
58. Beck AT. Depression: Clinical, experimental and theoretical aspects. New York: Harper and Row. 1967.
59. Beck AT, Haigh EA. Advances in cognitive theory and therapy: the generic cognitive model. Annu Rev Clin Psychol. 2014;10:1–24.
60. Rütgen M, Pletti C, Tik M, Kraus C, Pfabigan DM, Sladky R, et al. Antidepressant treatment, not depression, leads to reductions in behavioral and neural responses to pain empathy. Transl Psychiatry. 2019;9:164.
61. Radke S, Derntl B. Affective responsiveness is influenced by intake of oral contraceptives. Eur Neuropsychopharmacol. 2016;26:1014–9.
62. Pahnke R, Mau-Moeller A, Junge M, Wendt J, Weymar M, Hamm AO, et al. Oral contraceptives impair complex emotion recognition in healthy women. Front Neurosci. 2019;12:1041.
63. Spalek K, Loos E, Schicktanz N, Hartmann F, de Quervain D, Stier C, et al. Women using hormonal contraceptives show increased valence ratings and memory performance for emotional information. Neuropsychopharmacology. 2019;44:1258–64.
64. Wolkenstein L, Schönenberg M, Schirm E, Hautzinger M. I can see what you feel, but I can't deal with it: Impaired theory of mind in depression. J Affect Disord. 2011;132:104–11.
65. Wilbertz G, Brakemeier E-L, Zobel I, Härter M, Schramm E. Exploring preoperational features in chronic depression. J Affect Disord. 2010;124:262–9.
66. Davis MH. A multidimensional approach to individual differences in empathy. JSAS. 1980;10:85.

5 Effects of MR and NMDA-R Stimulation on Emotion Recognition and Selective Attention to Emotional Stimuli (Third Study)

This chapter has been published as: Nowacki, J., Wingenfeld, K., Kaczmarczyk, M., Chae, W. R., Salchow, P., Deuter, C. E., P., Piber, D., & Otte, C. (2021). Selective attention to emotional stimuli and emotion recognition in patients with major depression: The role of mineralocorticoid and glutamatergic NMDA receptors. *Journal of Psychopharmacology,* 35(8), 1017-1023.

DOI: 10.1177/02698811211009797
https://doi.org/10.1177/02698811211009797

6 Discussion

The previous sections addressed the theoretical and empirical rationale (section 1), the aims and design (section 2) of this research project, and the three studies that are included in this research project (section 3 to 5). The following sections will discuss the findings of this research project. The discussion starts with a summary of the main findings of this research project (section 6.1), followed by a detailed discussion of the individual results for each of the three studies (section 6.2 to 6.4). Subsequently, the discussion will address the strengths and limitations of this research project (section 6.5) and will integrate the main findings in an explanatory model (section 6.6). This section will finish with future research directions (section 6.7) and a conclusion (section 6.8). The discussion will answer the central research question of this research project:

What is the effect of MR and NMDA-R stimulation on steroid hormone secretion and social cognition in healthy individuals and patients with MDD?

6.1 Findings of the research project

This section summarizes the main findings of this research project, structured by the three studies that are included in this research project. The main findings are:

The first study showed that MR stimulation by fludrocortisone decreased cortisol secretion in healthy individuals and patients with MDD but showed no effect on the other steroid hormones (aldosterone and DHEA-S). Simultaneous MR and NMDA-R stimulation by fludrocortisone and DCS showed no (additive) effect on steroid hormone secretion in both groups when compared to separate MR stimulation or no stimulation (placebo). Independent of any receptor stimulation, MDD patients showed alterations in steroid hormone concentrations, as indicated by increased cortisol and aldosterone levels as well as decreased DHEA-S concentrations when compared to healthy individuals. For further information on the results, please see the corresponding publication (Nowacki et al., 2020b).

The second study revealed that MR stimulation by fludrocortisone enhanced cognitive empathy, but showed no effect on emotional empathy in healthy individuals or patients with MDD. Simultaneous MR and NMDA-R stimulation by fludrocortisone and DCS showed no (additive) effect on cognitive and emotional empathy in both groups when compared to single MR stimulation or no stimulation (placebo). Separate NMDA-R

stimulation decreased cognitive empathy in patients with MDD. Independent of any receptor stimulation, MDD patients showed difficulties in expressing emotional empathy for positive emotions but no difficulties in cognitive empathy when compared to healthy individuals. For further information on the results, please see the corresponding publication (Nowacki et al., 2020a)

The third study found that MR stimulation by fludrocortisone showed no effect on facial emotion recognition or selective attention to emotional stimuli, neither in healthy individuals nor patients with MDD. Simultaneous MR and NMDA-R stimulation by fludrocortisone and DCS showed no (additive) effect on facial emotion recognition and selective attention to emotional stimuli in both groups when compared to single MR stimulation or no stimulation (placebo). Separate NMDA-R stimulation improved emotion recognition in both groups. Independent of any receptor stimulation, MDD patients showed no difficulties in facial emotion recognition and no selective attention to emotional stimuli in comparison to healthy individuals. For further information on the results, please see the corresponding publication (Nowacki et al., 2021).

To sum up, this research project found evidence for an involvement of MR in the psycho-neuro-endocrinological stress response in healthy individuals and patients with MDD. The findings underline the involvement of MR in regulating the secretion of glucocorticoids in healthy individuals and patients with MDD. While the observations provide some evidence that MR stimulation might have beneficial effects on social cognition (cognitive empathy), in both groups, the research found no evidence that these effects are improvable by simultaneous MR and NMDA-R stimulation. Although the studies provide some evidence for NMDA-R involvement in social cognition (cognitive empathy and facial emotion recognition), further research is required to replicate these findings before firm conclusions on the role of NMDA-R in social cognitive processes can be drawn. The following sections will discuss the individual findings in more detail and separately for each of the three studies (first study in section 6.2, second study in section 6.3, & third study in section 6.4).

6.2 Discussion of the effects of MR and NMDA-R stimulation on steroid hormones

The first study of this research project examined the following research question and hypothesis:

Research question: What is the effect of MR and NMDA-R stimulation on steroid hormone secretion in healthy individuals and patients with MDD?

Hypothesis: MR stimulation by fludrocortisone leads to decreased cortisol concentrations in healthy individuals and patients with MDD in comparison to no stimulation (placebo).

The study revealed that administration of the MR agonist fludrocortisone decreased cortisol secretion in healthy individuals and patients with MDD but showed no effect on aldosterone and DHEA-S concentrations. Simultaneous MR and NMDA-R stimulation by fludrocortisone and DCS showed no (additive) effect on steroid hormone secretion in both groups when compared to single MR stimulation or no stimulation (placebo). Independent of any receptor stimulation, MDD patients showed increased cortisol and aldosterone and decreased DHEA-S concentrations when compared to healthy individuals. The observations are based on a large sample (n = 116/group) of relatively young (M = 34 years) and unmedicated patients with MDD and healthy individuals matched in age, sex, and education years (Nowacki et al., 2020b). Two points of discussion will be addressed in the following:

The first point of discussion refers to the finding that the MR agonist fludrocortisone decreased cortisol secretion in healthy individuals and relatively young and unmedicated patients with MDD (Nowacki et al., 2020b). The observation complements earlier research that observed intact HPA axis regulation after MR stimulation by fludrocortisone in younger (M = 26 years) patients (Otte et al., 2015a) and older (M > 60 years) patients (Otte et al., 2015b) with MDD. Considered together, the observations suggest that MR mediated negative feedback regulation of the HPA axis is intact in patients with MDD. However, other studies suggest that MR mediated regulation of the HPA axis is impaired in patients with psychotic MDD (Lembke et al., 2013) and patients with treatment resistant depression (Juruena et al., 2013). Thus, one might speculate that mainly patients who suffer from very severe MDD show impaired MR mediated negative feedback regulation of the HPA axis, however this needs to be confirmed by further research. In the field of MDD research, there is consistent evidence that patients with MDD suffer from *glucocorticoid resistance*. That is, patients with MDD show impaired GR functioning which leads to impaired GR mediated HPA axis negative feedback control and, as a result, to HPA axis hyperactivity (Pariante, 2017). In line with the idea of glucocorticoid resistance in MDD, this study found that relatively young and

unmedicated patients with MDD show increased cortisol concentrations when compared to healthy individuals (Nowacki et al., 2020b). Considered together, the observations provide evidence that patients with MDD suffer from disturbances in the neuro-endocrine response to stress. One might speculate that relatively young and unmedicated patients with MDD show intact MR mediated but impaired GR mediated HPA axis negative feedback control, contributing to overall increased cortisol concentrations in these patients when compared with healthy individuals. The observations are in line with the *MR:GR balance hypothesis,* which considers an imbalance in the interplay between MR and GR functioning to be mainly responsible for HPA axis dysregulation, which is associated with stress related disorders such as MDD (de Kloet, 2014).

The second point of discussion refers to the observation that relatively young and unmedicated MDD patients suffer from dysregulations in several steroid hormone systems (cortisol, aldosterone, and DHEA-S) when compared to healthy individuals (Nowacki et al., 2020b). The steroid hormone DHEA-S, for instance, was decreased in MDD patients in comparison to healthy individuals, both in the current study and in earlier research (Hu et al., 2015). Interestingly, DHEA-(S) appears to play an important role in mood regulation. Several studies showed that DHEA administration has beneficial effects on depressive symptoms (Peixoto et al., 2020; Schmidt et al., 2005; Wolkowitz et al., 1999). The exact mode of action remains unknown, but it has been suggested that the beneficial effect of DHEA-(S) on MDD might be related to its capability to down-regulate cortisol concentrations (Hu et al., 2015; Peixoto et al., 2020). This would be in line with the observation of this research project, that cortisol concentrations were increased in MDD patients compared with healthy individuals (Nowacki et al., 2020b). Thus, abnormally high cortisol concentrations and abnormally low DHEA-S concentrations may contribute to the symptomatology of MDD. All things considered, the observations of this research project confirm that several steroid hormone systems (cortisol, aldosterone, and DHEA-S) are involved in the pathophysiology of MDD (Emanuele et al., 2005; Hu et al., 2015; Stetler & Miller, 2011).

To summarize, the observations of the first study emphasize that relatively young and unmedicated patients with MDD show a well-functioning MR mediated HPA axis negative feedback inhibition, indicated by decreased cortisol concentrations in healthy individuals and patients with MDD after MR stimulation. However, the observations also emphasize that relatively young and unmedicated MDD patients suffer from impairments in several steroid hormone systems (cortisol, aldosterone, and DHEA-S) in comparison to

healthy individuals. One possible explanation is provided by the MR:GR balance hypothesis, which considers a disturbed interplay between MR and GR functioning to underlie the dysregulation of the neuro-endocrine stress response, which is associated with stress-related disorders, such as MDD. Based on the observations described above, one might speculate whether pharmacological manipulations of the steroid hormone systems positively impact the symptomatology of patients with MDD. For example, the observations of the current study emphasize that relatively young and unmedicated patients with MDD suffer from high cortisol and low DHEA-S concentrations. Therefore, research could examine whether long-term downregulation of cortisol concentrations, in combination with upregulation of DHEA-S concentrations, has antidepressant effects in patients with MDD.

6.3 Discussion of the effects of MR and NMDA-R stimulation on empathy

The second study of the current research project examined the following research question and hypotheses:

Research question: What is the effect of MR and NMDA-R stimulation on social cognition (cognitive and emotional empathy) in healthy individuals and patients with MDD?

Hypothesis (A): MR stimulation by fludrocortisone leads to higher scores in cognitive and emotional empathy in healthy individuals and patients with MDD compared to no stimulation (placebo).

Hypothesis (B): Simultaneous MR and NMDA-R stimulation by fludrocortisone and DCS leads to higher scores in cognitive and emotional empathy in healthy individuals and patients with MDD in comparison to separate MR stimulation and to no stimulation (placebo).

The study revealed that MR stimulation enhanced cognitive empathy but showed no effect on emotional empathy in healthy individuals and patients with MDD. Simultaneous MR and NMDA-R stimulation showed no (additive) effect on cognitive and emotional empathy in both groups when compared to single MR stimulation or no stimulation. Separate NMDA-R stimulation decreased cognitive empathy in MDD patients independently from MR stimulation. Independent of any receptor stimulation, MDD patients showed

decreased emotional empathy for positive emotions when compared to healthy individuals (Nowacki et al., 2020a). Three points of discussion will be addressed in the following:

The first point of discussion addresses the observation that MR stimulation enhanced cognitive empathy, but showed no effect on emotional empathy in healthy individuals and patients with MDD. The finding is partially in agreement with *hypothesis (A)*. The hypothesis was based on the observation that MR stimulation by fludrocortisone has cognitive-enhancing effects on verbal memory and executive functioning in both healthy individuals and patients with MDD (Otte et al., 2015a). Thus, the current research shows that the beneficial effects of MR stimulation are extendable to social cognition processes (cognitive empathy) in both MDD patients and healthy individuals. The observation is in line with other studies emphasizing that MR blockade decreases cognitive empathy in MDD patients (Wingenfeld et al., 2016) and that MR stimulation increases emotional empathy in healthy individuals and patients with borderline personality disorder (Wingenfeld et al., 2014). However, not all studies concur. Administration of the MR and GR agonist hydrocortisone showed no effect on cognitive and emotional empathy in healthy individuals (Duesenberg et al., 2016). Considered together, the studies provide some evidence that MR are involved in (social) cognition in health and disease, and that the receptor might serve as a treatment target to improve cognitive deficits in MDD.

