Freie Universität Berlin

CHARITÉ
UNIVERSITÄTSMEDIZIN BERLIN

DISSERTATION

MEASURES, MECHANISMS AND EFFECTS OF SPINAL AND CEREBRAL NOCICEPTIVE PROCESSING DURING GENERAL ANAESTHESIA

ZUR ERLANGUNG DES AKADEMISCHEN GRADES
DOKTOR DER NATURWISSENSCHAFT (DR. RER. NAT.)

AM FACHBEREICH ERZIEHUNGSWISSENSCHAFT UND PSYCHOLOGIE
DER FREIEN UNIVERSITÄT BERLIN

VORGELEGT VON
GREGOR LICHTNER, M. SC.

BERLIN 2018

Betreuer / Erstgutachter:	Priv.-Doz. Dr. Falk von Dincklage
Zweitgutachter:	Prof. Dr. Hauke Heekeren
Tag der Disputation:	2. Mai 2019

Contents

Abstract

Next to unconsciousness, the suppression of nociception – i. e. the neuronal processing of noxious stimuli – is a central component of general anaesthesia. While unconsciousness can be monitored fairly accurately using electroencephalography (EEG)-derived measures, there is no reliable measure that allows quantifying the level of nociception in unconscious humans available to this day.

Therefore, this dissertation aimed at developing a multimodal measure of nociceptive processing in humans and applying this measure to investigate the spinal and cerebral processing of innocuous and noxious somatosensory stimuli during general anaesthesia. Using a setup that combined functional magnetic resonance imaging (fMRI) with simultaneous EEG and spinal nociceptive reflex monitoring, we were able for the first time to (i) concurrently investigate spinal and cerebral effects of general anaesthetics on the processing of somatosensory stimuli and to (ii) investigate intense noxious stimuli at intensities comparable to surgical stimuli.

During unconsciousness, we found an anaesthetic dose-dependent change of nociceptive processing in a variety of brain regions including higher-order association cortices. The changes in processing were accompanied by changes in functional connectivity between nociceptive brain regions, in accordance with the notion that general anaesthetics induce unconsciousness by altering the information transfer patterns in the brain. We found that profound spinal and cerebral nociceptive-evoked activation persisted even at levels of general anaesthesia that are deeper than applied in clinical practice. Currently used clinical indicators of analgesic efficacy (e. g. haemodynamic responses to noxious stimuli) were absent at far lower levels of general anaesthesia, demonstrating that the absence of these clinical responses is not indicative of absent nociceptive processing.

Due to the unavailability of reliable measures of intraoperative nociception, it is not known whether persisting nociception during general anaesthesia contributes to adverse effects on patient outcomes such as pain chronification. We therefore supplemented the primary experimental research of this dissertation by a clinical study, in which we showed that the level of intraoperative analgesia was related to persistent postoperative pain. As the analgesic dosings were in the range in which we found profound persistent nociceptive processing in our experimental studies, these results suggest that persistent nociception during currently used levels of intraoperative analgesia indeed contributes to long-term harm on patient outcomes.

Zusammenfassung

Neben der Bewusstlosigkeit ist die Unterdrückung von Nozizeption – also der neuronalen Verarbeitung von potenziell gewebeschädigenden Reizen – eine zentrale Komponente der Allgemeinanästhesie. Während Bewusstlosigkeit relativ genau mittels Elektroenzephalographie (EEG) überwacht werden kann, existiert bis heute kein zuverlässiges Verfahren, um das Nozizeptionsniveau in bewusstlosen Menschen zu quantifizieren.

Ziel dieser Dissertation war es daher ein multimodales Maß der nozizeptiven Verarbeitung im Menschen zu entwickeln und dieses Maß zu verwenden, um die spinale und zerebrale Verarbeitung von nozizeptiven Reizen unter Allgemeinanästhesie zu untersuchen. Durch Kombination von funktioneller Magnetresonanztomographie (fMRT) mit simultaner EEG und spinalen nozizeptiven Reflexen waren wir erstmalig in der Lage (i) gleichzeitig spinale und zerebrale Effekte von Allgemeinanästhetika auf die Verarbeitung somatosensorischer Reize zu untersuchen und (ii) sehr starke nozizeptive Reize, deren Intensität vergleichbar mit der von chirurgischen Reizen ist, zu verwenden.

Unter Bewusstlosigkeit konnten wir eine dosisabhängige Veränderung der nozizeptiven Verarbeitung in einer Reihe von Hirnarealen, darunter Assoziationsareale, mit einhergehender Modulation der funktionellen Konnektivität zwischen nozizeptions-assoziierten Hirnarealen finden. Dies bestärkt die Vermutung, dass Allgemeinanästhetika Bewusstlosigkeit durch Veränderung der Informationsverbeitungspfade des Gehirns erzeugen. Unter allen untersuchten Narkosetiefen bis hin zu tieferer Narkose als in der derzeitigen klinischen Praxis verwendet konnten wir umfassende spinale und zerebrale nozizeptive Aktivierungen nachweisen. Klinisch verwendete Indikatoren überschießender Nozizeption (bspw. hämodynamische Reaktionen auf nozizeptive Reize) waren bereits bei wesentlich geringeren Narkosetiefen nicht mehr nachweisbar. Das Ausbleiben dieser klinischen Reaktionen bedeutet daher nicht ein Ausbleiben von nozizeptiver Verarbeitung.

Aufgrund des Fehlens von zuverlässigen Maßen intraoperativer Nozizeption ist bisher nicht bekannt, ob bestehende Nozizeption unter Allgemeinanästhesie zu klinisch relevanten Auswirkungen wie bspw. Schmerzchronifizierung beiträgt. In einer klinischen Patientenstudie konnten wir zeigen, dass das Niveau der intraoperativen Analgesie mit dem Auftreten von chronischen postoperativen Schmerzen assoziiert ist. Da die intraoperative Analgesie der Patienten in dem Bereich war, in dem wir noch umfassende nozizeptive Verarbeitung in den experimentellen Studien fanden, deuten diese Resultate darauf hin, dass persistierende Nozizeption bei heute gebräuchlicher intraoperativer Analgesie tatsächlich zu langfristigen Schäden von Patienten beitragen kann.

List of original articles

This dissertation is based on the following articles:

Primary work

Lichtner, G., Auksztulewicz, R., Velten, H., Mavrodis, D., Scheel, M., Blankenburg, F., and von Dincklage, F. (2018). Nociceptive activation in spinal cord and brain persists during deep general anaesthesia. *Br. J. Anaesth.*, 121(1):291–302.

Lichtner, G.*, Auksztulewicz, R.*, Kirilina, E., Velten, H., Mavrodis, D., Scheel, M., Blankenburg, F.†, and von Dincklage, F.† (2018). Effects of propofol anesthesia on the processing of noxious stimuli in the spinal cord and the brain. *NeuroImage*, 172:642–653.

*, †: contributed equally

Secondary work

Lichtner, G., Hösl, T. M., Jakuscheit, A., Jurth, C., and von Dincklage, F. (2016). Optimizing Nociceptive Flexion Reflex (NFR) Scoring Criteria by Adjusting for Noise and Reflex Properties and Sampling Rate. *Clin J Pain*, 32(9):773–783.

Jakuscheit, A., Weth, J., **Lichtner, G.**, Jurth, C., Rehberg, B., and von Dincklage, F. (2017). Intraoperative monitoring of analgesia using nociceptive reflexes correlates with delayed extubation and immediate postoperative pain: A prospective observational study. *Eur. J. Anaesthesiol.*, 34(5):297.‡

von Dincklage, F., Jakuscheit, A., Weth, J., **Lichtner, G.**, Jurth, C., and Rehberg-Klug, B. (2018). Higher doses of intraoperative analgesia are associated with lower levels of persistent pain and less analgesic consumption six months after total hip arthroplasty. *Eur. J. Pain*, 22(4):691–699.‡

‡: These papers are also part of the doctoral thesis of Axel Jakuscheit, Charité – Universitätsmedizin Berlin.

Full list of publications

Vorderwülbecke, B. J., **Lichtner, G.**, von Dincklage, F., and Holtkamp, M. (2018). Acute antiepileptic drug use in intensive care units. *J. Neurol. in press*.

Lichtner, G., Auksztulewicz, R., Velten, H., Mavrodis, D., Scheel, M., Blankenburg, F., and von Dincklage, F. (2018). Nociceptive activation in spinal cord and brain persists during deep general anaesthesia. *Br. J. Anaesth.*, 121(1):291–302.

Lichtner, G., Auksztulewicz, R., Kirilina, E., Velten, H., Mavrodis, D., Scheel, M., Blankenburg, F., and von Dincklage, F. (2018). Effects of propofol anesthesia on the processing of noxious stimuli in the spinal cord and the brain. *NeuroImage*, 172:642–653.

von Dincklage, F., Jakuscheit, A., Weth, J., **Lichtner, G.**, Jurth, C., and Rehberg-Klug, B. (2018). Higher doses of intraoperative analgesia are associated with lower levels of persistent pain and less analgesic consumption six months after total hip arthroplasty. *Eur. J. Pain*, 22(4):691–699.

von Dincklage, F., Jakuscheit, A., Weth, J., **Lichtner, G.**, Jurth, C., and Rehberg-Klug, B. (2018). Authors' reply to the comment by Kendall et al. *Eur. J. Pain*, 22(4):823–824.

Jurth, C., Dörig, T. M., **Lichtner, G.**, Golebiewski, A., Jakuscheit, A., and von Dincklage, F. (2018). Development, validation and utility of a simulation model of the nociceptive flexion reflex threshold. *Clin. Neurophysiol.*, 129(3):572–583.

von Dincklage, F., Suchodolski, K., **Lichtner, G.**, Friesdorf, W., Podtschaske, B., and Ragaller, M. (2017). Investigation of the Usability of Computerized Critical Care Information Systems in Germany. *J Intensive Care Med*, page 0885066617696848.

von Dincklage, F., **Lichtner, G.**, Suchodolski, K., Ragaller, M., Friesdorf, W., and Podtschaske, B. (2017). Design and validation of a questionnaire to evaluate the usability of computerized critical care information systems. *J Clin Monit Comput*, 31(4):833–844.

Schöneberg, J., Lehmann, M., Ullrich, A., Posor, Y., Lo, W.-T., **Lichtner, G.**, Schmoranzer, J., Haucke, V., and Noé, F. (2017). Lipid-mediated PX-BAR domain recruitment couples local membrane constriction to endocytic vesicle fission. *Nat. Commun.*, 8:15873.

Koch, S., Stegherr, A. M., Mörgeli, R., Kramer, S., Toubekis, E., **Lichtner, G.**, von Dincklage, F., and Spies, C. (2017). Electroencephalogram dynamics in children during different levels of anaesthetic depth. *Clinical Neurophysiology*, 128(10):2014–2021.

Jakuscheit, A., Weth, J., **Lichtner, G.**, Jurth, C., Rehberg, B., and von Dincklage, F. (2017). Intraoperative monitoring of analgesia using nociceptive reflexes correlates with delayed extubation and immediate postoperative pain: A prospective observational study. *Eur. J. Anaesthesiol.*, 34(5):297.

Jakuscheit, A., Posch, M. J., Gkaitatzis, S., Neumark, L., Hackbarth, M., Schneider, M., **Lichtner, G.**, Baars, J. H., and von Dincklage, F. (2017). Utility of nociceptive flexion reflex threshold and bispectral index to predict movement responses under propofol anaesthesia. *Somatosens. Mot. Res.*, 34(2):139–144.

Lichtner, G., Hösl, T. M., Jakuscheit, A., Jurth, C., and von Dincklage, F. (2016). Optimizing Nociceptive Flexion Reflex (NFR) Scoring Criteria by Adjusting for Noise and Reflex Properties and Sampling Rate. *Clin J Pain*, 32(9):773–783.

Lehmann, M., **Lichtner, G.**, Klenz, H., and Schmoranzer, J. (2016). Novel organic dyes for multicolor localization-based super-resolution microscopy. *J. Biophoton*, 9(1-2):161–170.

Lichtner, G., Golebiewski, A., Schneider, M. H., and von Dincklage, F. (2015). Introduction and validation of a less painful algorithm to estimate the nociceptive flexion reflex threshold. *Brain Res.*, 1608:147–156.

Albrecht, D., López-Murcia, F. J., Pérez-González, A. P., **Lichtner, G.**, Solsona, C., and Llobet, A. (2012). SPARC prevents maturation of cholinergic presynaptic terminals. *Molecular and Cellular Neuroscience*, 49(3):364–374.