The second point of discussion addresses the finding that simultaneous MR and NMDA-R stimulation showed no (additive) effect on cognitive and emotional empathy in in healthy individuals and patients with MDD when compared to single MR stimulation or no stimulation. The observation does not confirm *hypothesis (B)*. The assumption of *hypothesis (B),* that simultaneous MR and NMDA-R stimulation might have synergistic effects on social cognition, was based on the observation that glucocorticoid MR stimulation leads to glutamatergic NMDA-R activation (Popoli et al., 2012). The observations of this research emphasize that MR and NMDA-R contribute independently to social cognition processes (cognitive empathy). One explanation for the absence of synergistic effects of MR and NMDA-R stimulation on social cognition may lie in the observation that GR, in addition to MR, contributes to the effects of glucocorticoids on glutamate transmission. While both MR and GR contribute to rapid effects of glucocorticoids on glutamate transmission, it is mainly GR which contributes to delayed effects which can last for several hours (Karst et al., 2010; Karst et al., 2005; Treccani et

al., 2014; Wang & Wang, 2009). This research examined delayed, rather than rapid, effects of MR stimulation on social cognition, with reference to recent research models (de Kloet et al., 2019; de Kloet & Joëls, 2020; Hermans et al., 2014; Joëls et al., 2018; Joëls et al., 2012). Thus, one might speculate that the reason this research project found no synergistic effects of MR and NMDA-R on social cognition, was because the research examined social cognition in a timeframe in which delayed glucocorticoid effects on glutamate transmission are mainly mediated via GR rather than MR.

The third point of discussion addresses the observation that, independent of any receptor stimulation, MDD patients had difficulties to empathize with positive emotions of another person when compared to healthy individuals. In line with other studies, the finding shows that MDD is associated with difficulties in processing positive emotional information (Kellough et al., 2008; Leppanen, 2006; Sloan et al., 2002). Considered together, the observations confirm Beck's cognitive model of depression, which emphasizes that a mood-congruent bias in processing emotional information contributes to the development and persistence of depression (Disner et al., 2011). Further research should examine whether MR and NMDA-R modulation influences cognitive biases in MDD and improves symptomatology of patients with MDD.

In summary, the observations of this research project emphasize that MR stimulation has cognitive-enhancing effects on social cognition (cognitive empathy) in healthy individuals and in patients with MDD. Furthermore, the research provides some evidence that NMDA-R are involved in social cognition in MDD patients. Separate NMDA-R stimulation reduced cognitive empathy in patients with MDD, but not in healthy individuals. The observations of this research emphasize that MR and NMDA-R are independently involved in social cognition. In contrast to the expectations, simultaneous MR and NMDA-R stimulation had no (additional) effects on social cognition when compared to separate MR and no stimulation. One might speculate that this research examined delayed effects of glucocorticoids on glutamate transmission, which are mediated via GR rather than MR, and that this could explain the absence of synergistic effects of MR and NMDA-R stimulation on social cognition. This research also found that MDD patients appear to have a mood-congruent bias in processing emotional information, which contributes to difficulties in empathizing with positive emotions. Combined, the observations are promising to motivate future research on the role of MR and NMDA-R in social cognition in MDD, and the potential of both receptors to serve as treatment targets to improve symptomatology of patients with MDD.

6.4 Discussion of the effects of MR and NMDA-R stimulation on emotion recognition and selective attention to emotional stimuli

The third study of this research project examined the following research question and hypotheses:

Research question: What is the effect of MR and NMDA-R stimulation on social cognition (facial emotion recognition and selective attention to emotional stimuli) in healthy individuals and patients with MDD?

Hypothesis (A): MR stimulation by fludrocortisone leads to higher scores in facial emotion recognition and reduced selective attention to emotional stimuli in healthy individuals and patients with MDD in comparison to no stimulation (placebo).

Hypothesis (B): Simultaneous MR and NMDA-R stimulation by fludrocortisone and DCS leads to higher scores in facial emotion recognition and reduces selective attention to emotional stimuli in healthy individuals and patients with MDD in comparison to separate MR stimulation and to no stimulation (placebo).

The research showed that MR stimulation has no effect on facial emotion recognition nor selective attention to emotional stimuli in healthy individuals and patients with MDD. Simultaneous MR and NMDA-R stimulation also showed no (additive) effect on facial emotion recognition nor selective attention to emotional stimuli in healthy individuals and patients with MDD, when compared to separate MR stimulation or no stimulation. Separate NMDA-R stimulation improved emotion recognition in both groups. Independent of any receptor stimulation, MDD patients showed no difficulties in facial emotion recognition and no selective attention to emotional stimuli in comparison to healthy individuals (Nowacki et al., 2021). Three points of discussion will be addressed in the following:

The first point of discussion addresses the observation that MR stimulation has no effect on facial emotion recognition nor selective attention to emotional stimuli in healthy individuals and patients with MDD. The observation does not confirm *hypothesis (A)* and contradicts the finding of the second study that MR stimulation has beneficial effects on social cognition (cognitive empathy) in healthy individuals and patients with MDD (Nowacki et al., 2020a). The heterogeneity of the observations of this research

project is in line with the general inconclusive picture derived from research on the effect of MR and GR stimulation on social cognition (for a review, see von Dawans et al., 2020). For example, in line with the observations of this research project Schultebraucks et al. (2016) found no effect of MR stimulation by fludrocortisone on facial emotion recognition in healthy individuals and also Duesenberg et al. (2016) showed that the MR and GR agonist hydrocortisone had no effect on facial emotion recognition in healthy individuals. However, in contrast to the current observations, the same studies found that MR stimulation increased selective attention towards negative emotional stimuli in healthy individuals (Schultebraucks et al., 2016) and that hydrocortisone administration had no effect on cognitive and emotional empathy in healthy individuals (Duesenberg et al., 2016). Considering these together, the observations of the studies agree that MR and GR stimulation has no effect on facial emotion recognition in healthy individuals and patients with MDD, they are all inconclusive concerning the effect of MR and GR stimulation on selective attention to emotional stimuli and empathy in both groups. One explanation for the heterogeneity of the observations may be that the involvement of MR and GR in social cognitive processes depends on other psycho-neuro-endocrinological mechanisms of the human stress response. For instance, the glucocorticoid system appears to interact with the noradrenergic system to shape social behavior in humans. While hydrocortisone administration increased prosocial behavior in healthy individuals, the effect of glucocorticoid activation was offset after simultaneous noradrenergic activation by yohimbine (Margittai et al., 2018). Thus, one might speculate that influences from the noradrenergic system contribute to the heterogeneity of observations concerning the effect of MR and GR stimulation on social cognition in healthy individuals and patients with MDD. Therefore, future studies should further examine the interplay between MR and GR and other psycho-neuro-endocrinological processes which shape the relationship between the human stress response and social cognition in health and disease.

The second point of discussion addresses the finding that simultaneous MR and NMDA-R stimulation showed no (additive) effect on facial emotion recognition and selective attention to emotional stimuli in healthy individuals and patients with MDD when compared to single MR stimulation or no stimulation. The observation does not confirm *hypothesis (B)* which predicted synergistic effects of MR and NMDA-R stimulation on social cognition, based on the observation that glucocorticoid MR stimulation leads to glutamatergic NMDA-R activation (Popoli et al., 2012). Together, the second and third study of this research project emphasize that there are no synergistic effects of MR and

NMDA-R stimulation on social cognition in healthy individuals and patients with MDD. Referring to the explanation described above (section 6.3), this research project examined social cognition in a timeframe in which GR, rather than MR, contributed to glucocorticoid effects on glutamate transmission (Karst et al., 2010; Karst et al., 2005; Treccani et al., 2014; Wang & Wang, 2009). Thus, the design of this research project might not have captured the rapid MR mediated effects of glucocorticoids on glutamate transmission, which could explain the absence of synergistic effects of MR and NMDA-R stimulation on social cognition. Therefore, future studies should examine possible synergistic effects of MR and NMDA-R stimulation on social cognition in a timeframe in which glucocorticoid effects on glutamate transmission are mediated via MR and GR.

The third point of discussion addresses the finding that separate NMDA-R stimulation improved emotion recognition in healthy individuals and patients with MDD. The observation is in agreement with studies that show that NMDA-R stimulation has cognitive-enhancing effects on learning and memory in healthy individuals (Feld et al., 2013; Onur et al., 2010). The involvement of NMDA-R in processes of learning and memory has been thoroughly researched and is closely related to the receptor's important role in synaptic plasticity (Lee & Silva, 2009). For instance, NMDA-R manipulation in the hippocampus of mice is accompanied by changes in synaptic signal transmission and reduced learning (Sakimura et al., 1995). Furthermore, NMDA-R blockade enhances hippocampal synaptic plasticity and memory functioning (Amin et al., 2015). Considered together, the observations suggest that NMDA-R stimulation has cognitive-enhancing effects on learning and memory by enhancing synaptic plasticity. While the NMDA-R involvement in these higher-order cognitive processes is well-established, the receptor's role in social cognition processes remains largely unknown. One recent study found evidence for cognitive-enhancing effects of DCS administration on memory in healthy individuals, yet there was no effect on emotion recognition or selective attention to emotional stimuli (Chen et al., 2020). This observation might suggest that the beneficial effects of NMDAR stimulation on cognition are not attributable to processes of social cognition. However, the observations of the current study indicate that NMDA-R stimulation might enhance emotion recognition in healthy individuals and patients with MDD. Since the small effect size restricts firm conclusions, further research is required to confirm the findings.

Combined, the third study found that separate MR has no effect on emotion recognition and selective attention to emotional stimuli in healthy individuals and

patients with MDD, while separate NMDA-R stimulation increased emotion recognition in both groups. Simultaneous MR and NMDA-R stimulation showed no (additive) effect on facial emotion recognition and selective attention to emotional stimuli in healthy individuals and patients with MDD. While NMDA-R involvement in learning and memory is well-established and closely related to the role of the receptor in synaptic plasticity, this research provides some evidence for NMDA-R involvement in social cognition, in healthy individuals and patients with MDD. However, further research is required to confirm this observation and to examine whether MR and NMDA-R are involved in social cognition in MDD.

6.5 Strengths and limitations of the studies

At first, this section addresses the strengths of this research project (section 6.5.1). Then, the limitations of the studies will be addressed (section 6.5.2).

6.5.1 Strengths of the studies

Firstly, the current research project examined a well-characterized sample of MDD patients and healthy individuals which strengthened internal validity. There is evidence that antidepressant use influences social cognition (Rütgen et al., 2019) and that demographic characteristics such as age, sex, and education level are associated with social cognition performance (Dalili et al., 2015). In the current research project, the studies examined 116 unmedicated patients with MDD and 116 unmedicated healthy controls matched in age, sex, and education years. Therefore, the influence of medication intake and particular demographic characteristics can be ruled out as alternative explanations, which strengthens the results.

Secondly, the current research project used well-established and diverse social cognition tasks. Social cognition is a broad concept which describes processes of identification, perception, and interpretation of socially salient information in the environment (Weightman et al., 2019). This research project examined central processes of social cognition through well-established social cognition tasks: an empathy task, an emotion recognition task, and a task that measured selective attention to emotional stimuli (Duesenberg et al., 2016; Schultebraucks et al., 2016; Wingenfeld et al., 2014). This strengthens the observation of this research, that MDD patients show no difficulties in social cognitive processes when compared to healthy individuals, except for empathizing

with positive emotions. Thus, social cognition appears to be fairly unimpaired in relatively young and unmedicated patients with MDD. Future studies should use the same social cognitive tasks in older MDD patients to draw firm conclusions on social cognitive deficits in MDD.

Thirdly, this research project had a high standard methodological design, which increased internal validity of the results. The studies used a double-blind placebo-controlled design with block-randomization conducted by the pharmacy of the Charité – Universitätsmedizin Berlin (section 2.2.2). Hence, experimenter and participants were blinded regarding drug administration. Therefore, placebo and expectancy effects can be ruled out as alternative explanations, which strengthens the internal validity of the observations of the current research project.

6.5.2 Limitations of the studies

Firstly, the pharmacological MR stimulation by fludrocortisone represents a simplistic approach to examine the receptor's complex role in (social) cognition. Studies emphasize that MR are involved in (social) cognition as part of a complex interplay between several mechanisms of the psycho-neuro-endocrinological reaction to acute stress. Vogel et al. (2015), for example, found that psychophysiological stress increased amygdala and striatum connectivity in facial emotion vigilance processing in healthy individuals. Importantly, the change in neuronal resources appeared to depend on MR functioning because the stress effect was abolished after MR blockade (Vogel et al., 2015). Moreover, MR blockade by spironolactone has been found to impair selective attention in non-stressed healthy individuals but not in psychosocially stressed (TSST) healthy individuals. Working memory, in contrast, was reduced after MR blockade, but only in stressed and not in non-stressed individuals. Thus, the effect of MR manipulation on cognitive processes appears to be different when individuals are under stress or at rest (Cornelisse et al., 2011). Therefore, future studies should combine pharmacological MR manipulations with a psychosocial or psychophysiological stress induction to examine the receptor's complex role in (social) cognition.

Secondly, the use of the partial NMDA-R agonist DCS does not permit inferences on whether the effects of MR stimulation on social cognition in MDD depend on NMDA-R. This would require NMDA-R blockade, for example, by administration of the antagonist memantine. N-methyl-D-aspartate receptor blockade by memantine has been found to improve spatial working memory as well as associated synaptic plasticity in the

hippocampus of rats (Amin et al., 2015) and has been shown to protect from long-term adverse effects of corticosteroid on the human hippocampus (Brown et al., 2019). Thus, to draw conclusions on whether the beneficial effects of MR stimulation on (social) cognition depend on NMDA-R, future studies should examine social cognition after MR stimulation (administration of fludrocortisone) and simultaneous NMDA-R blockade (administration of memantine).

Thirdly, the sample of MDD patients examined in this research project was somatically relatively healthy. The sample characteristics indicate that patients were unmedicated, relatively young (M = 34 years), and did not suffer from severe medical conditions (Nowacki et al., 2020a; Nowacki et al., 2020b; Nowacki et al., 2021). This has implications for the interpretation and generalizability of the results. For instance, there is evidence that HPA axis activity dysregulations is especially pronounced in older compared to younger MDD patients (Murri et al., 2014). Furthermore, social cognition performance in MDD and bipolar disorder appears to be associated with age and education level (Kohler et al., 2011). Thus, the observations of this research project may particularly apply to MDD patients who are relatively young and physically healthy. Further research is required to examine the influence of MR and NMDA-R stimulation on steroid hormone secretion and social cognition in a sample of older and less physically healthy MDD patients. This would increase external validity.

6.6 Explanatory model for the effects of MR and NMDA-R stimulation on steroid hormone secretion and social cognition

The central finding of this research project is that MR stimulation by fludrocortisone decreased cortisol secretion and enhanced social cognition (cognitive empathy) in healthy individuals and patients with MDD. Moreover, MR stimulation showed no effect on emotional empathy, emotion recognition, or selective attention to emotional stimuli in both groups. Separate NMDA-R stimulation by DCS decreased cognitive empathy in MDD patients and improved emotion recognition in both groups. Lastly, simultaneous MR and NMDA-R stimulation showed no (additive) effect on all examined social cognitive processes in comparison to separate MR stimulation or no stimulation in both groups (Nowacki et al., 2020a; Nowacki et al., 2020b; Nowacki et al., 2021).

The following explanatory model aims to integrate the observations of this research project into existing models of the psycho-neuro-endocrinological reaction to stress (de Kloet, 2014; de Kloet et al., 2019; de Kloet & Joëls, 2020; Hermans et al., 2014).

The model represents a simplistic approach based on current research and it is important to highlight that the exact interplay between the psycho-neuro-endocrinological processes is far from understood. The explanatory model is based on the central assumption that this research project examined the late phase of the stress response (section 1.4), since social cognition was assessed four hours after MR and three hours after NMDA-R stimulation (de Kloet et al., 2019; de Kloet & Joëls, 2020; Hermans et al., 2014; Joëls et al., 2018; Joëls et al., 2012). The explanatory model is summarized in Figure 5 and outlined thereafter.

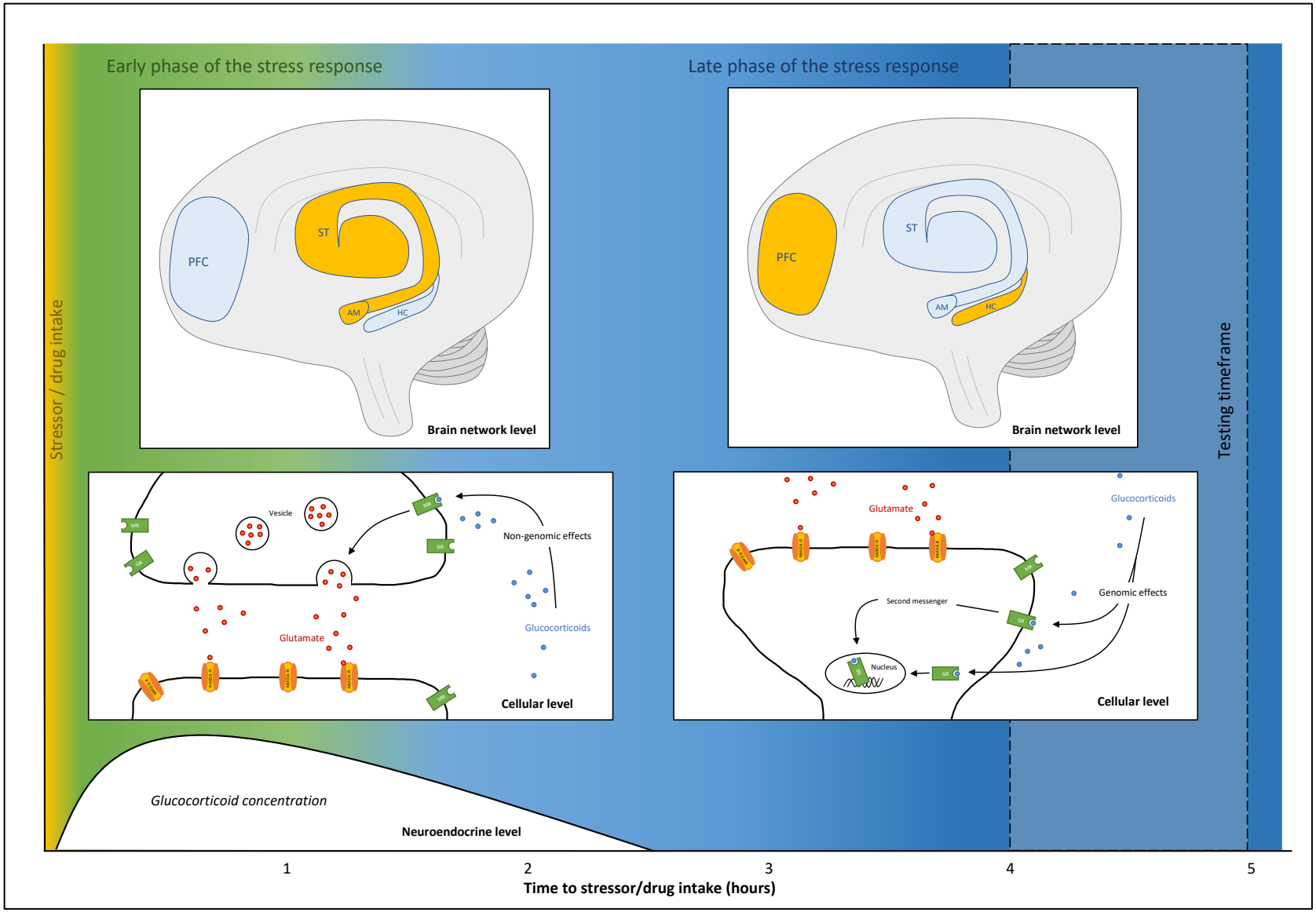

Figure 5. Schematic summary of the explanatory model.

Note: The figure illustrates the processes on the brain network, cellular, and neuroendocrine level, which take place after stress exposure or drug intake (yellow shaded) in the early phase of the stress response (green shaded) and late phase of the stress response (blue shaded) respectively. **In the early phase of the stress response**, on the brain network level (cutout of Figure 1), the amygdala (AM) and the striatum (ST) contribute to lower-order cognitive processes, which are less demanding and enable a quick response to the stressor. On the cellular level (cutout of Figure 3), rapid non-genomic processes take place via membrane mineralocorticoid receptors (MR) and glucocorticoid receptors (GR) which contribute to glutamatergic signal transmission via N-methyl-D-aspartate receptors (NMDA-R). On the neuroendocrine level, concentrations of glucocorticoids are rising and due to different binding affinities of cortisol for both receptors, MR become occupied before GR. **In the late phase of the stress response**, on the brain network level (cutout of Figure 1), the prefrontal cortex (PFC) and hippocampus (HC) contribute to higher-order cognitive processes, which are more demanding and that enable profound processing of the stressful situation. On the cellular level (cutout of Figure 3), delayed genomic MR and GR effects take place that contribute to glutamatergic signal transmission, which is prolonged via genomic GR. On the neuroendocrine level, the rise in glucocorticoid concentrations leads to increased GR occupation, which contributes to a down-regulation of cortisol secretion. The grey rectangle marks the approximate timeframe in which social cognitive processes were examined in this research project. Illustration based on existing models (de Kloet & Joëls, 2020; de Kloet et al., 2005; Hermans et al., 2014; Popoli et al., 2012; Vogel et al., 2016).

On the behavioral level, the early and late phase of the stress response are associated with different behavioral reactions. In the early phase of the stress response, the individual is focused on paying attention to and appraising the stressful information, these rapid behavioral reactions strongly involve emotional processes. In the late phase of the stress response, the individual is engaged in contextualization and rationalization to processes the stressful information, these behavioral reactions largely involve higher-order cognitive processes that are much more cognitive demanding (de Kloet, 2014; de Kloet et al., 2019; de Kloet & Joëls, 2020). Evidence for the difference in behavioral reactions between the early and late phases of the stress response stems from studies that examined (social) cognitive processes soon as well as long after stress exposure or drug administration respectively. For instance, one study found that directly after the induction of psychosocial stress (TSST), participants showed reduced contextualization of neutral information associated with cortisol secretion whereas long after stress induction (120min) contextualization of neutral information was enhanced (Sep et al., 2020). Moreover, in another study participants showed increased altruistic punishment directly after psychosocial stress (TSST), whereas long after stress induction (75min) participants showed less altruistic punishment but used more complex behavioral strategies that were more beneficial in the long-term (Vinkers et al., 2013). Importantly, the MR and GR appear to play an important role in this context as shown by research where hydrocortisone was administered, which serves as an MR and GR agonist, depending on the administered dosage (see section 1.6.1). For example, research found that soon after hydrocortisone administration (30min) participants showed decreased contextualization of emotional memories, while long after drug administration (210min) contextualization was enhanced (van Ast et al., 2013). Moreover, another study found that soon (15min) but not long (195min) after hydrocortisone administration, people used simple decision-making strategies when making intertemporal choices (Cornelisse et al., 2013). In brief, the studies emphasize that the effects of psychosocial stress and MR and GR stimulation on (social) cognitive processes are time-dependent. In the early phase of the stress response, people appear to use rapid, simple behavioral strategies and do not process much contextual information. In the late phase of the stress response, people appear to process more contextual information and show les reactive, but more complex, behavioral reactions. These findings are complemented by observations of research on the brain network level.

On the brain network level, the early and late phases of the stress response are linked to different brain networks. The early phase of the stress response is related to the salience network. The brain network is involved in fast, exogenous attention to and appraisal of emotional, salient information (e.g., facial emotion expression) linked to the amygdala, hypothalamus, and other brain areas. The late phase of the stress response is related to the executive control network. The brain network is associated with higher-order cognitive processes (e.g., endogenous attention, working memory, and decision making) linked to many brain regions allocated in the prefrontal cortex, among others (Hermans et al., 2014). Interestingly, several studies found evidence that MR are involved in allocating brain resources relevant for (social) cognitive processes in response to acute stress. The studies examined (social) cognition after the induction of psychophysiological stress (SECPT) while blocking or not blocking MR with spironolactone. Acute psychophysiological stress enhanced emotional vigilance processing and habitual learning (stimulus response learning) associated with a change in amygdala-striatum connectivity and, importantly, there was no stress effect after MR blockade (Vogel et al., 2015; Vogel et al., 2017). Moreover, in a similar study, acute psychophysiological stress induced a change from hippocampus-based declarative learning to striatum-based procedural learning, with associated altered connectivity between the amygdala and the two brain areas respectively and, again, the stress effect was prevented after MR blockade (Schwabe et al., 2013). Thus, in reaction to acute stress, MR appear to allocate brain resources to the amygdala and striatum to support lower-order (social) cognitive processes (emotional processing, stimulus response or procedural learning) in the early phase of the stress response and away from the hippocampus, which supports higher-order cognitive processes (declarative learning) relevant for the late phase of the stress response. These findings are complemented by four similar brain imaging studies that examined (social) cognitive processes soon as well as long after administration of the MR and GR agonist hydrocortisone to observe potential differences in the receptors' role between the early and late phase of the stress response. The first study showed that soon after drug administration (75min) amygdala reactivity to emotional stimuli was reduced, while long after hydrocortisone administration (258min) amygdala reactivity was normalized for negative emotional stimuli, which is associated with changes in amygdala connectivity with the medial prefrontal cortex (Henckens et al., 2010). The second study found that soon after administration of hydrocortisone (30min) there was no effect on working memory performance while long after drug administration (240min) working

memory performance was enhanced, which was associated with activity in the dorsolateral prefrontal cortex (Henckens et al., 2011). The third study showed that soon after hydrocortisone administration (30min) there was no effect on memory encoding, whereas long after drug administration (180min) reactivity of the prefrontal cortex and hippocampus was reduced and this downregulation might support fine-tuning of memory processes (Henckens et al., 2012a). The fourth study found that soon after hydrocortisone administration (60min) emotional interference and selective attention was increased, which was associated with reduced amygdala inhibition. Long after drug administration (270min), there was less bottom-up processing but enhanced sustained attention, which was associated with reduced activity in the cuneus (Henckens et al., 2012b). To sum up, the involvement of MR and GR in (social) cognitive processes appears to be different for the early and late phase of the stress response. In the early phase of the stress response, the receptors seem to be involved in lower-order (social) cognitive processes (emotional processing, emotional interference, and selective attention), which are linked to brain areas including the amygdala. In the late phase of the stress response, MR and GR might be relevant for higher-order (social) cognitive processes (sustained attention and memory processes), which are associated with the prefrontal cortex and hippocampus, among others.