1. Introduction

1.1. Clinical background

Pain is by definition a conscious experience, but the neuronal mechanisms that confer the processing of noxious stimuli, summarised under the term *nociception*, are still active during unconsciousness (Davis et al., 2017). And even without the conscious experience of pain, nociception in unconscious humans can still trigger a variety of adverse reactions, such as tachycardia (increased heart rate), hypertension (increased blood pressure), or neuroendocrine and metabolic stress responses (Liu et al., 1995; Borsook et al., 2010). Furthermore, high levels of nociception might lead to chronic pain through neuronal learning processes called central sensitisation (Kehlet et al., 2006; Kuner and Flor, 2017).

Accordingly, the suppression of nociception is, next to unconsciousness, the second key component of general anaesthesia (American Society of Anesthesiologists, 2002), deciding over the clinical outcome of hundreds of millions of patients undergoing surgical procedures each year (Weiser et al., 2015). But owing to the lack of validated sensitive and specific measures of nociception, anaesthesiologists commonly perform the dosing of anti-nociceptive drugs such as opioids based on the patients' clinical responses to noxious stimuli. These responses include blood pressure or heart rate elevations, movement responses, or lacrimation (Borsook et al., 2010; Gruenewald and Ilies, 2013). Once none of these clinical responses occur during interventions such as surgery, the depth of the general anaesthesia is considered sufficient and, consequently, it is assumed that nociception does not cause any further detrimental effects. However, it is unknown whether nociception persists despite the absence of these clinical responses as there is currently no way to reliably assess nociception during unconsciousness. Furthermore, without a way to reliably assess nociception during unconsciousness, it remains entirely uninvestigated whether ongoing nociception in absence of clinical responses causes adverse effects on patient outcomes such as acute or chronic postoperative pain.

However, simply increasing the dosing of anti-nociceptive drugs under the assumption that this would suppress all potential adverse effects caused by persistent nociception has significant side-effects on its own including bradycardia (low heart rate), hypotension (low blood pressure), nausea, opioid-induced hyperalgesia and prolonged time to emerge from anaesthesia (Guignard et al., 2000). Therefore, an objective measure of no-

ception might improve patient outcomes across the board by allowing for the optimal adjustment of anti-nociceptive dosing to prevent detrimental effects of both under- and overdosing. Additionally, a comprehensive measure of nociception that comprises all its components would allow insights into the spinal and brain neuronal mechanisms of nociception and pain perception and could lead to new approaches for the treatment of acute and chronic pain (Kragel et al., 2018).

1.2. Pain and nociception

Pain is defined by the *International Association for the Study of Pain* (IASP) as *"an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage"* (Loeser and Treede, 2008). The neuronal mechanisms that encode and process noxious stimuli, which may ultimately lead to the perception of pain, are called nociception. Pain is related to, but not entirely dependent on, nociception, as pain can emerge without obvious nociceptive input, e. g. in chronic pain conditions (Reddan and Wager, 2018). Conversely, nociceptive activation is not always accompanied by pain perception, for instance during unconsciousness or at low levels of nociception (Baliki and Apkarian, 2015). Thus, pain is not a direct readout of nociception (Wiech, 2016).

1.2.1. Physiological basis of nociception

The afferent neurons that respond to chemical, thermal or mechanical noxious stimuli are called nociceptors (Garland, 2012). The cell bodies of these neurons are located in the dorsal root ganglia (DRG) of the spinal cord for body nociceptors and in the trigeminal ganglia for face nociceptors (Julius and Basbaum, 2001). From the cell body, an axonal process bifurcates and one axonal branch enervates the peripheral target tissue whereas the other branch projects to the spinal dorsal horn (Fig. 1.1). Different from the prototypic neuron, which possesses one axonal process through which signals are sent to downstream cells and several dendritic processes via which the neuron receives input from other neurons, nociceptors form a unique class of neurons called pseudo-unipolar neurons, as both the peripheral and the central terminals of the axon branch are able to send and receive information. However, only the peripheral endings respond to chemical, thermal or mechanical noxious stimuli (Basbaum et al., 2009).

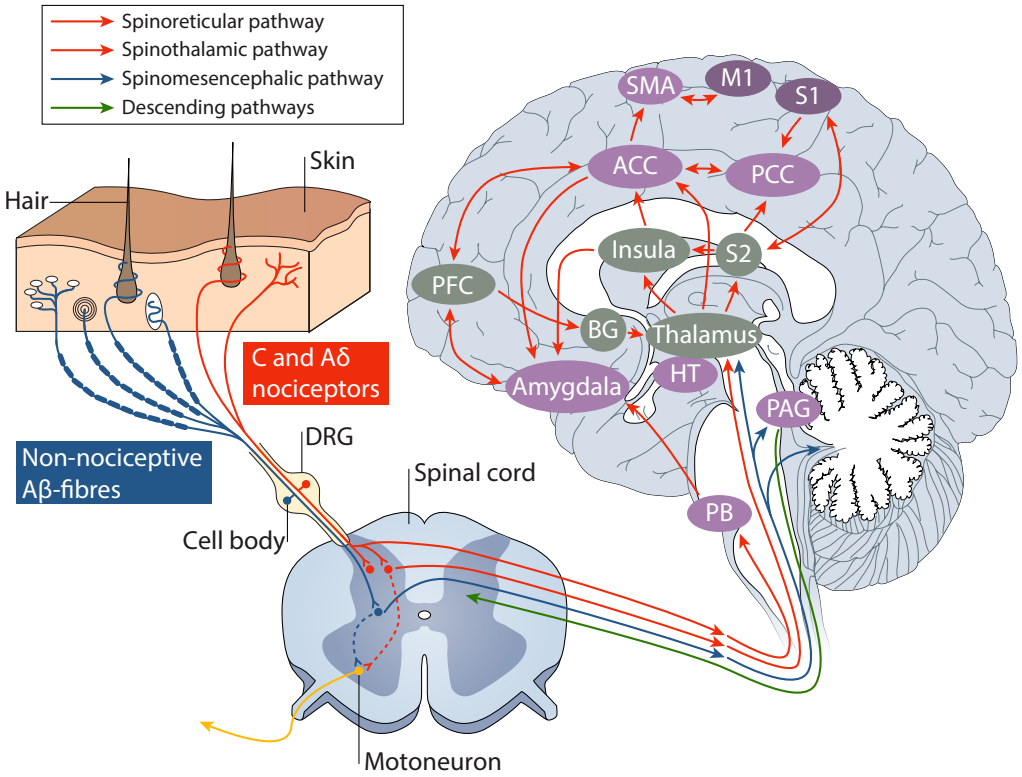

Figure 1.1: Human pain processing system. Peripheral nociceptive C- and Aδ-fibres (red) project to the spinal dorsal horn, from where projection neurons relay nociceptive information via different tracts to the brain, mainly to the thalamus, the periaqueductal grey (PAG) and the parabrachial nuclei (PB). From these brain regions, information is relayed to higher-order cortical and subcortical regions. Descending connections from the brain to the dorsal horn neurons also exist, mainly via the PAG-rostral ventromedial medulla (RVM) descending pain modulatory system (green). SMA, supplementary motor area; M1, primary motor cortex; S1, primary somatosensory cortex; ACC, anterior cingulate cortex; PCC, posterior cingulate cortex; PFC, prefrontal cortex; S2, secondary somatosensory cortex; BG, basal ganglia; PAG, periaqueductal grey; PB, parabrachial nuclei; DRG, dorsal root ganglion. Figure adapted from Kuner and Flor (2017).

Two major classes of nociceptors exist: (i) The fast transmitting (12–30 m/s), medium diameter (2–6 µm) myelinated Aδ-fibres that are responsible for the sharp and localized "first pain" after noxious stimulation, and (ii) the slowly transmitting (0.5–2.0 m/s), small diameter (0.4–1.2 µm) unmyelinated C-fibres that convey the less localized and longer lasting "second pain" (West et al., 2015). The central axonal terminals of nociceptors project to different laminae of the spinal dorsal horn. Specifically, Aδ-fibres project to laminae I and V, while C-fibres project to laminae I and II (Basbaum et al., 2009; D'Mello and Dickenson, 2008). In contrast, Aβ-fibres, which respond only to innocuous mechanical stimulation (i. e. touch), project to laminae III, IV and V. It follows that while neurons in laminae I/II or III/IV are responsive to either noxious or innocuous stimuli, the neur-

ons in lamina V are responsive to both stimulus modalities and are therefore called wide dynamic range (WDR) neurons. Prolonged input to these neurons leads to an increase of the magnitude of the output action potentials, a process called wind-up – a form of spinal cord synaptic plasticity that might also contribute to the development of chronic pain (Latremoliere and Woolf, 2009; West et al., 2015).

From the spinal dorsal horn, nociceptive information is relayed by projection neurons to the brain via different tracts: Lamina I projection neurons innervate the parabrachial nuclei (PB) in the pons via the spinobrachial (spinoreticular) tract and the periaqueductal grey (PAG) via the spinomesencephalic tract (McMahon et al., 2013). Lamina V projection neurons mainly innervate different nuclei of the thalamus via the spinothalamic tract (D'Mello and Dickenson, 2008). The thalamus is the central relay site for nociceptive information to cortical and subcortical structures (Tracey and Mantyh, 2007) and is connected to the amygdala, the hypothalamus, the PAG, the basal ganglia (BG) and cortical regions, such as the anterior cingulate cortex (ACC) and the insula (Garland, 2012).

A variety of cortical and subcortical regions are implied in nociceptive processing and have been historically summarised under the term "pain neuromatrix" or, more commonly today, under the term "pain matrix". These regions include the primary somatosensory cortex (S1), the secondary somatosensory cortex (S2), the ACC, the insula, the prefrontal cortex (PFC), and the thalamus (Apkarian et al., 2005). Several other regions such as the posterior cingulate cortex (PCC), the BG, the amygdala, the hypothalamus and the brainstem are also implicated in pain processing during different conditions (Tracey and Mantyh, 2007; Reddan and Wager, 2018). However, neither any of these regions alone nor the pain matrix as a whole is specific for pain or nociception (Legrain et al., 2011). For example, the pain matrix was shown to be similarly activated by mechanical noxious stimulation in pain-free individuals (Salomons et al., 2016), by hypnotically-induced pain (Raij et al., 2005) or by observing others in pain (Cheng et al., 2010).

Historically, the pain matrix was divided into a lateral system, comprising the S1, the S2, and lateral nuclei of the thalamus, which is attributed to the sensory-discriminative component of pain (localization, intensity, quality) and a medial system, comprising the ACC and the insular cortex, which is attributed to the cognitive-affective component of pain (Fig. 1.2). However, these brain areas cannot be distinctly assigned to either component, as for instance the sensory-discriminative regions are modulated by cognitive

processes (Wiech, 2016), and the cognitive-affective regions are responsive to moderate noxious stimuli despite unconsciousness (Warnaby et al., 2016).

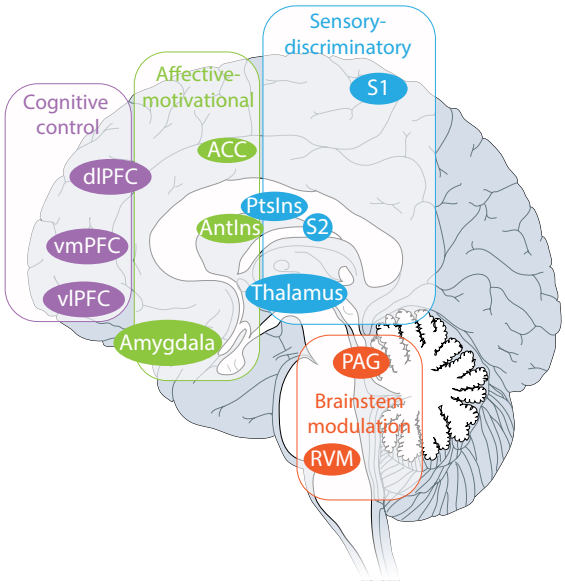

Figure 1.2: Pain components. Shown are the anatomical regions that are attributed to the different components of pain, the cognitive component (violet), the affective-motivational component (green) and the sensory-discriminative component (blue), as well as the brainstem modulatory regions ("vegetative component", red) according to Lee et al. (2014). dlPFC, dorsolateral prefrontal cortex; vmPFC, ventromedial prefrontal cortex; vlPFC, ventrolateral prefrontal cortex; ACC, anterior cingulate cortex; AntIns, anterior insula; PtsIns, posterior insula; S1, primary somatosensory cortex; S2, secondary somatosensory cortex; PAG, periaqueductal grey; RVM, rostral ventromedial medulla.