The observations on the behavioral and brain network level have implications for the interpretation of the results of the second study (Nowacki et al., 2020a) and third study (Nowacki et al., 2021) of this research project. Referring to the central assumption, this research project examined social cognitive processes in a timeframe associated with the late rather than early phase of the stress response. Accordingly, MR stimulation should have affected higher-order (social) cognitive processes, which are associated with the prefrontal cortex and hippocampus, among others, rather than lower-order (social) cognitive processes linked to the amygdala, among others. In line with this argumentation, the second study found that MR stimulation affected cognitive but not emotional empathy (Nowacki et al., 2020a). The social cognitive processes are related but independent processes: cognitive empathy is associated with higher-order cognitive processes (e.g., perspective taking), primarily linked to the medial and dorsolateral prefrontal cortex and emotional empathy is related to emotion-driven processes (e.g., emotional reactivity), primarily linked to the amygdala, hypothalamus, and hippocampus (Tone & Tully, 2014). The line of argumentation might also explain why the third study found no effect of MR stimulation on facial emotion recognition and selective attention to

emotional stimuli (Nowacki et al., 2021). The processes of facial emotion recognition and selective attention to emotional stimuli rely on quick emotional and attentional processes that are, as described above, associated with the early rather than the late phase of the stress response. Thus, one might speculate whether the timeframe in which this research project examined these social cognitive processes might have been too late to capture effects of MR stimulation on emotional empathy, facial emotion recognition, and selective attention to emotional stimuli. Based on this argumentation, MR stimulation should affect these social cognitive processes when examined in a timeframe associated with the early phase of the stress response. Indeed, when the social cognitive processes were examined in earlier timeframes, MR stimulation enhanced emotional but not cognitive empathy in healthy individuals and patients with borderline personality disorder (Wingenfeld et al., 2014) and increased selective attention to negative emotional information in healthy individuals (Schultebraucks et al., 2016). Future research should examine social cognitive processes early and late after MR stimulation to provide additional support for this line of argumentation.

On the neuroendocrine level, the involvement of MR and GR in the neuroendocrine response to stress differs between the early and late phase of the stress response. Cortisol has a higher binding affinity for MR than GR. Accordingly, in the early phase of the stress response, when cortisol concentrations are rising, predominantly MR are involved. With increasing cortisol concentrations, GR become occupied, which contributes to a down-regulation of the stress response and decreasing cortisol concentrations in the late phase of the stress response (de Kloet et al., 2018; Herman et al., 2016). The current research project observed that MR stimulation by the agonist fludrocortisone decreased cortisol concentration in healthy individuals and in patients with MDD; this observation is in line with earlier research (Buckley et al., 2007; Otte et al., 2003; Otte et al., 2015a). The observations suggest that the examined group of unmedicated and relatively young patients with MDD showed intact MR functioning, which contributed to accurate down-regulation of the HPA axis and decreased cortisol concentrations.

The MR:GR balance hypothesis, however, predicts that patients with MDD suffer from dysregulations in the interplay between MR and GR functioning, contributing to altered HPA axis functioning in MDD (de Kloet, 2014). In line with the MR:GR balance hypothesis, this research project found that the same group of MDD patients suffered from altered steroid hormone concentrations (cortisol, aldosterone, DHEA-S) when

compared to healthy individuals. Given the firm evidence for a glucocorticoid resistance in MDD (Pariante, 2017; Pariante & Lightman, 2008) and given that mainly GR are involved in the termination of the stress response (de Kloet et al., 2018; Herman et al., 2016), one might speculate that altered GR functioning contributed to the observed hypercortisolism in the group of examined patients with MDD. In contrast to patients with treatment resistant depression (Juruena et al., 2013) and patients with psychotic MDD (Lembke et al., 2013), the group of MDD patients examined showed intact MR mediated HPA axis regulation. In agreement with several researchers (Juruena et al., 2013; Pariante & Lightman, 2008), the observations might indicate that intact MR functioning can compensate for GR dysfunction in specific groups of patients with MDD. The results of this research project suggest that in unmedicated and relatively young patients with MDD, alterations in the neuroendocrine stress response are attributable to GR, rather than MR, dysfunction.

On the cellular level, in the early phase of the stress response mainly rapid non-genomic MR and GR mediated actions take place (de Kloet et al., 2019) and there is evidence that stimulation of membrane MR and GR leads to rapid glutamatergic signal transmission in the amygdala, hippocampus, and prefrontal cortex (Karst et al., 2010; Karst et al., 2005; Treccani et al., 2014; Wang & Wang, 2009). In the late phase of the stress response, mainly delayed genomic MR and GR mediated actions take place (de Kloet et al., 2019) and there is evidence that the rapid effects of glucocorticoids on glutamate transmission are prolonged for up to several hours via genomic GR in the amygdala (Karst et al., 2010). Furthermore, it was shown that this effect was associated with increased NMDA-R activity in the prefrontal cortex (Yuen et al., 2011). In brief, in the early phase of the stress response mainly rapid non-genomic MR and GR effects take place and glucocorticoids induce glutamatergic signal transmission via membrane MR and GR. In the late phase of the stress response, mainly delayed genomic MR and GR effects take place and the effects of glucocorticoids on glutamate transmission are prolonged via genomic GR. The observations have implications for the interpretation of the results of this research project. First, MR stimulation appears to increase cognitive empathy in healthy individuals and patients with MDD via delayed genomic effects in the late phase of the stress response. Second, the absence of synergistic effects of MR and NMDA-R stimulation on social cognition in the late phase of the stress response may be explained by the observation that delayed effects of corticosteroid on glutamatergic signal transmission are mediated via nuclear GR rather than MR.

To summarize the explanatory model, unmedicated and relatively young patients with MDD show intact MR mediated HPA axis down-regulation which leads to decreased cortisol concentrations, as in healthy individuals. Altered GR functioning, however, appears to change the interplay between MR and GR functioning, leading to altered steroid hormone concentrations in the group of patients with MDD. Furthermore, this research project examined the delayed effects of MR stimulation on social cognition in a timeframe that is associated with the late phase of the stress response. In the late phase of the stress response, genomic MR and GR mediated effects take place, which are linked to higher-order rational (social) cognitive processes associated with the prefrontal cortex and hippocampus among others. Accordingly, the increase in cognitive empathy in healthy individuals and patients with MDD after MR stimulation found in this research project may be associated with MR mediated genomic effects linked to brain areas including the prefrontal cortex. The early phase of the stress response is related to lower-order emotional (social) cognitive processes (attention to and appraisal of emotional information) which are linked to early non-genomic MR mediated effects and are associated with the amygdala and striatum among others. The current research project found no effect of MR stimulation on selective attention, emotion recognition, or emotional empathy. Accordingly, the timeframe in which social cognition was examined may have been too late to capture the rapid effects of MR stimulation on processes of attention to and appraisal of emotional information, which are associated with the amygdala and striatum, among others, in the early phase of the stress response. Simultaneous MR and NDMA-R stimulation showed no synergistic effects on social cognition in this research project, possibly due to the delayed effects of glucocorticoids on glutamate transmission are mainly mediated via nuclear GR rather than MR. Again, the explanatory model is simplistic and the exact interplay between the psycho-neuro-endocrinological processes is far from understood. Future research is required to provide further insight into the psycho-neuro-endocrinological stress response as described in the following.

6.7 Future directions

The observations of this research project have several implications for future research. First, the observations implicate, combined with earlier research (de Kloet, 2014; de Kloet et al., 2019; de Kloet & Joëls, 2020), that the late phase of the stress response is primarily associated with higher-order cognitive processes rather than emotional processes.

Therefore, it might be worth examining the delayed effects of MR stimulation on cognitive processes, which are high-demanding. For instance, there is evidence that MR stimulation by fludrocortisone affects risky decision making and spatial memory in healthy individuals (Deuter et al., 2017; Piber et al., 2016). Given that MDD patients suffer from impairments in several cognitive processes (Rock et al., 2014), it would be interesting to examine whether MR stimulation has beneficial delayed effects on higher-order cognitive domains, such as decision making and spatial memory, in patients with MDD.

Second, this research project examined the hypothesis that simultaneous MR and NMDA-R stimulation has synergistic effects on social cognition in MDD. The hypothesis was based on the observations that (1) MR stimulation has cognitive-enhancing effects in MDD (Otte et al., 2015a), that (2) glucocorticoids lead to MR mediated glutamate transmission via NMDA-R (Popoli et al., 2012), and that (3) NMDA-R stimulation has cognitive-enhancing effects in healthy individuals (Feld et al., 2013; Onur et al., 2010; Scholl et al., 2014). The absence of synergistic effects of MR and NMDA-R stimulation on social cognition observed in this research raised the question whether other mechanisms might underlie the long-term effects of glucocorticoids on glutamate transmission (section 6.6). There is evidence that the rapid MR mediated effects of glucocorticoids on glutamate transmission are prolonged by genomic GR (Karst et al., 2010; Yuen et al., 2011) and that GR play an important role, both in the stress response and in depression (Anacker et al., 2011; Gray et al., 2017). Therefore, future studies should examine the possible synergistic effects of GR and NMDA-R stimulation on social cognition in MDD in the late phase of the stress response.

Third, together with earlier research, the observations of this research project indicate that specific subgroups of patients with MDD may profit from the possible beneficial effects of MR stimulation on steroid hormone secretion and social cognition. For instance, in this research MR stimulation decreased cortisol secretion in healthy individuals and patients with MDD. The observation is in line with earlier research (Buckley et al., 2007; Otte et al., 2003; Otte et al., 2015a) and might indicate that HPA axis activity regulation via MR is intact in MDD patients. Other studies, however, report that MR mediated HPA axis regulation is impaired in patients with treatment-resistant MDD (Juruena et al., 2013) and psychotic MDD (Lembke et al., 2013) and that older MDD patients suffer from greater HPA axis dysregulation than younger patients with MDD (Murri et al., 2014). Moreover, this research revealed that relatively young and unmedicated patients with MDD show no alterations in social cognitive processes

compared with healthy individuals, except for expressing emotional empathy for positive emotions (Nowacki et al., 2020a; Nowacki et al., 2021). Other studies emphasize that patients with MDD suffer impairments in several (social) cognitive domains and it is debated that the impairments are associated with patient characteristics such as medication status and severity of depression (Dalili et al., 2015; Rock et al., 2014; Weightman et al., 2014). Thus, future studies should examine whether MR stimulation has delayed beneficial effects on steroid hormone secretion and social cognition in other samples of patients with MDD, such as, older patients with treatment resistant depression.

6.8 Conclusion

The aim of this research project was to examine the role of MR and NMDA-R in steroid hormone secretion and social cognitive processes in healthy individuals and patients with MDD. The central research question was:

What is the effect of MR and NMDA-R stimulation on steroid hormone secretion and social cognition in healthy individuals and patients with MDD?

The central observations of this research project were that MR stimulation decreased cortisol secretion and enhanced cognitive empathy in healthy individuals and patients with MDD. Furthermore, the research project provided evidence for NMDA-R involvement in social cognition in both groups. In addition, simultaneous MR and NMDA-R stimulation showed no (additional) effect on steroid hormone secretion and social cognition in healthy individuals and patients with MDD (Nowacki et al., 2020a; Nowacki et al., 2020b; Nowacki et al., 2021). Therefore, the observations provide new insights into the important role of MR and NMDA-R in the psycho-neuro-endocrine response to stress, in health and disease, they also provide knowledge for future research on the role of both receptors in steroid hormone secretion and social cognition in both healthy individuals and patients with MDD.

Importantly, the observations provide knowledge that could motivate future research to examine the potential of MR and NMDA-R to serve as treatment targets to improve the symptomatology of MDD. The current research shows that the cognitive-enhancing effects of MR stimulation (Otte et al., 2015a) are extendable to social cognition (cognitive empathy) in healthy individuals and patients with MDD and that the beneficial

effects of MR stimulation are not improvable by simultaneous NMDA-R stimulation. The explanatory model of this research project suggests that the beneficial effects may be induced by simultaneous GR and NMDA-R stimulation, this should also be examined further by future research. In addition, the explanatory model emphasizes, in line with the MR:GR balance hypothesis (de Kloet, 2014), that understanding the interplay between MR and GR functioning is crucial to gaining new insight into the psychopathology of MDD. New insight into the relationship between MR, GR, and NMDA-R in steroid hormone secretion and (social) cognition in patients with MDD may contribute to the development of new psychopharmacological methods to treat MDD. Major depressive disorder represents a psychiatric disorder with a high burden of disease (James et al., 2018) and high prevalence in the global population (World Health Organization, 2017).

In reference to the introduction, the observations of this research project support the notion of Lazarus, stress is the product of a transaction between an individual and its environment and that understanding this relationship is crucial to understand "diseases that have psychological determinants" (Lazarus, 1974, 1990; Lazarus & Folkman, 1984). This research project aimed to shed some more light on this relationship between the individual and its environment, it also emphasizes that each of the psychological, neurological, and endocrinological components combined shape the human stress response. To end where it began, it appears that decades ago Lazarus provided a profound description of stress when he stated that:

"Psychological stress is a particular relationship between the person and the environment that is appraised by the person as taxing or exceeding his or her resources and endangering his or her well-being"
(Lazarus & Folkman, 1984, p. 19)

References

Abel, K. M., Allin, M. P. G., Kucharska-Pietura, K., David, A., Andrew, C., Williams, S., . . . Phillips, M. L. (2003). Ketamine alters neural processing of facial emotion recognition in healthy men: an fMRI study. *Neuroreport*, *14*(3).

Agarwal, M., Coupry, F., & Philippe, M. (1977). Physiological activity and receptor binding of 9α fluorohydrocortisone. *Biochem Biophys Res Commun*, *78*(2), 747-753.

Ahima, R., Krozowski, Z., & Harlan, R. E. (1991). Type I corticosteroid receptor-like immunoreactivity in the rat CNS: distribution and regulation by corticosteroids. *Journal of Comparative Neurology*, *313*(3), 522-538.

Altamura, C. A., Mauri, M. C., Ferrara, A., Moro, A. R., D'Andrea, G., & Zamberlan, F. (1993). Plasma and platelet excitatory amino acids in psychiatric disorders. *The American journal of psychiatry*.

American Psychiatric Association. (2014). *Diagnostisches und Statistisches Manual Psychicher Störungen DSM-5*. Hogrefe.

Amin, S. N., El-Aidi, A. A., Ali, M. M., Attia, Y. M., & Rashed, L. A. (2015). Modification of hippocampal markers of synaptic plasticity by memantine in animal models of acute and repeated restraint stress: implications for memory and behavior. *Neuromolecular medicine*, *17*(2), 121-136.

Anacker, C., Zunszain, P. A., Carvalho, L. A., & Pariante, C. M. (2011). The glucocorticoid receptor: pivot of depression and of antidepressant treatment? *Psychoneuroendocrinology*, *36*(3), 415-425.

Arp, J. M., ter Horst, J. P., Kanatsou, S., Fernández, G., Joëls, M., Krugers, H. J., & Oitzl, M. S. (2014). Mineralocorticoid receptors guide spatial and stimulus-response learning in mice. *PLoS One*, *9*(1), e86236-e86236.

Arriza, J. L., Simerly, R. B., Swanson, L. W., & Evans, R. M. (1988). The neuronal mineralocorticoid eeceptor as a mediator of glucocorticoid response. *Neuron*, *1*(9), 887-900.

Berger, S., Wolfer, D. P., Selbach, O., Alter, H., Erdmann, G., Reichardt, H. M., . . . Lipp, H.-P. (2006). Loss of the limbic mineralocorticoid receptor impairs behavioral plasticity. *Proceedings of the National Academy of Sciences*, *103*(1), 195-200.

Brinks, V., Berger, S., Gass, P., De Kloet, E., & Oitzl, M. (2009). Mineralocorticoid receptors in control of emotional arousal and fear memory. *Hormones and Behavior*, *56*(2), 232-238.

Brown, E. S., Kulikova, A., Van Enkevort, E., Nakamura, A., Ivleva, E. I., Tustison, N. J., . . . Frol, A. (2019). A randomized trial of an NMDA receptor antagonist for reversing corticosteroid effects on the human hippocampus. *Neuropsychopharmacology*, *44*(13), 2263-2267.

Buckley, T. M., Mullen, B. C., & Schatzberg, A. F. (2007). The acute effects of a mineralocorticoid receptor (MR) agonist on nocturnal hypothalamic–adrenal–pituitary (HPA) axis activity in healthy controls. *Psychoneuroendocrinology*, *32*(8), 859-864.

Cain, D. W., & Cidlowski, J. A. (2017). Immune regulation by glucocorticoids. *Nature Reviews Immunology*, *17*(4), 233.

Caprio, M., Feve, B., Claës, A., Viengchareun, S., Lombes, M., & Zennaro, M.-C. (2007). Pivotal role of the mineralocorticoid receptor in corticosteroid-induced adipogenesis. *The FASEB Journal*, *21*(9), 2185-2194.

Chen, M.-H., Cheng, C.-M., Gueorguieva, R., Lin, W.-C., Li, C.-T., Hong, C.-J., . . . Krystal, J. H. (2019). Maintenance of antidepressant and antisuicidal effects by D-cycloserine

among patients with treatment-resistant depression who responded to low-dose ketamine infusion: a double-blind randomized placebo–control study. *Neuropsychopharmacology*, *44*(12), 2112-2118.
Chen, R., Capitão, L. P., Cowen, P. J., & Harmer, C. J. (2020). Effect of the NMDA receptor partial agonist, d-cycloserine, on emotional processing and autobiographical memory. *Psychological medicine*, 1-9.
Christian, K. M., Miracle, A. D., Wellman, C. L., & Nakazawa, K. (2011). Chronic stress-induced hippocampal dendritic retraction requires CA3 NMDA receptors. *Neuroscience*, *174*, 26-36.
Cornelisse, S., Joëls, M., & Smeets, T. (2011). A randomized trial on mineralocorticoid receptor blockade in men: effects on stress responses, selective attention, and memory. *Neuropsychopharmacology*, *36*(13), 2720-2728.
Cornelisse, S., Van Ast, V., Haushofer, J., Seinstra, M., & Joels, M. (2013). Time-dependent effect of hydrocortisone administration on intertemporal choice. *Available at SSRN 2294189.*
Dalili, M. N., Penton-Voak, I. S., Harmer, C. J., & Munafò, M. R. (2015). Meta-analysis of emotion recognition deficits in major depressive disorder. *Psychological medicine*, *45*(6), 1135-1144.
Davis, M., Ressler, K., Rothbaum, B. O., & Richardson, R. (2006). Effects of D-cycloserine on extinction: translation from preclinical to clinical work. *Biological psychiatry*, *60*(4), 369-375.
de Kloet, A. D., Krause, E. G., Solomon, M. B., Flak, J. N., Scott, K. A., Kim, D.-H., . . . Seeley, R. J. (2015). Adipocyte glucocorticoid receptors mediate fat-to-brain signaling. *Psychoneuroendocrinology*, *56*, 110-119.
de Kloet, E. (2014). From Receptor Balance to Rational Glucocorticoid Therapy. *Endocrinology*, *155*(8), 2754-2769.
de Kloet, E., Meijer, O., de Nicola, A., de Rijk, R., & Joëls, M. (2018). Importance of the brain corticosteroid receptor balance in metaplasticity, cognitive performance and neuro-inflammation. *Frontiers in neuroendocrinology*.
de Kloet, E. R., de Kloet, S. F., de Kloet, C. S., & de Kloet, A. D. (2019). Top-down and bottom-up control of stress-coping. *Journal of neuroendocrinology*, *31*(3), e12675.
de Kloet, E. R., & Joëls, M. (2020). Mineralocorticoid Receptors and Glucocorticoid Receptors in HPA Stress Responses During Coping and Adaptation. In *Oxford Research Encyclopedia of Neuroscience*.
de Kloet, E. R., Joëls, M., & Holsboer, F. (2005). Stress and the brain: from adaptation to disease. *Nature Reviews Neuroscience*, *6*(6), 463-475.
Deuter, C. E., Nowacki, J., Wingenfeld, K., Kuehl, L. K., Finke, J. B., Dziobek, I., & Otte, C. (2018). The role of physiological arousal for self-reported emotional empathy. *Autonomic Neuroscience*, *214*, 9-14.
Deuter, C. E., Wingenfeld, K., Schultebraucks, K., Hellmann-Regen, J., Piber, D., & Otte, C. (2017). Effects of mineralocorticoid-receptor stimulation on risk taking behavior in young healthy men and women. *Psychoneuroendocrinology*, *75*, 132-140.
Disner, S. G., Beevers, C. G., Haigh, E. A., & Beck, A. T. (2011). Neural mechanisms of the cognitive model of depression. *Nature Reviews Neuroscience*, *12*(8), 467.
Duesenberg, M., Weber, J., Schulze, L., Schaeuffele, C., Roepke, S., Hellmann-Regen, J., . . . Wingenfeld, K. (2016). Does cortisol modulate emotion recognition and empathy? *Psychoneuroendocrinology*, *66*, 221-227.
Dziobek, I., Rogers, K., Fleck, S., Bahnemann, M., Heekeren, H. R., Wolf, O. T., & Convit, A. (2008). Dissociation of cognitive and emotional empathy in adults with Asperger

syndrome using the Multifaceted Empathy Test (MET). *Journal of autism and developmental disorders*, *38*(3), 464-473.
Ebert, A., Haussleiter, I. S., Juckel, G., Brüne, M., & Roser, P. (2012). Impaired facial emotion recognition in a ketamine model of psychosis. *Psychiatry Res*, *200*(2), 724-727.
Emanuele, E., Geroldi, D., Minoretti, P., Coen, E., & Politi, P. (2005). Increased Plasma Aldosterone in Patients with Clinical Depression. *Archives of Medical Research*, *36*(5), 544-548.
Feld, G. B., Lange, T., Gais, S., & Born, J. (2013). Sleep-dependent declarative memory consolidation—unaffected after blocking NMDA or AMPA receptors but enhanced by NMDA coagonist D-cycloserine. *Neuropsychopharmacology*, *38*(13), 2688.
Ferguson, D., & Sapolsky, R. (2008). Overexpression of mineralocorticoid and transdominant glucocorticoid receptor blocks the impairing effects of glucocorticoids on memory. *Hippocampus*, *18*(11), 1103-1111.
Feyissa, A. M., Chandran, A., Stockmeier, C. A., & Karolewicz, B. (2009). Reduced levels of NR2A and NR2B subunits of NMDA receptor and PSD-95 in the prefrontal cortex in major depression. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *33*(1), 70-75.
Fitzsimons, C. P., Herbert, J., Schouten, M., Meijer, O. C., Lucassen, P. J., & Lightman, S. (2016). Circadian and ultradian glucocorticoid rhythmicity: Implications for the effects of glucocorticoids on neural stem cells and adult hippocampal neurogenesis. *Frontiers in neuroendocrinology*, *41*, 44-58.
Fleischer, J., Wingenfeld, K., Kuehl, L. K., Hinkelmann, K., Roepke, S., & Otte, C. (2015). Does fludrocortisone influence autobiographical memory retrieval? A study in patients with major depression, patients with borderline personality disorder and healthy controls. *Stress*, *18*(6), 718-722.
Flood, J. F., Morley, J. E., & Lanthorn, T. H. (1992). Effect on memory processing by D-cycloserine, an agonist of the NMDA/glycine receptor. *European journal of pharmacology*, *221*(2-3), 249-254.
Funder, J. W., & Reincke, M. (2010). Aldosterone: A cardiovascular risk factor? *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*, *1802*(12), 1188-1192.
Gray, J. D., Kogan, J. F., Marrocco, J., & McEwen, B. S. (2017). Genomic and epigenomic mechanisms of glucocorticoids in the brain. *Nature Reviews Endocrinology*, *13*(11), 661.
Groch, S., Wilhelm, I., Lange, T., & Born, J. (2013). Differential contribution of mineralocorticoid and glucocorticoid receptors to memory formation during sleep. *Psychoneuroendocrinology*, *38*(12), 2962-2972.
Grossmann, C., Scholz, T., Rochel, M., Bumke-Vogt, C., Oelkers, W., Pfeiffer, A., . . . Bahr, V. (2004). Transactivation via the human glucocorticoid and mineralocorticoid receptor by therapeutically used steroids in CV-1 cells: a comparison of their glucocorticoid and mineralocorticoid properties. *European Journal of Endocrinology*, *151*(3), 397-406.
Henckens, M. J., Pu, Z., Hermans, E. J., van Wingen, G. A., Joëls, M., & Fernández, G. (2012a). Dynamically changing effects of corticosteroids on human hippocampal and prefrontal processing. *Hum Brain Mapp*, *33*(12), 2885-2897.
Henckens, M. J., van Wingen, G. A., Joëls, M., & Fernández, G. (2010). Time-dependent effects of corticosteroids on human amygdala processing. *Journal of Neuroscience*, *30*(38), 12725-12732.
Henckens, M. J., van Wingen, G. A., Joëls, M., & Fernández, G. (2011). Time-dependent corticosteroid modulation of prefrontal working memory processing. *Proceedings of the National Academy of Sciences*, *108*(14), 5801-5806.