1.2.2. Top-down modulation of pain and nociception

The PAG and the rostral ventromedial medulla (RVM) are at the core of the opioidergic descending pain modulatory system that can exert both facilitating (pro-nociceptive) and inhibiting (anti-nociceptive) effects on nociceptive processing (Fig. 1.3). The PAG receives ascending inputs from the spinal cord and descending inputs from higher-order cortical and subcortical structures including the rostral anterior cingulate cortex (rACC), the amygdala, the hypothalamus and the PFC (Bushnell et al., 2013). Direct electrical stimulation of the PAG has been shown to produce anti-nociceptive effects in humans (Hosobuchi et al., 1977), suggesting a direct involvement of PAG neurons in creating analgesia. The PAG projects to the RVM, which additionally receives connections from subcortical regions such as the thalamus and locus coeruleus (Vanegas and Schaible, 2004).

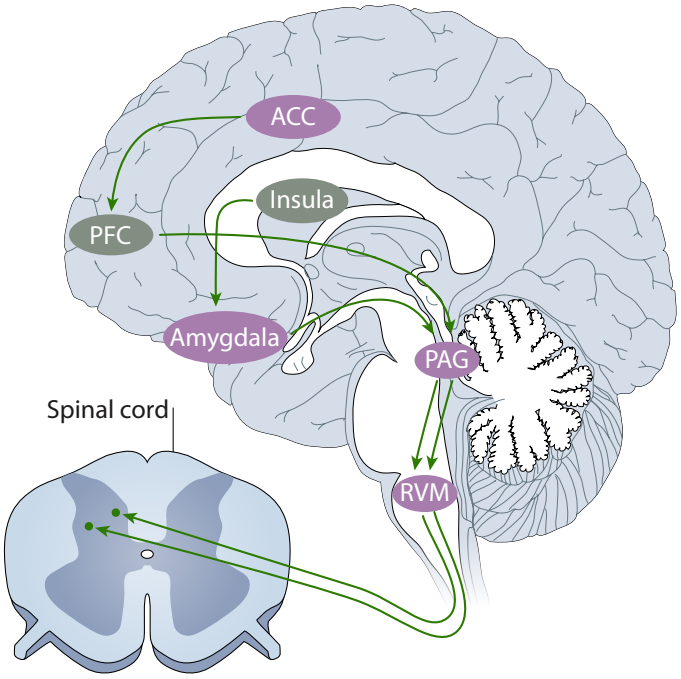

Figure 1.3: The descending pain modulatory system. Major descending pathways that modulate nociception in the human central nervous system (CNS) (Davis et al., 2017). ACC, anterior cingulate cortex; PFC, prefrontal cortex; PAG, periaqueductal grey; RVM, rostral ventromedial medulla.

The RVM comprises so-called on- and off-cells – so named because they are either active or inactive prior to the occurrence of nociceptive reflexes – that project to spinal dorsal horn neurons (Mason, 2012). Opioids inhibit on-cells and activate off-cells and the latter has been found to be critical to produce anti-nociceptive effects (Heinricher et al., 1994). The descending pain control system exerts its function mainly through the release of endogenous opioids (Eippert et al., 2009). As noted, the descending pain modulatory system can also produce pro-nociceptive effects on spinal nociception (Gebhart, 2004), which might contribute to the development of chronic pain (Porreca et al., 2002).

1.2.3. Experimental pain and nociception stimulation paradigms

Human and animal experimental pain research is commonly conducted using thermal or electrical noxious stimulation. The most common thermal noxious stimulation paradigms are performed using laser-generated radiant heat pulses, contact heat stimulators or warm/cold water baths (Arendt-Nielsen and Chen, 2003). Thermal stimulation paradigms are limited in their maximal intensity due to the risk of skin burns and thus cannot be used to mimic the intense noxious stimuli that occur during surgical interventions.

In contrast, transcutaneous or direct electrical stimulation of nociceptive nerve afferents provides the possibility of very high pain intensities without the risk of tissue damage.

However, in contrast to the pure nociceptive thermal noxious stimuli that activate both Aδ- and C-fibres via thermosensitive nociception-receptors, electrical noxious stimuli activate nerve fibres directly, synchronously and by bypassing the nociception-receptors in the cells. Additionally, electrical stimuli activate different fibre types with different preference depending on the stimulus characteristics (Merrill et al., 2005). Thus, electrical stimulation, in contrast to noxious thermal stimulation, is not a specific activator of nociceptors (Disbrow et al., 1998; Reddy et al., 2012).

A particularly useful site for electrical stimulation is the sural nerve at the ankle, as its painful stimulation evokes the nociceptive flexion reflex (NFR), a protective withdrawal reflex that can be detected and quantified using electromyography (EMG) at the ipsilateral biceps femoris muscle of the thigh (Fig. 1.4; Lichtner et al., 2015). In contrast to the withdrawal reflex of the upper extremities, stimulation of the purely sensory sural nerve does not activate motoneurons directly, which would confound electromyographic measurements of the spinally conferred motoneuronal activation. Two components of the NFR can be distinguished in the EMG: The short latency RII-component (40-60 ms after the stimulus) and the nociception-specific RIII-component (90-150 ms after the stimulus). The RII-component is attributed to the activation of non-nociceptive Aβ-fibres and the RIII-component to the activation of Aδ-fibres (Sandrini et al., 2005). Thus, the RIII-component compensates for the non-nociception specific activation of the electrical stimulation paradigm. The RIII-component has two properties that make it particularly suitable for pain research: First, its magnitude as quantified using EMG correlates with the perceived pain intensity and second, the threshold current required to elicit the reflex – the nociceptive flexion reflex threshold (NFRT) – correlates with the individual pain threshold (Willer, 1977). Moreover, the NFRT has been shown to correlate with nociceptive responsiveness in unconscious humans during general anaesthesia (von Dincklage et al., 2010a,b, 2012).

Besides the electrical stimulation of the sural nerve to elicit the NFR, a second commonly used electrical stimulation paradigm is the tetanic (i. e. high frequency) stimulation of the ulnar nerve at the upper extremities. This stimulation paradigm is the standard experimental paradigm for mimicking surgical stimuli in anaesthesiological research, as it has

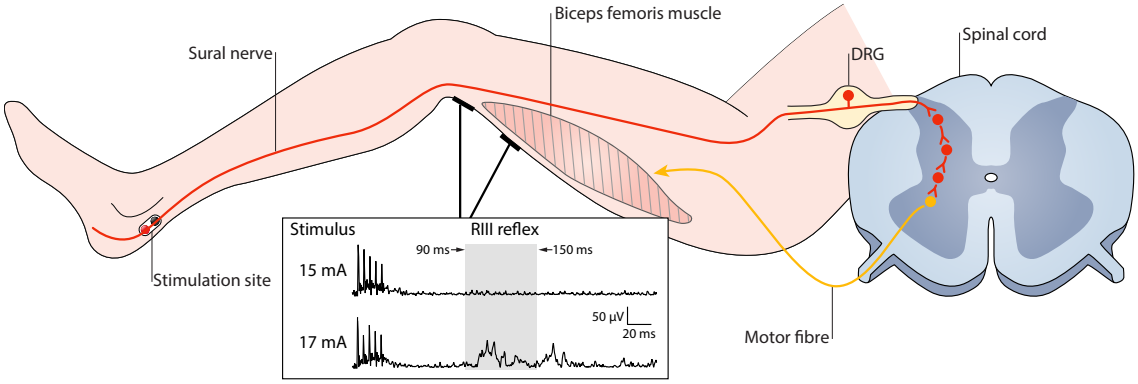

Figure 1.4: Experimental setting for the recording of the nociceptive flexion reflex. The nociceptive flexion reflex is a polysynaptic spinal withdrawal reflex that can be elicited by transcutaneous electrical stimulation of the sural nerve at the ankle and quantified using electromyography of the ipsilateral biceps femoris muscle. Inset: Occurrence and magnitude of the nociceptive flexion reflex are dependent on the stimulus intensity. DRG, dorsal root ganglion.

been shown to evoke clinical responses (e. g. body movement) in a comparable fashion to clinical noxious stimuli (e. g. skin incision; Rantanen et al., 2007). It is therefore used as a standardised stimulus to determine the key figure that defines the potency of anaesthetic drugs, which is the concentration at which half of the patients respond to a defined noxious stimulus and which is referred to as the half maximal effective concentration (EC_{50}) for intravenous anaesthetics or the minimum alveolar concentration (MAC) for inhalational anaesthetics. Additionally, this stimulus paradigm is used for eliciting the pupillary dilation reflex (PDR), which is another nociceptive reflex that can be utilised for pain research (Larson, 2008; Guglielminotti et al., 2015). Similar to the NFR, the PDR has been shown to correlate with the subjective pain intensity in awake subjects (Ellermeier and Westphal, 1995) and with intraoperative nociceptive responses during general anaesthesia (Guglielminotti et al., 2015).

1.2.4. Pain and nociception assessment using functional neuroimaging

Pain has been a cognitive process of enormous interest for neuroimaging research ever since the advent of functional magnetic resonance imaging (fMRI) and other imaging modalities (Davis, 2011; Moayedi et al., 2018). Pain-related imaging studies have identified a set of brain regions that are activated during the processing of noxious stimuli (the pain matrix; cf. Sec. 1.2.1). However, none of these regions are specific to pain and activation of these areas is not exclusive to nociceptive processing. There are two reasons

that might contribute to this non-specificity of activations seen in neuroimaging studies: First, as there are indeed nociception-specific neurons in several brain regions (e. g. Shyu et al., 2010) a problem might be the coarse spatial resolution of current neuroimaging modalities, in which large voxel sizes in the millimetre range result in the averaging of the signals of a high number of different neurons and/or anatomical regions, such that the local specificity is masked by the spatial averaging. Second, rather than consisting of locally distinct functional modules, the brain might encode and process in a distributed fashion, such that local brain activations are not specific for the process in question (Kragel et al., 2018).

The latter assumption of a distributed representation of pain processing is supported by the seminal work of Wager and colleagues, in which they used an approach called multivariate pattern analysis (MVPA) that uses a combination of the signals from multiple voxels to fit an experimental model, rather than the classical mass-univariate approach, in which a model is fit to the signal from each voxel independently (Wager et al., 2013). By a combination of different techniques, Wager and colleagues created the *neurologic pain signature* (NPS) that can be used to quite reliably predict individual pain ratings of nociceptive evoked pain, including pain induced by thermal, electrical, mechanical and visceral distension noxious stimuli. In contrast, the NPS is not responsive to socially and emotionally induced pain, to vicarious pain, nor to placebo or cognitive modulation of pain perception (Reddan and Wager, 2018). Thus, the NPS seems to capture a nociception-dependent component of pain but is clearly no comprehensive measure of pain perception. Although further approaches were taken to create a neural signature that better captures the self-regulation of pain (Woo et al., 2017), no comprehensive measure of nociception and pain perception exists to this day. Importantly, while the NPS was shown to respond to opioid analgesia (Zunhammer et al., 2018), it is not known whether the NPS could be a reliable measure of nociception in unconscious subjects such as during general anaesthesia, as general anaesthetics might change the signal processing patterns of the brain in a way that is not captured by the NPS.

1.3. General Anaesthesia

A modern definition describes general anaesthesia as a state of reversible drug-induced non-responsiveness to external stimuli (Shafer and Stanski, 2008). Following this definition, the clinical requirements of a general anaesthesia, which are unconsciousness, amnesia, immobility and vegetative stability are achieved by suppressing the responsiveness to both noxious and innocuous stimuli using anaesthetic drugs. While unconsciousness (suppressed purposeful responses) and amnesia (suppressed memory formation) are achieved primarily through the effects of hypnotic drugs on the brain, immobility (suppressed movement responses) and vegetative stability (suppressed responses of the autonomic nerve system) are achieved primarily through the effects of analgesic drugs on the spinal cord (Antognini and Carstens, 2002). However, each of these anaesthetic requirements is also affected by secondary effects of the respective other drug class. For instance, already low doses of analgesic drugs vastly reduce the required doses of hypnotic drugs to prevent arousal from unconsciousness due to noxious stimuli (Mertens et al., 2003; Kern et al., 2004).

Analgesia if often included in the list of clinical requirements of general anaesthesia, however, it is strictly speaking not a requirement, as analgesia refers to the absence of pain, a conscious experience that is by definition absent during unconsciousness. Anti-nociception, on the other hand, is also not (yet) a clinical requirement *per se*, as it is currently not known whether persistent nociception during general anaesthesia causes adverse effects on patient outcomes other than by triggering movement or vegetative responses. Thus, to the current state of knowledge, anti-nociceptive drugs are used only to prevent movement and haemodynamic or other vegetative responses to noxious stimuli and not to prevent nociception *per se*.