Henckens, M. J., van Wingen, G. A., Joëls, M., & Fernández, G. (2012b). Time-dependent effects of cortisol on selective attention and emotional interference: a functional MRI study. *Frontiers in integrative neuroscience*, *6*, 66.

Heresco-Levy, U., Gelfin, G., Bloch, B., Levin, R., Edelman, S., Javitt, D. C., & Kremer, I. (2013). A randomized add-on trial of high-dose D-cycloserine for treatment-resistant depression. *International Journal of Neuropsychopharmacology*, *16*(3), 501-506.

Heresco-Levy, U., Javitt, D. C., Gelfin, Y., Gorelik, E., Bar, M., Blanaru, M., & Kremer, I. (2006). Controlled trial of D-cycloserine adjuvant therapy for treatment-resistant major depressive disorder. *Journal of Affective Disorders*, *93*(1-3), 239-243.

Herman, J. P., McKlveen, J. M., Ghosal, S., Kopp, B., Wulsin, A., Makinson, R., . . . Myers, B. (2016). Regulation of the Hypothalamic-Pituitary-Adrenocortical Stress Response. *Compr Physiol*, *6*(2), 603-621.

Hermans, E. J., Henckens, M. J., Joëls, M., & Fernández, G. (2014). Dynamic adaptation of large-scale brain networks in response to acute stressors. *Trends in neurosciences*, *37*(6), 304-314.

Hinkelmann, K., Moritz, S., Botzenhardt, J., Riedesel, K., Wiedemann, K., Kellner, M., & Otte, C. (2009). Cognitive impairment in major depression: association with salivary cortisol. *Biological psychiatry*, *66*(9), 879-885.

Hu, Q., Zhang, S.-Y., Liu, F., Zhang, Y.-L., Zhu, D.-M., & Zang, Y.-Y. (2015). Clinical significance of decreased protein expression of dehydroepiandrosterone sulfate in the development of depression: a meta-analysis. *Journal of Affective Disorders*, *174*, 416-423.

James, S. L., Abate, D., Abate, K. H., Abay, S. M., Abbafati, C., Abbasi, N., . . . Murray, C. J. L. (2018). Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. *The Lancet*, *392*(10159), 1789-1858.

Joëls, M., & Baram, T. Z. (2009). The neuro-symphony of stress. *Nature Reviews Neuroscience*, *10*(6), 459.

Joëls, M., & de Kloet, E. R. (2017). 30 YEARS OF THE MINERALOCORTICOID RECEPTOR: The brain mineralocorticoid receptor: a saga in three episodes. *Journal of endocrinology*, *234*(1), T49-T66.

Joëls, M., Karst, H., & Sarabdjitsingh, R. A. (2018). The stressed brain of humans and rodents. *Acta Physiologica*, *223*(2), e13066.

Joëls, M., Sarabdjitsingh, R. A., & Karst, H. (2012). Unraveling the time domains of corticosteroid hormone influences on brain activity: rapid, slow, and chronic modes. *Pharmacological reviews*, *64*(4), 901-938.

Juruena, M. F., Bocharova, M., Agustini, B., & Young, A. H. (2018). Atypical depression and non-atypical depression: is HPA axis function a biomarker? A systematic review. *Journal of Affective Disorders*, *233*, 45-67.

Juruena, M. F., Pariante, C. M., Papadopoulos, A. S., Poon, L., Lightman, S., & Cleare, A. J. (2013). The role of mineralocorticoid receptor function in treatment-resistant depression. *Journal of Psychopharmacology*, *27*(12), 1169-1179.

Kamin, H. S., & Kertes, D. A. (2017). Cortisol and DHEA in development and psychopathology. *Hormones and Behavior*, *89*, 69-85.

Karst, H., Berger, S., Erdmann, G., Schütz, G., & Joëls, M. (2010). Metaplasticity of amygdalar responses to the stress hormone corticosterone. *Proceedings of the National Academy of Sciences*, *107*(32), 14449-14454.

Karst, H., Berger, S., Turiault, M., Tronche, F., Schütz, G., & Joëls, M. (2005). Mineralocorticoid receptors are indispensable for nongenomic modulation of

hippocampal glutamate transmission by corticosterone. *Proceedings of the National Academy of Sciences*, *102*(52), 19204-19207.
Karst, H., & Joëls, M. (2016). Severe stress hormone conditions cause an extended window of excitability in the mouse basolateral amygdala. *Neuropharmacology*, *110*, 175-180.
Keller, J., Flores, B., Gomez, R. G., Solvason, H. B., Kenna, H., Williams, G. H., & Schatzberg, A. F. (2006). Cortisol circadian rhythm alterations in psychotic major depression. *Biological psychiatry*, *60*(3), 275-281.
Kellough, J. L., Beevers, C. G., Ellis, A. J., & Wells, T. T. (2008). Time course of selective attention in clinically depressed young adults: An eye tracking study. *Behav Res Ther*, *46*(11), 1238-1243.
Kimonides, V., Spillantini, M., Sofroniew, M., Fawcett, J., & Herbert, J. (1999). Dehydroepiandrosterone antagonizes the neurotoxic effects of corticosterone and translocation of stress-activated protein kinase 3 in hippocampal primary cultures. *Neuroscience*, *89*(2), 429-436.
Kirschbaum, C., Pirke, K.-M., & Hellhammer, D. H. (1993). The 'Trier Social Stress Test'–a tool for investigating psychobiological stress responses in a laboratory setting. *Neuropsychobiology*, *28*(1-2), 76-81.
Klok, M. D., Alt, S. R., Lafitte, A. J. I., Turner, J. D., Lakke, E. A., Huitinga, I., . . . DeRijk, R. H. (2011). Decreased expression of mineralocorticoid receptor mRNA and its splice variants in postmortem brain regions of patients with major depressive disorder. *Journal of psychiatric research*, *45*(7), 871-878.
Knorr, U., Vinberg, M., Kessing, L. V., & Wetterslev, J. (2010). Salivary cortisol in depressed patients versus control persons: a systematic review and meta-analysis. *Psychoneuroendocrinology*, *35*(9), 1275-1286.
Kohler, C. G., Hoffman, L. J., Eastman, L. B., Healey, K., & Moberg, P. J. (2011). Facial emotion perception in depression and bipolar disorder: a quantitative review. *Psychiatry Res*, *188*(3), 303-309.
Krystal, J. H., Abdallah, C. G., Sanacora, G., Charney, D. S., & Duman, R. S. (2019). Ketamine: A Paradigm Shift for Depression Research and Treatment. *Neuron*, *101*(5), 774-778.
Kubzansky, L. D., & Adler, G. K. (2010). Aldosterone: A forgotten mediator of the relationship between psychological stress and heart disease. *Neuroscience & Biobehavioral Reviews*, *34*(1), 80-86.
Lai, M., Horsburgh, K., Bae, S. E., Carter, R. N., Stenvers, D. J., Fowler, J. H., . . . Kenyon, C. J. (2007). Forebrain mineralocorticoid receptor overexpression enhances memory, reduces anxiety and attenuates neuronal loss in cerebral ischaemia. *European Journal of Neuroscience*, *25*(6), 1832-1842.
Lazarus, R. S. (1974). Psychological stress and coping in adaptation and illness. *The International journal of psychiatry in medicine*, *5*(4), 321-333.
Lazarus, R. S. (1990). Theory-based stress measurement. *Psychological inquiry*, *1*(1), 3-13.
Lazarus, R. S., & Folkman, S. (1984). *Stress, appraisal, and coping*. Springer Publishing Company.
Lee, Y.-S., & Silva, A. J. (2009). The molecular and cellular biology of enhanced cognition. *Nature Reviews Neuroscience*, *10*(2), 126-140.
Lembke, A., Gomez, R., Tenakoon, L., Keller, J., Cohen, G., Williams, G. H., . . . Schatzberg, A. F. (2013). The mineralocorticoid receptor agonist, fludrocortisone, differentially inhibits pituitary–adrenal activity in humans with psychotic major depression. *Psychoneuroendocrinology*, *38*(1), 115-121.

Leppanen, J. M. (2006). Emotional information processing in mood disorders: a review of behavioral and neuroimaging findings. *Curr Opin Psychiatry*, *19*(1), 34-39.
Levine, S. (2005). Developmental determinants of sensitivity and resistance to stress. *Psychoneuroendocrinology*, *30*(10), 939-946.
MacLeod, C., Mathews, A., & Tata, P. (1986). Attentional bias in emotional disorders. *Journal of abnormal psychology*, *95*(1), 15.
Margittai, Z., Van Wingerden, M., Schnitzler, A., Joëls, M., & Kalenscher, T. (2018). Dissociable roles of glucocorticoid and noradrenergic activation on social discounting. *Psychoneuroendocrinology*.
Martin, K. P., & Wellman, C. L. (2011). NMDA receptor blockade alters stress-induced dendritic remodeling in medial prefrontal cortex. *Cereb Cortex*, *21*(10), 2366-2373.
Mataix-Cols, D., De La Cruz, L. F., Monzani, B., Rosenfield, D., Andersson, E., Pérez-Vigil, A., . . . Dunlop, B. W. (2017). D-cycloserine augmentation of exposure-based cognitive behavior therapy for anxiety, obsessive-compulsive, and posttraumatic stress disorders: a systematic review and meta-analysis of individual participant data. *JAMA Psychiatry*, *74*(5), 501-510.
McEwen, B. S., Bowles, N. P., Gray, J. D., Hill, M. N., Hunter, R. G., Karatsoreos, I. N., & Nasca, C. (2015). Mechanisms of stress in the brain. *Nat Neurosci*, *18*(10), 1353.
McEwen, B. S., Weiss, J. M., & Schwartz, L. S. (1968). Selective retention of corticosterone by limbic structures in rat brain. *Nature*, *220*(5170), 911-912.
McKeon, G. L., Scott, J. G., Spooner, D. M., Ryan, A. E., Blum, S., Gillis, D., . . . Robinson, G. A. (2016). Cognitive and social functioning deficits after anti-N-methyl-D-aspartate receptor encephalitis: an exploratory case series. *Journal of the International Neuropsychological Society: JINS*, *22*(8), 828.
McKlveen, J. M., Myers, B., Flak, J. N., Bundzikova, J., Solomon, M. B., Seroogy, K. B., & Herman, J. P. (2013). Role of prefrontal cortex glucocorticoid receptors in stress and emotion. *Biological psychiatry*, *74*(9), 672-679.
Medina, A., Seasholtz, A. F., Sharma, V., Burke, S., Bunney Jr, W., Myers, R. M., . . . Watson, S. J. (2013). Glucocorticoid and mineralocorticoid receptor expression in the human hippocampus in major depressive disorder. *Journal of psychiatric research*, *47*(3), 307-314.
Mikasova, L., Xiong, H., Kerkhofs, A., Bouchet, D., Krugers, H. J., & Groc, L. (2017). Stress hormone rapidly tunes synaptic NMDA receptor through membrane dynamics and mineralocorticoid signalling. *Scientific reports*, *7*(1), 1-12.
Miserendino, M. J., Sananes, C. B., Melia, K. R., & Davis, M. (1990). Blocking of acquisition but not expression of conditioned fear-potentiated startle by NMDA antagonists in the amygdala. *Nature*, *345*(6277), 716-718.
Mitani, H., Shirayama, Y., Yamada, T., Maeda, K., Ashby Jr, C. R., & Kawahara, R. (2006). Correlation between plasma levels of glutamate, alanine and serine with severity of depression. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *30*(6), 1155-1158.
Monahan, J. B., Handelmann, G. E., Hood, W. F., & Cordi, A. A. (1989). D-cycloserine, a positive modulator of the N-methyl-D-aspartate receptor, enhances performance of learning tasks in rats. *Pharmacology Biochemistry and Behavior*, *34*(3), 649-653.
Murck, H., Braunisch, M. C., Konrad, C., Jezova, D., & Kircher, T. (2019). Markers of mineralocorticoid receptor function: changes over time and relationship to response in patients with major depression. *International clinical psychopharmacology*, *34*(1), 18-26.

Murri, M. B., Pariante, C., Mondelli, V., Masotti, M., Atti, A. R., Mellacqua, Z., . . . Zanetidou, S. (2014). HPA axis and aging in depression: systematic review and meta-analysis. *Psychoneuroendocrinology*, *41*, 46-62.