1.3.1. Molecular targets of hypnotic drugs

Two major classes of hypnotic drugs exist: (i) intravenous drugs, such as propofol, barbiturates, or ketamine, and (ii) volatile (i. e. gaseous) drugs, such as sevoflurane, desflurane, isoflurane, xenon, or nitrous oxide. How these drugs generate unconsciousness remains one of the most important unresolved scientific questions (Kennedy and Norman, 2005). With the advent of molecular biological and neuroimaging techniques, however, it is now

well established that hypnotic drugs have specific molecular targets and act specifically on distinct regions of the brain. While there are two main molecular targets of hypnotic drugs (discussed below), the effects on brain activity patterns are diverse amongst different drugs, although eventually all generate the same state, which is unconsciousness.

The two most important molecular targets of hypnotic drugs are γ-aminobutyric acid type A ($GABA_A$)- and N-Methyl-D-aspartic acid (NMDA)-receptors in the cerebral cortex, the thalamus and the brainstem (MacDonald et al., 2015). Additionally, hypnotic drugs show effects on several other ligand-gated ion channels (Rudolph and Antkowiak, 2004). $GABA_A$-receptors are the main inhibitory receptors in the central nervous system (CNS). The binding of γ-aminobutyric acid (GABA) to these receptors results in the influx of chloride anions that lead to a membrane hyperpolarisation, thereby reducing the excitability of the neuron. Almost all hypnotic drugs have been shown to increase GABA-mediated Cl^- currents, and at high concentrations even directly activate $GABA_A$-receptors in the absence of GABA, except for small and apolar anaesthetics such as xenon and nitrous oxide and the notable exception ketamine (Franks, 2008). These act on the second main target of hypnotic drugs, NMDA-receptors. NMDA-receptors are one of the main types of glutamate receptors, which are the main excitatory receptors of the CNS. The binding of glutamate to an NMDA-receptor leads to the influx of calcium cations, thereby depolarising the neuron and increasing the likelihood of evoking an action potential. NMDA-receptor antagonistic hypnotic drugs include xenon, nitrous oxide, and ketamine.

In summary, it is assumed that the molecular action of hypnotic drugs that ultimately leads to induced unconsciousness is the decrease of neuronal excitability, mainly by activating inhibitory $GABA_A$-receptors or by inhibiting excitatory NMDA-type glutamate receptors in the CNS.

1.3.2. Molecular targets of analgesic drugs

Although a wide range of analgesic drugs exist that can be used during general anaesthesia, strong opioids, such as fentanyl, sufentanil, alfentanil or remifentanil, are the most used analgesic drugs for general anaesthesia (Brown et al., 2011; Sury et al., 2014). The molecular targets for opioids are μ-, κ-, and δ-opioid receptors that are expressed in brain

regions including the PAG, the RVM, the amygdala, the BG and the spinal cord (Brown et al., 2011). Activation of all three types of opioid-receptors leads to the suppression of Ca^{2+} influx, thereby decreasing the neuronal excitability. Opioid receptor binding also leads to opening of G protein–coupled inwardly rectifying K^{2+} (GIRK) channels, thereby inhibiting the generation and propagation of action potentials. Additionally, opioids inhibit Na^{+} channels in DRG neurons and postsynaptic excitatory currents evoked by glutamate receptors in the spinal cord (Stein, 2016). The result is a decreased transmission of nociceptive information at all levels of the nociceptive afferent system.

1.3.3. Possible mechanisms of drug-induced unconsciousness

Most general anaesthetics lead to an overall reduced neuronal activity of the brain and it was early thought that this could be the mechanism of drug-induced unconsciousness. However, different anaesthetics have different regional effects on the brain, and ketamine, for instance, even increases brain activation in certain areas such as the thalamus (Långsjö et al., 2004), rendering the theory of global brain depression as a cause of unconsciousness unlikely.

Many neuroimaging studies showed that a drug-induced loss of consciousness leads to a reduced activity in the thalamus (Alkire et al., 2008; with the notable exception of ketamine-induced unconsciousness). It was therefore speculated that the thalamus, which is a central relay site of ascending and descending information to and from the cortex, could be a prime target of general anaesthetics to induce unconsciousness. However, several studies showed that the decrease of thalamic activity is preceded by a decrease of cortical activity after drug-induced loss of consciousness (e. g. Velly et al., 2007). Thus, the decrease of thalamic activity might rather be caused by a decrease of cortico-thalamic input rather than by direct anaesthetic effects on the thalamus itself (Alkire et al., 2008).

More recent neuroimaging and EEG studies suggested instead that unconsciousness is a consequence of reduced functional connectivity between brain networks and of diminished information integration capabilities of the brain (Hudetz and Mashour, 2016). Light propofol-induced sedation before the loss of consciousness was found to impair the connectivity of subcortical thalamo-regulating systems while leaving thalamo-cortical

connectivity relatively intact (Ní Mhuircheartaigh et al., 2010). Propofol-induced unconsciousness was shown to reduce the activity within two important resting state networks, the default mode network (DMN) and the executive control network, and that the anti-correlation between both networks, which is seen in awake subjects, was absent (Boveroux et al., 2010). In contrast, functional connectivity in low-level sensory networks was largely preserved, suggesting that a disruption of the interaction between low-level and higher-level functional networks impairs the conscious experience of external stimuli (Liu et al., 2012).

Parieto-frontal and occipito-frontal feedforward brain connectivities are thought to represent the transfer of primary sensory information to higher-order cortices and feedback connections are thought to be responsible for selecting and interpreting this information (Uhrig et al., 2014). Several neuroimaging studies found that loss of consciousness using propofol, ketamine or sevoflurane reduces fronto-parietal and fronto-occipital feedback connectivity, while leaving feedforward connections relatively preserved (Jordan et al., 2013; Lee et al., 2013; Untergehrer et al., 2014), suggesting that the attenuation of feedback connectivity contributes to unconsciousness.

In summary, the current state of knowledge is the assumption that the breakdown of within- and between-network functional connectivity between low-level sensory networks and higher-level association networks as well as the attenuation of feedback connectivity might prevent the integration of sensory information and thereby generate unconsciousness (Hudetz and Mashour, 2016).

1.3.4. Arousal reduction by general anaesthetics

Arousal describes the state of general cortical activation in response to sensory stimulation mediated via the ascending reticular activating system (Yeo et al., 2013; Satpute et al., 2018). For example, a sleeping person may be aroused (and thereby awoken) by mild innocuous stimuli, while a person under shallow levels of general anaesthesia around the loss of consciousness may be aroused only by a much stronger, usually noxious, stimulus, as both hypnotic and analgesic drugs have attenuating effects on arousal (Fig. 1.5). The GABA-agonist propofol inhibits arousal nuclei in the pons, midbrain, hypothalamus and basal forebrain (Saper et al., 2005) as well as cortical pyramidal neurons by enhancing

the activity of local GABAergic inhibitory interneurons. As local inhibitory interneurons control a large number of cortical pyramidal neurons, propofol can effectively reduce activation of large cortical areas (Brown et al., 2011). However, although propofol reduces brain metabolism globally (Alkire et al., 1995), the reduction is not uniform across brain regions, with specific large decrease of cerebral blood flow in the thalamus, the cuneus and precuneus, the PCC, in the orbitofrontal cortex and the right angular gyrus (Fiset et al., 1999).

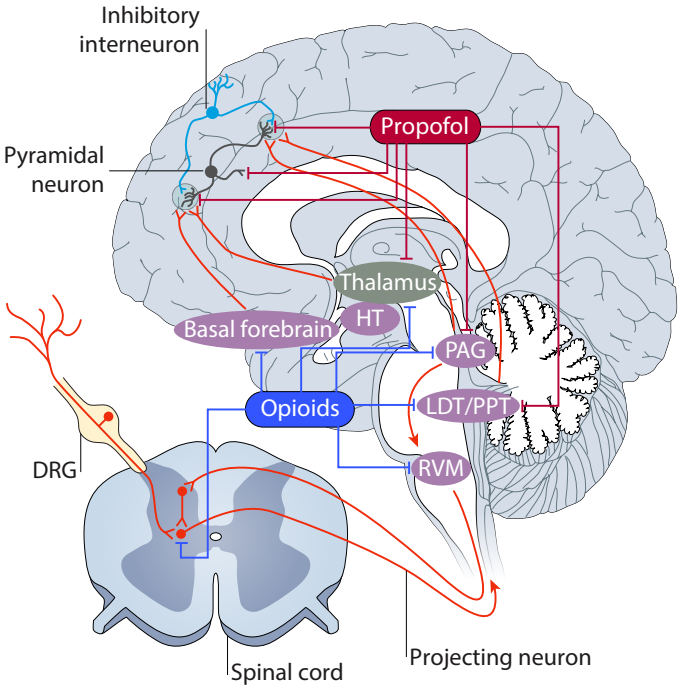

Figure 1.5: Effects of the hypnotic drug propofol and opioids on the central nervous system (CNS). Propofol (red) is a $GABA_A$-receptor agonist that increases the activity of inhibitory interneurons (light blue), thereby inhibiting pyramidal neurons (grey) in the cortex as well as inhibiting excitatory projections from arousal nuclei in the basal forebrain, the hypothalamus, the PAG and in the reticular formation, including the LDT and the PPT, amongst others (see Brown et al. (2010, 2011) for details). Opioids (blue), such as remifentanil, reduce arousal by inhibiting nuclei of the ascending arousal system, as well as by attenuated ascending nociceptive input in the PAG and the RVM and by binding to opioid receptors directly at synapses between peripheral afferent nociceptive neurons and projection neurons in the spinal dorsal horn (see Brown et al. (2010, 2011) for details). HT, hypothalamus; PAG, periaqueductal grey; LDT, laterodorsal tegmental area; PPT, pedunculopontine tegmental area; RVM, rostral ventromedial medulla; DRG, dorsal root ganglion.

Opioids reduce arousal by inhibiting ascending nociceptive signal transmission directly at synapses in the spinal dorsal horn (Fig. 1.5). Additionally, they activate the descending pain modulatory system by binding to opioid receptors in the PAG and the RVM,

resulting in an overall attenuation of nociceptive processing (Millan, 2002). They also lead to the inhibition of arousal nuclei in the laterodorsal tegmental area (LDT), pedunculopontine tegmental area (PPT) and in the basal forebrain, which send excitatory projections to the thalamus and the cortex (Brown et al., 2011).

1.3.5. Effects of general anaesthetics on nociceptive processing

The effects of general anaesthetics have been studied extensively using functional neuroimaging techniques, however, the focus of most – if not all – research was on changes of brain activity around the loss of consciousness for hypnotic drugs or at low analgesic dosages for analgesic drugs. An early study that investigated the effects of propofol on the processing of thermal noxious stimuli of moderate intensity in the brain found at mild propofol sedation before the loss of consciousness an increased pain-evoked activity in the thalamus and the ACC compared to alert control condition and a complete suppression of activity in these regions after propofol-induced unconsciousness (Hofbauer et al., 2004). However, pain-evoked activity was still found in the insular cortex in this condition. Interestingly, this study found increased subjective pain ratings at mild propofol sedation compared to alert control, which was affirmed in later studies (Frölich et al., 2005). Another study found that propofol reduced pain-evoked activity in the S2, the insula and the ACC after the loss of behavioural responses, defined as the loss of reactions to verbal commands. After further increasing propofol dosage, a loss of responses to painful stimulation in the thalamus and in the primary sensory cortex was found, while activation in the precuneus, the PFC and parietal cortices persisted (Ní Mhuircheartaigh et al., 2013). The same authors later concluded that the dorsal anterior insular cortex might be a potential gate responsible for the loss of behavioural responses, as this region showed responses to both auditory and moderate noxious stimuli in the awake subjects and was specifically suppressed around the propofol-induced loss of behavioural responses (Warnaby et al., 2016).

Neuroimaging studies of the effects of the opioid remifentanil have found a dose-dependent reduction of pain-related activations (Wise et al., 2002, 2004) along with a general baseline activation of pain matrix regions (Petrovic et al., 2002). Pain-related activations in the insular cortex were found to be particularly susceptible to remifentanil (Lee et al., 2014). However, only the posterior insula, together with other brain regions that are

attributed to the sensory-discriminative component of pain (Fig. 1.2), was found to be reduced dose-dependently with remifentanil. The anterior insula and other brain regions attributed to the affective component of pain such as the ACC and the amygdala were suppressed completely at low remifentanil doses (Oertel et al., 2008). A remifentanil-induced increase of pain-related activity was found in the cingulofrontal cortex and the PAG, suggesting that remifentanil has an activating effect on the endogenous pain inhibitory system (Wagner et al., 2007).

1.4. Assessment of nociception during general anaesthesia

The current gold standard for the assessment of nociception in unconscious patients during general anaesthesia is the monitoring of physiological responses to noxious stimuli (Guignard, 2006). These responses are conferred by nociceptive activation of projection neurons in the spinal cord and include body movement, heart rate and blood pressure increases, lacrimation, sweating and pupil dilation (Brown et al., 2010). In current clinical practice, these responses serve as indicators of insufficient anti-nociception during general anaesthesia. Thus, nociceptive neuronal activation is not assessed directly in current clinical practice, but instead indirectly through monitoring of the physiological responses to these activations. This indirect monitoring has the drawback that all influences that modulate the efferent part of the response also confound the monitored quantity. For example, both muscle relaxants, which are often administered additionally during general anaesthesia, and the reduction of motoneuronal excitability caused by general anaesthetics attenuate body movement responses to noxious stimuli, although the underlying afferent nociceptive activation might remain unchanged. Another problem with physiological responses is that they are often not specific to nociceptive activation but, in contrast, can likewise be triggered by a variety of other causes (Gruenewald and Ilies, 2013). Therefore, monitoring of physiological responses as indicators of nociceptive processing has an inherently limited sensitivity, specificity and reliability.

A variety of technical surrogate measures of nociception have been developed to improve the measurement sensitivity (Cowen et al., 2015). However, all these surrogate measures are validated against the gold standard, which are the above described physiological responses to noxious stimuli. As these responses are only indirect measures of nociception, surrogate measures validated against them inherit the same inaccuracy. Consequently,

clinical responses and surrogate measures validated against them do not allow to determine whether nociception at a level that does not evoke these clinical responses persists during general anaesthesia.

Accordingly, the direct measurement of afferent nociceptive activation would be preferable over the measurement of efferent responses. Potential approaches for the direct measurement include EEG-derived measures as well as neuroimaging techniques. Stimulus-free EEG measures are used in clinical practice to monitor general anaesthesia, however, they indicate the level of hypnosis rather than the level of (anti-)nociception (von Dincklage et al., 2012). Stimulus-evoked EEG potentials scale with the intensity of the applied stimulus, but they are generally not nociception-specific (Cowen et al., 2015). Neuroimaging techniques such as fMRI, positron emission tomography (PET) and near-infrared spectroscopy (NIRS) have been used extensively to study pain, and a rigorous attempt was made by Wager and colleagues to develop a neurologic pain signature (NPS) that can quite sensitively and specifically predict perceived acute, nociceptive-evoked pain intensity of awake subjects (Wager et al., 2013). However, it is currently not known whether the NPS is also a valid measure of nociception during general anaesthesia, as general anaesthetics have already at low doses a profound impact on the activation patterns and the functional connectivity of the brain.

In summary, technical surrogate measures of nociceptive processing may be more sensitive than monitoring of clinical physiological responses but inherit the same fundamental limitations of these responses as they are validated against them. Additionally, technical surrogate measures of indirect responses to nociception (e. g. haemodynamic, movement or reflex responses) are confounded by influences on the efferent part of the response. No reliable sensitive and specific measure of nociception in humans currently exists.

1.5. Aim of the thesis

The primary aim of this thesis was to develop a measure of spinal and cerebral nociception and apply it to advance the understanding of nociceptive processing in unconscious humans during general anaesthesia. To this end, we devised and validated a multimodal experimental setup that allows to simultaneously quantify both spinal and cerebral processing of nociception in unconscious humans using functional magnetic resonance imaging (fMRI), electroencephalography (EEG) and spinal nociceptive reflexes. In the first main study of this dissertation, ***"Effects of propofol anesthesia on the processing of noxious stimuli in the spinal cord and the brain"*** **(Study 1)**, it was tested whether this setup was indeed able to reliably quantify spinal and cerebral nociceptive processing in awake subjects and during general anaesthesia. We then applied this setup to investigate the hypothesis that the hypnotic drug propofol induces a gradual attenuation and disruption of nociceptive processing as has been shown for other stimulus modalities.

Having established the experimental setup, we next investigated in the second main study of this dissertation, ***"Nociceptive activation in spinal cord and brain persists during deep general anaesthesia"*** **(Study 2)**, the hypotheses that (i) the opioid remifentanil alters spinal and cerebral processing in a dose-dependent fashion and that (ii) noxious stimuli at intensities comparable to surgical stimuli evoke cerebral responses despite a general anaesthesia that is considered clinically sufficient, which would suggest that nociception persists in patients receiving a general anaesthesia that is in accordance with current clinical practice. Persisting nociceptive processing during general anaesthesia might in turn be associated with adverse effects on patient outcomes such as the development of chronic pain through neuronal learning processes. To the best of my knowledge, this has never been investigated before, partly because there is currently no or few evidence that nociception during a general anaesthesia that is considered clinically sufficient has clinically relevant effects on patient outcomes and partly due to the lack of technical measures to quantify spinal and cerebral nociception during general anaesthesia – which, in turn, precludes evidence for clinically relevant effects of possibly persisting nociception during general anaesthesia.

This main experimental line of research that intended to investigate the spinal and cerebral processing of noxious stimuli and their modulation by general anaesthetics was supplemented by clinical research on patient outcomes to investigate whether the levels of in-

traoperative anti-nociception during general anaesthesia may be associated with adverse patient outcomes. To that end, we investigated in ***"Higher doses of intraoperative analgesia are associated with lower levels of persistent pain and less analgesic consumption six months after total hip arthroplasty"*** **(Study 3)** the hypothesis that low levels of intraoperative analgesia are associated with chronic postoperative pain six months after the surgery.

As an fMRI-based measure of nociception is much too complex to be used in clinical practice, simpler surrogate measures have to be developed. Nociceptive reflexes such as the nociceptive flexion reflex (NFR) and the pupillary dilation reflex (PDR) are among these potential surrogate measures. So far it has only been shown that these reflexes correlate with other surrogate markers of excessive nociception during general anaesthesia, but it has neither been investigated whether the mechanisms underlying these reflexes during general anaesthesia correspond to the mechanisms of subjective pain perception in awake subjects nor whether these reflexes are also able to indicate excessive anti-nociceptive drug dosing. We therefore investigated in ***"Intraoperative monitoring of analgesia using nociceptive reflexes correlates with delayed extubation and immediate postoperative pain: A prospective observational study"*** **(Study 4)** the hypotheses that nociceptive reflexes during general anaesthesia correlate with immediate postoperative pain, which would indicate that the mechanisms of these nociceptive reflexes during general anaesthesia correspond to the mechanisms that lead to pain perception in awake subjects and that they correlate with the time to extubation after surgery as a surrogate marker of excessive anti-nociception during surgery.

A weakness of the NFR that currently limits its clinical applicability as a technical surrogate measure of anti-nociception is the insufficient standardisation of its measurement procedure and the resulting inaccuracy of the reflex detection. In the last study of this work, ***"Optimizing nociceptive flexion reflex (NFR) scoring criteria by adjusting for noise and reflex properties and sampling rate"*** **(Study 5)**, we therefore investigated the hypothesis that machine learning techniques can help to build better reflex detection models.

2. Summary and discussion of publications

This dissertation is based on a primary research part, in which a spinal and cerebral measure of nociception in unconscious humans is developed and applied to investigate the spinal and cerebral mechanisms of nociceptive processing during general anaesthesia (Sec. 2.1) and a secondary research part, in which the effects of low levels of anti-nociception during general anaesthesia on patient outcomes and the relationship between surrogate markers of the level of anti-nociception and postoperative outcomes as well as their standardisation are investigated (Sec. 2.2).

2.1. Primary research: Spinal and cerebral neuronal mechanisms of sensory processing during general anaesthesia

In the primary research of this dissertation, we developed and validated an experimental setup that allowed to simultaneously monitor spinal and cerebral nociceptive processing and applied this setup to investigate the processing during clinically relevant general anaesthesia using the hypnotic drug propofol (**Study 1**, Sec. 2.1.1) and the analgesic drug remifentanil (**Study 2**, Sec. 2.1.2). Different from previous work, we investigated (i) anaesthesia at clinically relevant depths and not only around the loss of consciousness, (ii) spinal and cerebral processing simultaneously to be able to tell spinal and cerebral effects of general anaesthetics apart and (iii) we applied intense noxious stimuli at intensities comparable to surgical stimuli – much more intense than the moderate noxious stimuli used by previous studies.

2.1.1. Study 1: Effect of propofol anaesthesia on the processing of noxious stimuli in the spinal cord and the brain

Drug-induced unconsciousness is an exquisite tool for the controlled manipulation of consciousness in an experimental setting. A variety of neuroimaging studies thus have investigated the effects of drug-induced unconsciousness on the cerebral processing of sensory stimuli using hypnotic drugs like propofol to induce the loss of consciousness (LOC). These studies have found as a general mechanism that propofol-induced sedation and unconsciousness first leads to the impairment of higher-order cortices that are associated with complex signal processing (e. g. word processing for auditory stimuli)

and, at deeper levels of anaesthesia, an attenuation or complete elimination of responses in lower-order sensory cortices (Colon et al., 2017).

Different from other sensory modalities, responses to innocuous tactile and noxious stimuli were found to be already diminished or absent in primary and secondary somatosensory cortices at shallow levels of propofol-induced anaesthesia, while activations in higher-order cortices persisted (Bonhomme et al., 2001; Hofbauer et al., 2004). However, propofol (and other anaesthetics) exerts an inhibiting effect on the spinal processing of innocuous and noxious somatosensory stimuli (Matute et al., 2004; Hudetz, 2012). Thus, previous neuroimaging studies on the cerebral effects of propofol-induced unconsciousness are confounded by spinal inhibitory effects.

We therefore developed an experimental setup that allowed for the simultaneous measurement of both spinal and cerebral effects of drug-induced unconsciousness on the processing of noxious somatosensory stimuli (Fig. 2.1; Lichtner et al., 2018a). Using transcutaneous electrical stimulation of the sural nerve at the ankle, we could monitor spinal nociception through quantification of the amplitude of the NFR (cf. Sec. 1.2.3). To monitor cerebral responses to innocuous and noxious stimulation, we used blood-oxygen-level-dependent (BOLD)-fMRI. To ensure that the effects observed using fMRI are indeed of neuronal origin and not confounded by effects of the anaesthetics on the neurovascular coupling, which is the basis of the BOLD-effect, we simultaneously recorded the electroencephalogram as a direct measure of neuronal activity.

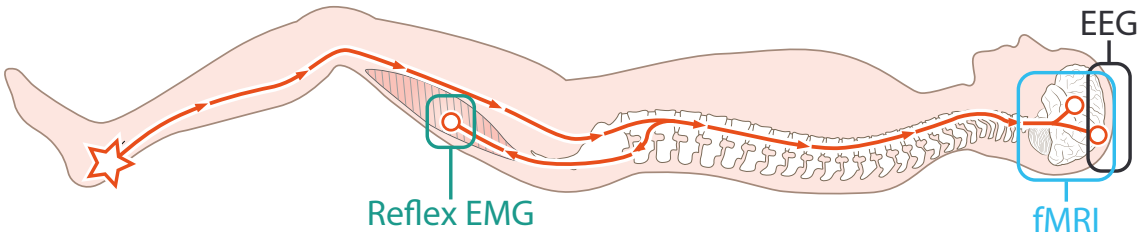

Figure 2.1: Experimental setup for the simultaneous measurement of spinal and cerebral nociception during general anaesthesia. Electrical innocuous and noxious stimuli were applied transcutaneously to the sural nerve at the ankle. Spinal nociception was assessed using reflex electromyography (EMG) at the ipsilateral biceps femoris muscle. Cerebral nociception was assessed using simultaneous blood-oxygen-level-dependent (BOLD)-fMRI and electroencephalography (EEG). Illustration of the spine: Designed by Freepik.

Research question 1.1: *Is it possible to reliably and concomitantly detect spinal and cerebral processing of noxious stimuli during general anaesthesia using a combined fMRI/EEG/EMG setup?*

We showed that our highly complex setup could reliably differentiate innocuous from moderate noxious stimuli in volunteer awake subjects, both in the spinal measure of nociception (reflex EMG) as well as in the cerebral measures (somatosensory evoked potentials (SSEPs) in the EEG and BOLD-fMRI activations). To test our setup during drug-induced unconsciousness, we administered propofol to the subjects at a dose that ensured stable unconsciousness. At that concentration level, a laryngeal mask airway – a medical device to keep open the patients' airway during anaesthesia – was inserted to facilitate monitoring and assistance of ventilation, which is required as most general anaesthetics including propofol and especially remifentanil have respiratory depressing effects that would otherwise strongly affect the dynamics of the BOLD responses via altered end-tidal CO_2 concentration levels (Brown et al., 2011; Kemna and Posse, 2001).

During propofol-induced unconsciousness, we additionally administered intense noxious stimuli at intensities comparable to surgical stimuli to the subjects, which would not have been endured by awake subjects. The possibility of using such intense noxious stimuli is unique to the electrical stimulation paradigm because it does not have the risk of tissue damage that is highly present with thermal or mechanical stimulation. To the best of my knowledge, such intense noxious stimuli have not been used before in neuroimaging studies to investigate nociceptive processing during general anaesthesia.