Murrough, J. W., Abdallah, C. G., & Mathew, S. J. (2017). Targeting glutamate signalling in depression: progress and prospects [Review]. *Nat Rev Drug Discov*, *16*(7), 472-486.

Nowacki, J., Wingenfeld, K., Kaczmarczyk, M., Chae, W. R., Abu-Tir, I., Deuter, C. E., . . . Otte, C. (2020a). Cognitive and emotional empathy after stimulation of brain mineralocorticoid and NMDA receptors in patients with major depression and healthy controls. *Neuropsychopharmacology*, *45*(13), 2155-2161.

Nowacki, J., Wingenfeld, K., Kaczmarczyk, M., Chae, W. R., Salchow, P., Abu-Tir, I., . . . Otte, C. (2020b). Steroid hormone secretion after stimulation of mineralocorticoid and NMDA receptors and cardiovascular risk in patients with depression. *Translational Psychiatry*, *10*(1), 109.

Nowacki, J., Wingenfeld, K., Kaczmarczyk, M., Chae, W. R., Salchow, P., Deuter, C. E., . . . Otte, C. (2021). Selective attention to emotional stimuli and emotion recognition in patients with major depression: The role of mineralocorticoid and glutamatergic NMDA receptors. *Journal of Psychopharmacology*, *35*(8), 1017-1023.

Nowak, G., Ordway, G. A., & Paul, I. A. (1995). Alterations in the N-methyl-d-asparatate (NMDA) receptor complex in the frontal cortex of suicide victims. *Brain Res*, *675*(1-2), 157-164.

Oitzl, M. S., Fluttert, M., & Ron de Kloet, E. (1994). The effect of corticosterone on reactivity to spatial novelty is mediated by central mineralocorticosteroid receptors. *European Journal of Neuroscience*, *6*(7), 1072-1079.

Oitzl, M. S., van Haarst, A. D., & Ron de Kloet, E. (1997). Behavioral and neuroendocrine responses controlled by the concerted action of central mineralocorticoid (MRS) and glucocorticoid receptors (GRS). *Psychoneuroendocrinology*, *22*, S87-S93.

Onur, O. A., Schlaepfer, T. E., Kukolja, J., Bauer, A., Jeung, H., Patin, A., . . . Kendrick, K. M. (2010). The N-methyl-D-aspartate receptor co-agonist D-cycloserine facilitates declarative learning and hippocampal activity in humans. *Biological psychiatry*, *67*(12), 1205-1211.

Otte, C., Gold, S. M., Penninx, B. W., Pariante, C. M., Etkin, A., Fava, M., . . . Schatzberg, A. F. (2016). Major depressive disorder [Primer]. *Nature Reviews Disease Primers*, *2*, 16065.

Otte, C., Hinkelmann, K., Moritz, S., Yassouridis, A., Jahn, H., Wiedemann, K., & Kellner, M. (2010). Modulation of the mineralocorticoid receptor as add-on treatment in depression: a randomized, double-blind, placebo-controlled proof-of-concept study. *Journal of psychiatric research*, *44*(6), 339-346.

Otte, C., Jahn, H., Yassouridis, A., Arlt, J., Stober, N., Maass, P., . . . Kellner, M. (2003). The mineralocorticoid receptor agonist, fludrocortisone, inhibits pituitary-adrenal activity in humans after pre-treatment with metyrapone. *Life sciences*, *73*(14), 1835-1845.

Otte, C., Moritz, S., Yassouridis, A., Koop, M., Madrischewski, A. M., Wiedemann, K., & Kellner, M. (2007). Blockade of the mineralocorticoid receptor in healthy men: effects on experimentally induced panic symptoms, stress hormones, and cognition. *Neuropsychopharmacology*, *32*(1), 232-238.

Otte, C., Wingenfeld, K., Kuehl, L. K., Kaczmarczyk, M., Richter, S., Quante, A., . . . Wiedemann, K. (2015a). Mineralocorticoid receptor stimulation improves cognitive function and decreases cortisol secretion in depressed patients and healthy individuals. *Neuropsychopharmacology*, *40*(2), 386-393.

Otte, C., Wingenfeld, K., Kuehl, L. K., Richter, S., Regen, F., Piber, D., & Hinkelmann, K. (2015b). Cognitive function in older adults with major depression: Effects of mineralocorticoid receptor stimulation. *Journal of psychiatric research*, *69*, 120-125.

Otto, M. W., Basden, S. L., McHugh, R. K., Kantak, K. M., Deckersbach, T., Cather, C., . . . Smits, J. A. (2009). Effects of D-cycloserine administration on weekly nonemotional memory tasks in healthy participants. *Psychotherapy and Psychosomatics*, *78*(1), 49-54.

Otto, M. W., Kredlow, M. A., Smits, J. A., Hofmann, S. G., Tolin, D. F., de Kleine, R. A., . . . Pollack, M. H. (2016). Enhancement of psychosocial treatment with d-cycloserine: models, moderators, and future directions. *Biological psychiatry*, *80*(4), 274-283.

Pariante, C. M. (2017). Why are depressed patients inflamed? A reflection on 20 years of research on depression, glucocorticoid resistance and inflammation. *European Neuropsychopharmacology*, *27*(6), 554-559.

Pariante, C. M., & Lightman, S. L. (2008). The HPA axis in major depression: classical theories and new developments. *Trends in neurosciences*, *31*(9), 464-468.

Peixoto, C., José Grande, A., Gomes Carrilho, C., Nardi, A. E., Cardoso, A., & Barciela Veras, A. (2020). Dehydroepiandrosterone for depressive symptoms: A systematic review and meta-analysis of randomized controlled trials. *J Neurosci Res*.

Peyrovian, B., Rosenblat, J. D., Pan, Z., Iacobucci, M., Brietzke, E., & McIntyre, R. S. (2019). The glycine site of NMDA receptors: A target for cognitive enhancement in psychiatric disorders. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*.

Piber, D., Schultebraucks, K., Mueller, S. C., Deuter, C. E., Wingenfeld, K., & Otte, C. (2016). Mineralocorticoid receptor stimulation effects on spatial memory in healthy young adults: A study using the virtual Morris Water Maze task. *Neurobiology of learning and memory*, *136*, 139-146.

Popoli, M., Yan, Z., McEwen, B. S., & Sanacora, G. (2012). The stressed synapse: the impact of stress and glucocorticoids on glutamate transmission. *Nature Reviews Neuroscience*, *13*(1), 22.

Qi, X.-R., Kamphuis, W., Wang, S., Wang, Q., Lucassen, P. J., Zhou, J.-N., & Swaab, D. F. (2013). Aberrant stress hormone receptor balance in the human prefrontal cortex and hypothalamic paraventricular nucleus of depressed patients. *Psychoneuroendocrinology*, *38*(6), 863-870.

Quinkler, M., Oelkers, W., Remde, H., & Allolio, B. (2015). Mineralocorticoid substitution and monitoring in primary adrenal insufficiency. *Best Practice & Research Clinical Endocrinology & Metabolism*, *29*(1), 17-24.

Rainey, W. E., Carr, B. R., Sasano, H., Suzuki, T., & Mason, J. I. (2002). Dissecting human adrenal androgen production. *Trends in Endocrinology & Metabolism*, *13*(6), 234-239.

Reul, J., & Kloet, E. D. (1985). Two receptor systems for corticosterone in rat brain: microdistribution and differential occupation. *Endocrinology*, *117*(6), 2505-2511.

Rock, P., Roiser, J., Riedel, W., & Blackwell, A. (2014). Cognitive impairment in depression: a systematic review and meta-analysis. *Psychological medicine*, *44*(10), 2029-2040.

Rütgen, M., Pletti, C., Tik, M., Kraus, C., Pfabigan, D. M., Sladky, R., . . . Lamm, C. (2019). Antidepressant treatment, not depression, leads to reductions in behavioral and neural responses to pain empathy. *Translational Psychiatry*, *9*(1), 164.

Sahu, P., Gidwani, B., & Dhongade, H. (2020). Pharmacological activities of dehydroepiandrosterone: a review. *Steroids*, *153*, 108507.

Sakimura, K., Kutsuwada, T., Ito, I., Manabe, T., Takayama, C., Kushiya, E., . . . Sugiyama, H. (1995). Reduced hippocampal LTP and spatial learning in mice lacking NMDA receptor ε1 subunit. *Nature*, *373*(6510), 151-155.

Sanacora, G., Gueorguieva, R., Epperson, C. N., Wu, Y.-T., Appel, M., Rothman, D. L., . . . Mason, G. F. (2004). Subtype-specific alterations of γ-aminobutyric acid and glutamatein patients with major depression. *Archives of general psychiatry*, *61*(7), 705-713.

Sanacora, G., Zarate, C. A., Krystal, J. H., & Manji, H. K. (2008). Targeting the glutamatergic system to develop novel, improved therapeutics for mood disorders [Review Article]. *Nature Reviews Drug Discovery*, *7*, 426.

Sandi, C., & Haller, J. (2015). Stress and the social brain: behavioural effects and neurobiological mechanisms [Review Article]. *Nature Reviews Neuroscience*, *16*, 290.

Schade, S., & Paulus, W. (2016). D-Cycloserine in neuropsychiatric diseases: a systematic review. *International Journal of Neuropsychopharmacology*, *19*(4).

Schatzberg, A. F. (2015). Anna-Monika Award Lecture, DGPPN Kongress, 2013: the role of the hypothalamic–pituitary–adrenal (HPA) axis in the pathogenesis of psychotic major depression. *The World Journal of Biological Psychiatry*, *16*(1), 2-11.

Schmidt, P. J., Daly, R. C., Bloch, M., Smith, M. J., Danaceau, M. A., Clair, L. S. S., . . . Rubinow, D. R. (2005). Dehydroepiandrosterone monotherapy in midlife-onset major and minor depression. *Archives of general psychiatry*, *62*(2), 154-162.

Scholl, J., Günthner, J., Kolling, N., Favaron, E., Rushworth, M. F., Harmer, C. J., & Reinecke, A. (2014). A role beyond learning for NMDA receptors in reward-based decision-making—a pharmacological study using d-cycloserine. *Neuropsychopharmacology*, *39*(12), 2900.

Schultebraucks, K., Deuter, C. E., Duesenberg, M., Schulze, L., Hellmann-Regen, J., Domke, A., . . . Wingenfeld, K. (2016). Selective attention to emotional cues and emotion recognition in healthy subjects: the role of mineralocorticoid receptor stimulation. *Psychopharmacology (Berl)*, *233*(18), 3405-3415.

Schwabe, L., Haddad, L., & Schachinger, H. (2008). HPA axis activation by a socially evaluated cold-pressor test. *Psychoneuroendocrinology*, *33*(6), 890-895.

Schwabe, L., Tegenthoff, M., Höffken, O., & Wolf, O. T. (2013). Mineralocorticoid receptor blockade prevents stress-induced modulation of multiple memory systems in the human brain. *Biological psychiatry*, *74*(11), 801-808.

Sebastian, V., Estil, J. B., Chen, D., Schrott, L. M., & Serrano, P. A. (2013). Acute physiological stress promotes clustering of synaptic markers and alters spine morphology in the hippocampus. *PLoS One*, *8*(10), e79077.

Sep, M. S., Joëls, M., & Geuze, E. (2020). Individual differences in the encoding of contextual details following acute stress: An explorative study. *European Journal of Neuroscience*.

Sloan, D. M., Bradley, M. M., Dimoulas, E., & Lang, P. J. (2002). Looking at facial expressions: Dysphoria and facial EMG. *Biol Psychol*, *60*(2-3), 79-90.

Stárka, L., Dušková, M., & Hill, M. (2015). Dehydroepiandrosterone: A neuroactive steroid. *J Steroid Biochem Mol Biol*, *145*, 254-260.

Stetler, C., & Miller, G. E. (2011). Depression and hypothalamic-pituitary-adrenal activation: a quantitative summary of four decades of research. *Psychosomatic Medicine*, *73*(2), 114-126.

Taylor, V. A., Ellenbogen, M. A., Washburn, D., & Joober, R. (2011). The effects of glucocorticoids on the inhibition of emotional information: a dose–response study. *Biol Psychol*, *86*(1), 17-25.

Ter Horst, J. P., van der Mark, M., Kentrop, J., Arp, M., van der Veen, R., De Kloet, R., & Oitzl, M. S. (2014). Deletion of the forebrain mineralocorticoid receptor impairs social discrimination and decision-making in male, but not in female mice. *Frontiers in behavioral neuroscience*, *8*, 26.

Thompson, L. T., Moskal, J. R., & Disterhoft, J. F. (1992). Hippocampus-dependent learning facilitated by a monoclonal antibody or D-cycloserine. *Nature*, *359*(6396), 638-641.

Tone, E. B., & Tully, E. C. (2014). Empathy as a "risky strength": A multilevel examination of empathy and risk for internalizing disorders. *Development and psychopathology*, *26*(4pt2), 1547-1565.