We could show that during general anaesthesia our setup reliably differentiated intense noxious from moderate noxious and innocuous stimuli in both the spinal and the cerebral measures of nociception. Thus, our setup could reliably detect the processing of noxious stimuli both in awake subjects and during general anaesthesia (Research question 1.1).

Research question 1.2: *Does propofol alter the spinal transmission of nociceptive stimuli after the loss of consciousness?*

As propofol has been shown to inhibit spinal nociceptive transmission, we next investigated the dose-dependent effects of propofol on the spinal transmission of moderate and

intense noxious stimuli. To that end, we increased the propofol dosage in a stepwise fashion and performed measurements at each predefined concentration level. We found that propofol severely attenuated the spinal processing of moderate noxious stimuli already at sub-hypnotic doses. In contrast, the spinal processing of intense noxious stimuli was not dependent on the propofol concentration level after the loss of consciousness (Research question 1.2). Intense noxious stimuli might therefore be a valid tool to investigate the cerebral effects of propofol, as these stimuli are not confounded by further spinal effects of propofol.

Research question 1.3: *Does propofol dose-dependently induce a gradual attenuation and disruption of nociceptive processing?*

We next used our setup to investigate the hypothesis that propofol induces a gradual attenuation and disruption of the cerebral processing of noxious somatosensory stimuli as has been shown for other stimulus modalities, such as auditory, visual and tactile stimuli (MacDonald et al., 2015). We found that cerebral responses to innocuous stimuli as measured by BOLD-fMRI were abolished already at sub-hypnotic propofol doses, while responses to moderate noxious stimuli persisted in multiple brain regions including primary cortices at that concentration level. After the loss of consciousness, we could not detect any cerebral responses to innocuous or moderate noxious stimuli using fMRI. In contrast, significant SSEPs of the primary somatosensory cortex could still be detected at medium propofol concentration levels after the loss of consciousness, indicating further sensory processing in the primary somatosensory cortex. As EEG is more sensitive than fMRI, the lack of significant activations in the latter modality might be a consequence of the rather small sample size in our study.

Using intense noxious stimulation at intensities comparable to surgical stimuli, we could detect significant BOLD-fMRI activations in a variety of cortical and subcortical areas at all propofol concentration levels, even at profound anaesthetic depth (Fig. 2.2). Based on the dose-response characteristics and their functional connectivity, we identified four groups of brain areas:

1. The *temporal group* consisted of areas in the right insular cortex, in the right S2 and in the right middle temporal gyrus. These areas showed strong responses to intense

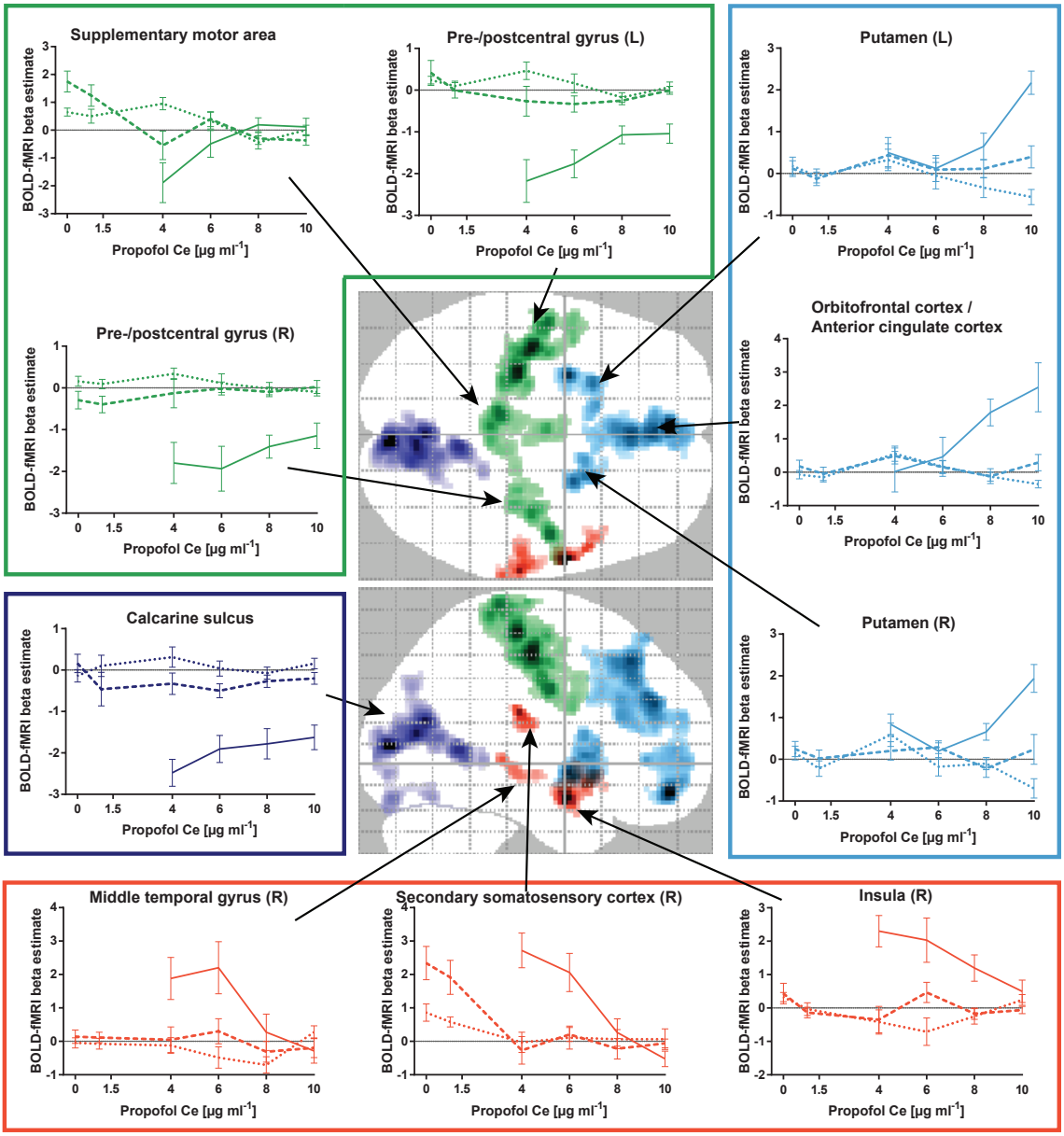

Figure 2.2: FMRI activations triggered by the intense noxious stimuli in dependency of the propofol concentration. The figure shows the fMRI activations in all brain regions with significant differences in activity between the intense noxious and the innocuous control stimuli during any propofol level after the loss of consciousness, projected onto SPM's glass brain. The graphs show the fMRI activations triggered by innocuous (dotted lines), moderate painful (dashed lines) and intense noxious stimuli (solid lines) in each brain region quantified by the regression coefficient ("beta") estimates of random-effects general linear models, plotted against propofol effect-site concentrations. The brain regions are arranged in four groups (purple, blue, red and green colour) based on the high levels of within-group functional connectivity throughout all propofol levels. FMRI analysis methods are described in Lichtner et al. (2018a). Ce, effect-site concentration; R, right; L, left.

noxious stimulation at the lowest propofol concentration levels and decreasing response amplitudes with increasing propofol dosage until no responses could be detected at the highest propofol concentration level investigated.

2. The *anterior group* consisted of areas in the bilateral putamen and in the orbitofrontal cortex (OFC)/ACC. Intriguingly, these areas showed no responses to intense noxious stimulation after the loss of consciousness but increasing activations with increasing propofol dosage.

3. The *central group* consisted of areas in the supplementary motor area (SMA) and in the bilateral pre- and postcentral gyri. They showed profound deactivations following intense noxious stimuli during all levels of propofol anaesthesia, except for the SMA, in which no activation/deactivation could be detected during the deepest levels of anaesthesia.

4. The calcarine sulcus showed a dose-response relationship similar to that of the central group, but – in contrast to the other brain regions of that group – no consistent within-group functional connectivity.

In conclusion, we found a gradual breakdown of the processing of intense noxious stimuli with increasing propofol dosage in the sense that deepening propofol-induced unconsciousness abolished nociceptive activations the S2 and the insular cortex and largely rearranged the functional connectivity between nociceptive brain regions (Research question 1.3). However, in contrast to studies of other stimulus modalities that showed that propofol preferentially reduced impaired activation of higher-order cortices, we found that the ACC – a higher-order association cortex associated with the affective-motivational component of pain – and the bilateral putamen were not activated by intense noxious stimuli after the loss of consciousness, but were profoundly activated at the highest propofol concentration levels investigated.

Research question 1.4: *Is EEG burst suppression associated with an abolished cerebral processing of noxious stimuli?*

The highest propofol dosage that we applied in our study caused profound EEG burst suppression, which corresponds to the deepest levels of anaesthesia that are reached in

clinical practice. Burst suppression is commonly attributed to an inactivated brain (Purdon et al., 2015). Despite this deep level of anaesthesia, we could still detect profound cerebral responses to intense noxious stimuli even in higher-order cortices. This is in accordance with the notion that burst suppression is not really associated with an inactive brain (Pilge et al., 2014). EEG burst suppression might therefore be only the epiphenomenon of an altered cerebral functional circuitry, as it occurs in the same propofol concentration range as the profound changes in functional connectivity that accompany the vastly altered noxious stimuli-induced activation patterns. In conclusion, EEG burst suppression does not preclude the cerebral processing of noxious stimuli (Research question 1.4).

2.1.2. Study 2: Nociceptive activation in the spinal cord and the brain during general anaesthesia

Using the setup established in **study 1**, we next aimed to investigate the responses to noxious stimuli in the spinal cord and the brain during general anaesthesia at clinically relevant depths, which is performed using a hypnotic drug, such as propofol, and a strong analgesic, such as remifentanil. We therefore investigated nociceptive processing in ten volunteer subjects during general anaesthesia (Lichtner et al., 2018b). Like in **study 1**, we first performed a propofol mono-anaesthesia at a propofol concentration level that ensured stable unconsciousness during assisted ventilation using a laryngeal mask airway. We then additionally administered the opioid remifentanil in a stepwise fashion and performed measurements using innocuous, moderate noxious and intense noxious electrical stimulation at an intensity that is comparable to surgical stimuli.

Research question 2.1: *Does remifentanil alter spinal and cerebral processing in a dose-dependent fashion?*

Spinal nociceptive responses to intense noxious stimuli as assessed by the amplitude of the NFR slightly decreased with increasing remifentanil concentration, but were significantly detected throughout all concentration levels, indicating that even very high doses of remifentanil (higher than usually used in clinical practice; cf. von Dincklage et al., 2018) do not completely block spinal nociceptive processing. Responses to moderate and intense noxious stimuli quantified by the SSEPs of the primary somatosensory cortex in

the EEG were also detected through most (moderate noxious) or all concentration levels (intense noxious) of remifentanil, showing a persistent primary cortical responsiveness to noxious stimuli. While we could not detect subcortical or cortical responses to moderate noxious stimuli in the BOLD-fMRI, intense noxious stimuli evoked significant responses even at the highest remifentanil concentration levels in a variety of brain regions including the putamen, the bilateral insular cortex, the S2 and the ACC. We found a significant remifentanil-induced reduction of responses to intense noxious stimuli only in frontal brain regions. However, due to our limited sample size, negative findings such as non-significant decreases of activations have to be interpreted with caution.

Consistent with the dose-dependent attenuation of cerebral responses in multiple brain areas, we found a significant dose-dependent decrease of functional connectivity between all brain regions that were activated by intense noxious stimuli during unconsciousness. This could indicate that reduced connectivity between brain areas causes reduced processing of noxious information. However, from our data we cannot conclude whether the reduced functional connectivity causes the reduced processing or the other way round, or whether both effects are generated through the action of remifentanil on an underlying common process.

In conclusion, remifentanil dose-dependently attenuates spinal and cerebral responses but does not completely suppress them – even not at the highest investigated dose of remifentanil, which is above the highest commonly used doses in clinical practice (Research question 2.1).

Research question 2.2: *Do noxious stimuli evoke cerebral responses despite a general anaesthesia that is considered clinically sufficient?*

During surgical and other noxious procedures, anaesthesiologists monitor the anaesthetised patients for clinical responses to noxious stimuli (e. g. movement, blood pressure or heart rate elevations). The occurrence of such responses indicates an insufficient anti-nociceptive component of the general anaesthesia, requiring intervention by the anaesthesiologist. Once none of these responses can be observed during noxious procedures, the level of anti-nociception of the general anaesthesia is considered clinically sufficient. We therefore aimed to relate our findings to these clinical indicators of insufficient anti-nociception during general anaesthesia. To that end, we tested for clinical responsiveness

to a noxious stimulus that is comparable to a surgical stimulus (tetanic stimulation of the ulnar nerve; cf. Sec. 1.2.3) before and after the series of measurements at each remifentanil concentration level. During and after administration of that stimulus, we closely monitored the subjects for any observable arm, leg, body or head movement, and for heart rate or blood pressure increases. We found that blood pressure and heart rate elevations were already abolished at the lowest dose of remifentanil applied and that body movement responses could not be detected at medium doses of remifentanil, similar to those used in clinical practice. In contrast, as described above, spinal and cerebral measures showed profound responses to intense noxious stimulation even at much deeper levels of general anaesthesia despite the complete suppression of clinical responses (Research question 2.2).

2.2. Secondary research: Studies on the association of intraoperative anti-nociception with postoperative outcomes and on nociception assessment

In the secondary research part of this dissertation, the possible association between the level of intraoperative anti-nociception during general anaesthesia and persistent effects on patient outcomes is explored. To that end, we performed a clinical study on patients undergoing surgery, where we assessed surrogate markers of intraoperative anti-nociception and their relationship to persistent postoperative pain (**Study 3**, Sec. 2.2.1).

To validate nociceptive reflexes as surrogate measures of intraoperative nociception, we additionally assessed intraoperative nociceptive reflexes in the aforementioned study and examined their relationship to immediate postoperative pain and delayed extubation as surrogate markers of low and high levels of intraoperative anti-nociception, respectively (**Study 4**, Sec. 2.2.2).

Furthermore, we investigated whether we could improve the precision of one of these surrogate markers of intraoperative anti-nociception, the nociceptive flexion reflex threshold (NFRT), by application of supervised machine learning techniques for signal detection (**Study 5**, Sec. 2.2.3).

2.2.1. Study 3: Association between intraoperative anti-nociceptive dosing and persistent postoperative pain

Chronic postoperative pain is a major health problem that affects up to 50% of all surgical patients (Kehlet et al., 2006) and up to 30% of all patients undergoing total hip arthroplasty (Nikolajsen et al., 2006). While it has been shown that immediate postoperative pain is associated with pain chronification, it has so far not been investigated whether the level of intraoperative anti-nociception is related to the development of chronic pain – which is, at least partly, a consequence of the lack of reliable measures of nociception in unconscious humans.

Excessive activity of neurons in nociceptive pathways is known to lead to increased excitability of these neurons, a process called central sensitisation, resulting in changes of cerebral activity and contributing to the development of chronic pain (Woolf, 2011). It is possible that persistent nociception that is not suppressed by anti-nociceptive dosing during general anaesthesia triggers the same neuronal learning processes, which can then facilitate the development of chronic pain. We thus aimed to investigate whether the level of intraoperative anti-nociceptive dosing is associated with persistent postoperative pain.

Research question 3.1: *Does anti-nociceptive dosing during general anaesthesia relate to persistent postoperative pain?*

To that end, we performed a clinical prospective observational study in 110 patients undergoing primary hip arthroplasty, a highly standardised surgical procedure (von Dincklage et al., 2018). During the surgery, we monitored the anti-nociceptive dosings as a surrogate measure of intraoperative anti-nociceptive levels. We then contacted the patients six months after their surgery and asked them to report their currently perceived pain intensity on a numerical rating scale (NRS) from 0–10 as well as to indicate whether they used pain medication on a regular basis. Using univariate correlations and multivariate models that adjusted for a variety of possible confounding variables, we found that the average intraoperative opioid administration rate as a surrogate measure of the intraoperative anti-nociceptive level correlated negatively with both the pain intensity and the use of pain medication six months after surgery (Research question 3.1). To the best of

our knowledge, this was the first study to investigate the association between intraoperative anti-nociceptive dosing and persistent postoperative pain. These results suggest that persisting nociception during general anaesthesia that is not sufficiently suppressed by anti-nociceptive dosing contributes, at least partly, to the development of chronic postoperative pain.

2.2.2. Study 4: Validation of nociceptive reflexes as measures of intraoperative anti-nociceptive levels

Nociceptive reflexes such as the NFR and the pupillary dilation reflex (PDR) are potential surrogate measures of the level of the intraoperative nociception/anti-nociception balance, as they have been shown to correlate with physiological responses to noxious stimuli during general anaesthesia (cf. Sec. 1.2.3). However, it is not known whether the mechanisms underlying the nociceptive reflexes during general anaesthesia correspond to the mechanisms that lead to pain perception in awake subjects.

Additionally, the correlation between nociceptive reflexes and physiological responses to excessive intraoperative nociception might support the use of these reflexes as measures of insufficient anti-nociception but does not imply their applicability as measures of excessive anti-nociception. Excessive anti-nociception during general anaesthesia causes adverse effects on patient outcomes on its own, including opioid-induced hyperalgesia (Kim et al., 2014), nausea (Apfel et al., 2012) and prolonged time to emergence from anaesthesia (Macintyre et al., 2011).

Research question 4.1: *Does the level of intraoperative anti-nociception during general anaesthesia relate to immediate postoperative pain?*

We aimed to investigate whether nociceptive reflex thresholds as surrogate markers of the level of intraoperative anti-nociception are related to immediate postoperative pain. To that end, we assessed the intraoperative nociceptive flexion reflex threshold (NFRT) and the pupillary dilation reflex threshold (PDRT) of the patients who participated in **study 3** at the end of the surgical procedure while the patients were still anaesthetised (Jakuscheit et al., 2017). Immediately after anaesthesia emergence, the patients were asked to indicate their currently perceived pain intensity on a numerical rating scale (NRS) from 0–10.

We found that the PDRT measured at the end of the surgery correlated with the immediate postoperative pain, which suggests that the mechanisms underlying this nociceptive reflex correspond to the mechanism that leads to pain perception in awake subjects.

Research question 4.2: *Is the level of intraoperative anti-nociception during general anaesthesia associated with delayed extubation?*

We additionally assessed the time between the end of the surgical procedure and extubation of the patients as a surrogate indicator of anti-nociceptive overdosing, as this is known to delay the emergence from anaesthesia (Macintyre et al., 2011). We found that both the NFRT and the PDRT correlated with the time to extubation, suggesting that nociceptive reflexes are indeed able to indicate excessive levels of anti-nociception in unconscious humans.

In conclusion, our study was the first to show that nociceptive reflexes measured during general anaesthesia indeed correlate with immediate postoperative pain, which suggests that the pathways of nociceptive reflexes during general anaesthesia and subjective pain perception during wakefulness are similarly affected by anti-nociceptive drugs and which, in turn, might suggest that both pathways at least partly coincide (Research question 4.1). Additionally, adding to previous studies, we validated nociceptive reflexes as indicators of excessive anti-nociceptive dosing during general anaesthesia (Research question 4.2).

2.2.3. Study 5: Optimising nociceptive flexion reflex scoring criteria

The nociceptive flexion reflex threshold (NFRT) is a potential tool to assess the level of nociceptive processing in unconscious humans during general anaesthesia. No such measure that is sufficiently validated and reliable currently exists, but it would allow for the patient- and stimulus-specific titration of anti-nociceptive drugs, thereby potentially improving patient outcomes across the board.

One problem of the NFRT is that its measurement has not been sufficiently standardised (Cowen et al., 2015). The NFR is evoked experimentally by electrical stimulation over the sural nerve and quantified using EMG recordings at the ipsilateral biceps femoris muscle (Lichtner et al., 2015; cf. Sec. 1.2.3). The threshold is then defined as the stimulus intensity

that evokes a reflex response with 50% probability. However, the gold standard to decide whether an EMG recording contains a reflex response or not is the manual analysis by an expert (Rhudy and France, 2007). Previous studies have tried to derive automated scoring parameters for whether an EMG recording contains a reflex or not (Rhudy and France, 2007; France et al., 2009), but these were biased towards large reflex responses, did not take into account the technical variability of measurement setups (e. g. noise level and sampling rate) and did not explore multivariate models of their predefined features. We thus aimed to investigate whether we can improve the classification accuracy of reflex responses from EMG recordings (Lichtner et al., 2016).

Research question 5.1: *Can currently used detection criteria for nociceptive flexion reflex responses be improved using machine learning techniques?*

To that end, a total of 5400 EMG recordings of NFR stimuli from two studies (3600 recordings in the training dataset and 1800 recordings in the test dataset) were presented to each of four experts familiar with the NFR measurement procedure. Logistic regression models and support vector machines were then trained on a set of manually engineered features and validated on the test dataset (the latter were not shown in the publication due to their inferior performance). We found that for our dataset multiple logistic regression models performed superior to all other used methods and could reduce the number of wrongly classified recordings by 25%–37% compared to the previous standard model for automatic classification by Rhudy and France (2007). I have additionally used convolutional neural networks trained directly on the raw EMG recordings (as opposed to training on manually engineered features) using a variety of architectures and data augmentation strategies (not shown in the publication), but could not achieve a higher accuracy compared to the multiple logistic regression models, which might be due to the rather small dataset for a convolutional neural network or because the expert raters unintentionally focussed on rating the recordings in accordance with the same features that were used for the multivariate logistic regression models.

In summary, we could significantly improve the classification accuracy of nociceptive flexion reflex responses from EMG recordings using supervised machine learning techniques (Research question 5.1). This should help to reduce the variability of NFRT measurements and improve its applicability in both the clinical and experimental context.

3. General discussion

Our studies were the first to investigate spinal and cerebral nociceptive processing concomitantly during deep general anaesthesia and using intense noxious stimuli at intensities comparable to surgical stimuli. Using a multimodal measurement setup that combined spinal nociceptive reflexes with simultaneous fMRI and EEG, we could reliably detect spinal and cerebral nociceptive responses in awake subjects and during general anaesthesia. We found that despite drug-induced unconsciousness at clinically relevant levels using the hypnotic drug propofol, intense noxious stimuli evoked profound cerebral responses in a variety of regions including higher-order association cortices. As we identified groups of brain regions that showed similar dose-response relationships to increasing hypnotic drug doses and consistent within-group functional connectivity but changing between-group functional connectivity, we concluded that the altered cerebral processing of noxious stimuli during propofol-induced unconsciousness might be related to changes in functional connectivity between these brain regions (**study 1**). Using propofol in combination with the opioid remifentanil to induce a clinical general anaesthesia and analgesia, we could show that intense noxious stimuli evoked profound cerebral responses despite a deep general anaesthesia that is considered clinically sufficient. Surrogate markers of nociception that are used by anaesthesiologists as indicators of insufficient analgesia during general anaesthesia were already abolished at far lower doses and seem therefore not indicative of nociceptive cerebral processing (**study 2**).

As cerebral nociceptive activations do not imply any clinically relevant effects *per se*, we performed a clinical observational study, in which we could show for the first time that the level of intraoperative anti-nociception is associated with the development of persistent postoperative pain six months after the surgery (**study 3**). Thus, an insufficient nociception/anti-nociception balance during general anaesthesia seems to indeed influence patient outcomes through a mechanism for which we provided a pathophysiological foundation in our experimental studies. Consequently, the current practice of adjusting the depth of general anaesthesia according to clinical surrogate markers – which we found to be not indicative of cerebral nociceptive processing in our experimental study – might result in analgesic underdosing leading to persistent nociception that causes adverse patient outcomes.

As a measurement setup for nociception assessment that includes fMRI is much too complex to be used in clinical routine, simpler surrogate measures have to be developed that allow to investigate the relationship between anti-nociception and patient outcomes in detail as well as to titrate anti-nociceptive dosings individually to prevent adverse patient outcomes. We therefore aimed to validate nociceptive reflexes as surrogate measures of the intraoperative nociception/anti-nociception balance. To that end, we demonstrated that the intraoperative reflex thresholds correlated with immediate postoperative pain, suggesting a correspondence between nociceptive reflex pathways during general anaesthesia and pain perception during wakefulness, and with delayed extubation, suggesting that the reflexes are not only able to indicate anti-nociceptive under- but also overdosing (**study 4**).

One of these nociceptive reflexes, the nociceptive flexion reflex (NFR), is at its current stage of development too inaccurate to be used to predict clinical events. We therefore investigated in the last study of this work whether we could improve the measurement procedure of the NFR. Using supervised machine learning techniques, we could profoundly increase the detection accuracy of the NFR, which should help to enhance its applicability in clinical and experimental research (**study 5**).