Treccani, G., Musazzi, L., Perego, C., Milanese, M., Nava, N., Bonifacino, T., . . . Racagni, G. (2014). Stress and corticosterone increase the readily releasable pool of glutamate vesicles in synaptic terminals of prefrontal and frontal cortex. *Molecular Psychiatry*, *19*(4), 433.

van Ast, V. A., Cornelisse, S., Meeter, M., Joels, M., & Kindt, M. (2013). Time-dependent effects of cortisol on the contextualization of emotional memories. *Biol Psychiatry*, *74*(11), 809-816.

van Marle, H. J., Hermans, E. J., Qin, S., & Fernández, G. (2009). From specificity to sensitivity: how acute stress affects amygdala processing of biologically salient stimuli. *Biological psychiatry*, *66*(7), 649-655.

Vinkers, C. H., Zorn, J. V., Cornelisse, S., Koot, S., Houtepen, L. C., Olivier, B., . . . Joëls, M. (2013). Time-dependent changes in altruistic punishment following stress. *Psychoneuroendocrinology*, *38*(9), 1467-1475.

Vogel, S., Fernández, G., Joëls, M., & Schwabe, L. (2016). Cognitive Adaptation under Stress: A Case for the Mineralocorticoid Receptor. *Trends Cogn Sci*, *20*(3), 192-203.

Vogel, S., Gerritsen, L., van Oostrom, I., Arias-Vásquez, A., Rijpkema, M., Joëls, M., . . . Fernández, G. (2014). Linking genetic variants of the mineralocorticoid receptor and negative memory bias: interaction with prior life adversity. *Psychoneuroendocrinology*, *40*, 181-190.

Vogel, S., Klumpers, F., Krugers, H. J., Fang, Z., Oplaat, K. T., Oitzl, M. S., . . . Fernández, G. (2015). Blocking the mineralocorticoid receptor in humans prevents the stress-induced enhancement of centromedial amygdala connectivity with the dorsal striatum. *Neuropsychopharmacology*, *40*(4), 947-956.

Vogel, S., Klumpers, F., Schröder, T. N., Oplaat, K. T., Krugers, H. J., Oitzl, M. S., . . . Fernández, G. (2017). Stress induces a shift towards striatum-dependent stimulus-response learning via the mineralocorticoid receptor. *Neuropsychopharmacology*, *42*(6), 1262-1271.

von Dawans, B., Strojny, J., & Domes, G. (2020). The effects of acute stress and stress hormones on social cognition and behavior: current state of research and future directions. *Neuroscience & Biobehavioral Reviews*.

Walker, D. L., Ressler, K. J., Lu, K.-T., & Davis, M. (2002). Facilitation of conditioned fear extinction by systemic administration or intra-amygdala infusions of D-cycloserine as assessed with fear-potentiated startle in rats. *Journal of Neuroscience*, *22*(6), 2343-2351.

Wang, C. C., & Wang, S. J. (2009). Modulation of presynaptic glucocorticoid receptors on glutamate release from rat hippocampal nerve terminals. *Synapse*, *63*(9), 745-751.

Wang, S., Kamphuis, W., Huitinga, I., Zhou, J., & Swaab, D. (2008). Gene expression analysis in the human hypothalamus in depression by laser microdissection and real-time PCR: the presence of multiple receptor imbalances. *Molecular Psychiatry*, *13*(8), 786-799.

Weightman, M. J., Air, T. M., & Baune, B. T. (2014). A Review of the Role of Social Cognition in Major Depressive Disorder [Review]. *Frontiers in Psychiatry*, *5*(179).

Weightman, M. J., Knight, M. J., & Baune, B. T. (2019). A systematic review of the impact of social cognitive deficits on psychosocial functioning in major depressive disorder and opportunities for therapeutic intervention. *Psychiatry Res*, *274*, 195-212.

Wingenfeld, K., Kuehl, L. K., Dziobek, I., Roepke, S., Otte, C., & Hinkelmann, K. (2016). Effects of mineralocorticoid receptor blockade on empathy in patients with major depressive disorder [journal article]. *Cognitive, Affective, & Behavioral Neuroscience*, *16*(5), 902-910.

Wingenfeld, K., Kuehl, L. K., Janke, K., Hinkelmann, K., Dziobek, I., Fleischer, J., . . . Roepke, S. (2014). Enhanced emotional empathy after mineralocorticoid receptor stimulation in women with borderline personality disorder and healthy women. *Neuropsychopharmacology*, *39*(8), 1799-1804.

Wingenfeld, K., & Otte, C. (2019). Mineralocorticoid receptor function and cognition in health and disease. *Psychoneuroendocrinology*, *105*, 25-35.

Wolf, O. T., Schulte, J. M., Drimalla, H., Hamacher-Dang, T. C., Knoch, D., & Dziobek, I. (2015). Enhanced emotional empathy after psychosocial stress in young healthy men. *Stress*, *18*(6), 631-637.

Wolkowitz, O. M., Reus, V. I., Keebler, A., Nelson, N., Friedland, M., Brizendine, L., & Roberts, E. (1999). Double-blind treatment of major depression with dehydroepiandrosterone. *Am J Psychiatry*, *156*(4), 646-649.

World Health Organization. (2017). *Depression and other common mental disorders: global health estimates*.

Yongue, B. G., & Roy, E. J. (1987). Endogenous aldosterone and corticosterone in brain cell nuclei of adrenal-intact rats: regional distribution and effects of physiological variations in serum steroids. *Brain Res*, *436*(1), 49-61.

Yuen, E. Y., Liu, W., Karatsoreos, I. N., Ren, Y., Feng, J., McEwen, B. S., & Yan, Z. (2011). Mechanisms for acute stress-induced enhancement of glutamatergic transmission and working memory. *Molecular Psychiatry*, *16*(2), 156-170.

APPENDIX

List of Abbreviations

AC	–	Adrenal cortex
ACTH	–	Adrenocorticotrophic hormone
AMPA	–	α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor
AM	–	Amygdala
AP	–	Anterior pituitary
CRH	–	Corticotropin-releasing hormone
Ca	–	Calcium
DCS	–	D-cycloserine
DHEA	–	Dehydroepiandrosterone
DHEA-S	–	Sulfated dehydroepiandrosterone
DNA	–	Deoxyribonucleic acid
DSM-5	–	Diagnostic and Statistical Manual of Mental Disorders fifth edition
GR	–	Glucocorticoid receptor
HC	–	Hippocampus
HPA axis	–	Hypothalamic-pituitary-adrenal axis
HT	–	Hypothalamus
MDD	–	Major depressive disorder
MET	–	Multifaceted Empathy Test
mGluR	–	Metabotropic glutamate receptor
MR	–	Mineralocorticoid receptor
NMDA-R	–	N-methyl-D-aspartate receptor
Na	–	Natrium
PFC	–	Prefrontal cortex
PVN	–	Paraventricular nucleus
SECPT	–	Socially Evaluated Cold-Pressor Test
ST	–	Striatum
TSST	–	Trier Social Stress Test

List of Figures

Curriculum Vitae

For reasons of data protection, the curriculum vitae is not published in this version of the dissertation.

List of Publications

Nowacki, J., Wingenfeld, K., Kaczmarczyk, M., Chae, W. R., Salchow, Deuter, C. E., P., Piber, D., & Otte, C. (2021). Selective attention to emotional stimuli and emotion recognition in patients with major depression: the role of mineralocorticoid and glutamatergic NMDA receptors. *Journal of Psychopharmacology, 35*(8), 1017-1023.

Otte, C., Chae, W. R., **Nowacki**, J., Kaczmarczyk, M., Piber, D., Roepke, S., . . . Ettrich, B. (2020). Simvastatin add-on to escitalopram in patients with comorbid obesity and major depression (SIMCODE): study protocol of a multicentre, randomised, double-blind, placebo-controlled trial. *BMJ Open*, 10(12), e040119.

Nowacki, J., Wingenfeld, K., Kaczmarczyk, M., Chae, W. R., Abu-Tir, I., Deuter, C. E., Piber, D., Hellmann-Regen, J., & Otte, C. (2020). Cognitive and emotional empathy after stimulation of brain mineralocorticoid and NMDA receptors in patients with major depression and healthy controls. Neuropsychopharmacology, 45(13), 2155-2161.

Nowacki, J., Wingenfeld, K., Kaczmarczyk, M., Chae, W. R., Salchow, P., Abu-Tir, I., Piber, D., Hellmann-Regen, J., & Otte, C. (2020). Steroid hormone secretion after stimulation of mineralocorticoid and NMDA receptors and cardiovascular risk in patients with depression. Translational Psychiatry, 10(1), 109.

Chae, W. R., Metz, S., Weise, J., **Nowacki**, J., Piber, D., Mueller, S. C., . . . Otte, C. (2019). Effects of glucocorticoid and noradrenergic activity on spatial learning and spatial memory in healthy young adults. Behavioural brain research, 373, 112072.

Nowacki, J., Duesenberg, M., Deuter, C. E., Otte, C., & Wingenfeld, K. (2019). Delayed effects of psychosocial stress on risk taking. Stress, 1-9.

Nowacki, J., Heekeren, H. R., Deuter, C. E., Joerißen, J. D., Schröder, A., Otte, C., & Wingenfeld, K. (2019). Decision making in response to physiological and combined physiological and psychosocial stress. Behavioral neuroscience, 133(1), 59-67.

Hasselmann, H., Gamradt, S., Taenzer, A., **Nowacki**, J., Zain, R., Patas, K., . . . Piber, D. (2018). Pro-inflammatory monocyte phenotype and cell-specific steroid signaling alterations in unmedicated patients with major depressive disorder. Frontiers in immunology, 9.

Deuter, C. E., **Nowacki**, J., Wingenfeld, K., Kuehl, L. K., Finke, J. B., Dziobek, I., & Otte, C. (2018). The role of physiological arousal for self-reported emotional empathy. Autonomic Neuroscience, 214, 9-14.

Piber, D., **Nowacki**, J., Mueller, S. C., Wingenfeld, K., & Otte, C. (2018). Sex effects on spatial learning but not on spatial memory retrieval in healthy young adults. Behavioural brain research, 336, 44-50.

Congress contributions

Nowacki, J., Kaczmarczyk, M., Chae, W. R., Wingenfeld, K., Otte, C. (2019). Effekte einer separaten und kombinierten Mineralocorticoid- und NMDA Rezeptorstimulation auf die Empahtiefähigkeit depressiver Patienten. Poster: DGPPN congress 2019, Berlin.

Nowacki, J., Chae, W. R., Kaczmarczyk, M., Wingenfeld, K., Otte, C. (2019). Effects of mineralocorticoid and NMDA receptor stimulation on stress hormone secretion in depressed patients and healthy controls. Poster: ECNP congress 2019, Copenhagen.

Nowacki, J., Kaczmarczyk, M., Chae, W. R., Wingenfeld, K., Otte, C. (2019). Effects of mineralocorticoid and NMDA receptor stimulation on empathy in depressed patients and healthy individuals. Poster: ISPNE congress 2019, Milan.

Nowacki, J., Kaczmarczyk, M., Chae, W. R., Abu-Tir, I., Salchow, P., Wingenfeld, K., Otte, C. (2019). Kardiovasculäres Risiko in jungen Patienten mit einer depressiven Störung. Poster: AGNP congress 2019, Berlin.

Nowacki, J., Duesenberg, M., Deuter, C. E., Otte, C., & Wingenfeld, K. (2018). Späte Effekte von psychosozialem Stress auf die Entscheidungsfindung: die Vertrautheit mit der Entscheidungssituation macht den Unterschied. Poster: Psychologie und Gehirn congress 2018, Gießen.

Nowacki, J., Duesenberg, M., Deuter, C. E., Otte, C., & Wingenfeld, K. (2018). Späte Effekte von psychosozialem Stress auf das Risikoverhalten. Poster: DGPPN congress 2018, Berlin.

Nowacki, J., Heekeren, H. R., Wingenfeld, K., & Otte, C. (2017). Decision making under risk in response to psychophysiological stress. Poster: ISPNE congress 2017, Zürich.

Nowacki, J., Wingenfeld, K., Mueller, S. C., Otte, C., & Piber, D. (2017). Sex effects on spatial learning and spatial memory performance in healthy young adults. WPA XVII World Congress of Psychiatry 2017, Berlin.

Nowacki, J., Wingenfeld, K., & Otte, C. (2016). Entscheidungsfindung nach psychophysiologischem Stress. Poster: DGPPN congress 2016, Berlin.

Eidesstattliche Versicherung (statement of authorship)

Ich versichere, dass ich die vorliegende Arbeit selbstständig verfasst habe und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt wurden. Alle Zitate wurden kenntlich gemacht. Die vorliegende Dissertation wurde in keinem vorhergehenden Promotionsverfahren eingereicht und ich besitze keinen Doktorgrad im Fach Psychologie. Die Promotionsordnung der Freien Universität Berlin vom 08.08.2016, veröffentlicht im Amtlichen Mitteilungsblatt Nr. 35/2016, ist mir bekannt.

Berlin, den

_____

Jan Nowacki