3.1. Altered nociceptive processing during general anaesthesia

3.1.1. Dose-dependent effects of propofol

In **study 1** we found that propofol-induced sedation before the loss of consciousness reduced the cerebral responses to moderate noxious stimuli compared to the fully awake subjects. After propofol-induced loss of consciousness, innocuous and moderate noxious stimuli did not evoke any significant cerebral responses as measured by BOLD-fMRI. Overall, these results are consistent with findings from previous studies which showed that propofol dose-dependently decreased cerebral responses to such stimuli (Hofbauer et al., 2004; Ní Mhuircheartaigh et al., 2010, 2013). However, in contrast to previous studies, we concurrently monitored the spinal nociceptive responsiveness by quantification of the NFR amplitude and found that propofol profoundly attenuated nociceptive processing already at the spinal level. Therefore, the changes in cerebral processing cannot be ascribed to cerebral effects of propofol alone and thus results from the previous studies are severely confounded by the spinal inhibitory effect of propofol. In contrast to the innocuous and moderate noxious stimuli, which were used by previous studies, we found that spinal processing of intense noxious stimuli was not significantly affected by propofol after the loss of consciousness. Intense noxious stimuli seem therefore suitable to investigate dose-dependent effects of propofol on cerebral nociceptive processing after the loss of consciousness.

Using stimulus-evoked BOLD-fMRI responses and functional connectivity analyses, we identified four groups of brain areas related to nociceptive processing during general anaesthesia (cf. Sec. 2.1.1). We found that the level of stimulus-evoked activity of two of these groups – the "anterior group", comprising the OFC/ACC and the bilateral putamen, and the "temporal group", comprising the right S2 and the right insular cortex – correlated with their functional connectivity to the "central group", comprising the pre- and postcentral gyri (including the S1) and the SMA. As the central group includes the primary areas of cerebral nociceptive processing, we speculate that under increasing anaesthetic depths the nociceptive information is directed away from the temporal areas and towards anterior areas. Interestingly, the S2 and the insular cortex that are part of the "temporal group" have been previously suggested to be downstream recipients of somatosensory information from the primary somatosensory cortex in a ventral pathway of somatosensory processing (Preusser et al., 2015). This would suggest that increas-

ing depth of propofol-induced anaesthesia is associated with a breakdown of the ventral pathway of nociceptive somatosensory processing and a subsequent establishment of a more anterior pathway. However, the hypothesis that the altered functional connectivity is responsible for the altered pattern of evoked brain activity is speculative at the moment, as from our data we cannot conclude whether the altered functional connectivity drives the changes of altered evoked activity or the other way round, or whether both are the epiphenomenon of an underlying process.

Increasing propofol doses have been described to cause a gradual attenuation and disruption of the processing of other sensory modalities such as auditory, visual and tactile stimuli (MacDonald et al., 2015), first affecting higher-order cortices associated with more complex integrative processing and at higher doses resulting in the impairment of primary sensory cortices. We found a gradual breakdown of functional connectivity between nociceptive regions and an accompanying dose-dependent disruption of evoked activity patterns. However, we additionally found that high doses of propofol recruited the ACC, a brain region implicated in higher-order cognitive processes and part of the aforementioned anterior group, to the processing of intense noxious stimuli. As the dose-dependent activation of the ACC was concordant with an increasing EEG burst suppression ratio – an indicator of the deepest levels of anaesthesia reached in clinical practice and traditionally seen as a sign of an inactived brain – the nociceptive activation of the ACC as observed in BOLD-fMRI might rather be a consequence of the breakdown of physiological nociceptive processing pathways and consolidation of the aforementioned potential "anterior pathway", than a reintegration of the ACC in the standard pathway. We therefore argue that despite the nociceptive activation of the ACC at deep anaesthesia, propofol induces a gradual breakdown of nociceptive processing as has been shown for other stimulus modalities.

In conclusion, propofol dose-dependently induces a gradual attenuation and disruption of the processing of noxious stimuli. As propofol has a strong attenuating effect on spinal nociceptive processing, moderate noxious stimuli, as have been used by previous studies, are not appropriate for the investigation of cerebral effects of propofol. In contrast, intense noxious stimuli are not further attenuated at the spinal level during unconsciousness and evoke profound cerebral activations even during very deep anaesthesia.

3.1.2. Dose-dependent effects of remifentanil

Using the opioid remifentanil in addition to propofol (**study 2**), we observed a gradual decrease of nociceptive-evoked activity in the spinal cord and the brain, including a decreased functional connectivity between areas responsive to noxious stimuli, but not a restructured cerebral pattern of nociceptive processing as in deepening levels of propofol anaesthesia. It has to be noted that a parametric contrast across remifentanil concentration levels showed significant decreases of cerebral responses to noxious stimuli only in frontal brain regions. However, the lack of statistically significant decreases in the other nociceptive brain regions might be due to our rather small sample sizes and must therefore be interpreted cautiously.

The dose-dependent reduction of cortical activity is consistent with the inhibitory action of remifentanil on both spinal cord neurons and on arousal-promoting nuclei that send excitatory projections to the thalamus and the cortex (cf. Sec. 1.3.4). Our findings are also in line with previous studies using low-dose remifentanil in conscious subjects that found a dose-dependent reduction of nociceptive activation (Wise et al., 2002, 2004). However, different from previous findings that showed that responses in the anterior insula are absent already at low doses of remifentanil while responses in the posterior insula were dose-dependently attenuated (Oertel et al., 2008), we could detect significant responses in the anterior insula even at high doses of remifentanil. This might be again attributed to different stimulus intensities, as moderate noxious stimuli (as were used by Oertel et al.) are attenuated already at the spinal level, while the intense noxious stimuli at intensities comparable to surgical stimuli that we used were intense enough to evoke responses in higher-order cerebral areas.

As remifentanil dose-dependently also attenuated the spinal processing of intense noxious stimuli, we cannot conclude whether attenuated cerebral nociceptive processing is caused by cerebral effects of remifentanil or by reduced spinal input. As it was suggested that remifentanil activates the endogenous pain inhibitory system, the reduction of spinal nociceptive processing could be the effect of both direct spinal and indirect cerebral effects of remifentanil (cf. Sec. 1.2.2). However, our finding that the functional connectivity between brain areas responsive to noxious stimuli was also reduced dose-dependently advocates for a cerebral effect of remifentanil, as the stimulus-independent functional connectivity should not be affected by spinal inhibitory effects of remifentanil.

3.1.3. Comparison of the effects of propofol and remifentanil

We found distinct dose-dependent effects of propofol and remifentanil on cerebral nociceptive processing. Increasing doses of propofol changed the cerebral response patterns to noxious stimuli, revealing distinct groups of brain regions in which the responses to noxious stimuli either increased, decreased or remained unchanged with increasing propofol concentration. This is consistent with the notion that unconsciousness, which is the main effect of the hypnotic drug propofol, is induced by altered information transfer and integration pathways of the brain (cf. Sec. 1.3.3). In contrast, increasing remifentanil doses primarily attenuated the cerebral responses of noxious stimuli in all nociceptive brain regions and did not seem to recruit other brain regions to nociceptive processing at increasing doses as was the case for propofol.

A key difference between our findings from propofol mono-anaesthesia and joint propofol/remifentanil general anaesthesia was the profound activation of the anterior cingulate cortex (ACC) at higher levels of propofol anaesthesia, but not during any level of remifentanil analgesia. Activation of the ACC during deep propofol anaesthesia is intriguing as the ACC is a higher-order cortex that is – in addition to its known role in pain processing – associated with cognitive processes such as memory, attention, cognitive control and emotion (Wager et al., 2016) and is generally considered to function in an affective component of pain perception (Rainville et al., 1997; Wiech, 2016). Although it is not possible to deduce any precise cognitive process from activation of the ACC – as due to its described non-specificity this reverse inference is invalid – and despite our lack of certainty whether activation during deep general anaesthesia even indicates any kind of directed information processing towards a specific goal, it is still an unusual finding to see an association cortex activated by noxious stimulation during such deep anaesthesia. Even more so as the ACC is known to play an important role in chronic pain through synaptic learning processes called long-term potentiation (LTP). A special form of LTP is also implied in the generation and maintenance of pain-related anxiety and fear memory (Bliss et al., 2016). Persistent nociceptive activation during general anaesthesia could trigger the same learning processes and thereby contribute to the development of chronic pain (cf. Sec. 3.2.1). However, it has to be noted that propofol and other $GABA_A$-agonistic anaesthetics have been shown to inhibit LTP, which might attenuate nociception-induced synaptic learning processes (Nagashima et al., 2005).

3.1.4. The nociception matrix

The "pain matrix" is defined as a variety of brain regions that are consistently activated by noxious stimuli in awake subjects (cf. Sec. 1.2.1; Fig. 3.1, top row). As during general anaesthesia the nociceptive-evoked activation patterns changed dose-dependently and were different from the pain matrix of awake subjects, we generated an analogous "nociception matrix" from our data, consisting of all regions that were activated by intense noxious stimuli vs innocuous stimuli during any concentration level of general anaesthesia using propofol and remifentanil (Fig. 3.1, middle row). In this way, we intended to generate a single nociception matrix that captures all brain regions activated during any concentration level of propofol and remifentanil, instead of generating separate differential nociception matrices for every concentration level of propofol and remifentanil.

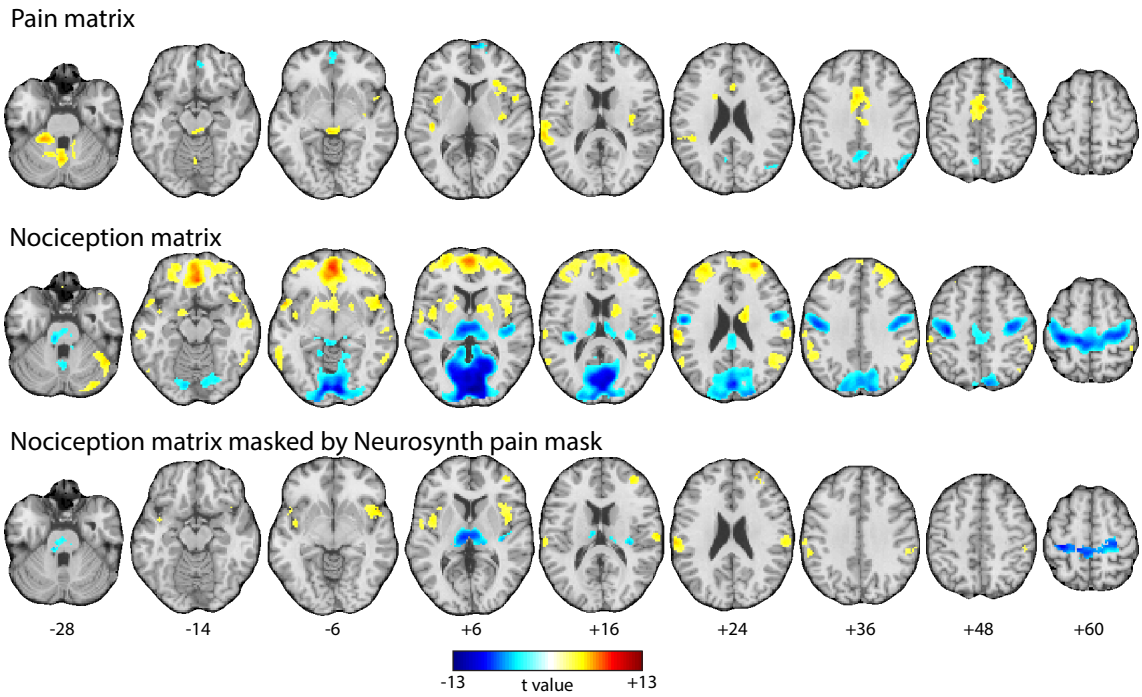

Figure 3.1: Nociceptive brain activations in awake subjects and during general anaesthesia. Shown are the brain activations by moderate noxious vs innocuous stimuli in awake subjects ("pain matrix"; top row), the average activations by intense noxious vs innocuous stimuli during general anaesthesia at all remifentanil concentration levels ("nociception matrix", middle row) and the nociception matrix masked by the Neurosynth pain mask derived from a search for the term "pain" in the Neurosynth meta-analytic database (www.neurosynth.org; corrected at an FDR of 0.01; Yarkoni et al., 2011). Pain and nociception matrices are thresholded using an uncorrected voxel threshold of $p < 0.001$ and a family-wise error corrected cluster threshold of $p < 0.05$ (two-sided). Analyses were performed as described in Lichtner et al. (2018b).

The pain matrix and the nociception matrix from our data are not directly comparable, as the former was generated using moderate noxious stimuli while the latter was generated using intense noxious stimuli, which were not administered to the awake subjects as they would not have been endurable. Indeed, the activation from both conditions shows little overlap with each other, which could be caused either by the difference of mental states (awake vs general anaesthesia) or stimulus intensity (moderate vs intense noxious) or a synergistic combination of both. When comparing the nociception matrix with a mask derived from the Neurosynth meta-analytic database using the search term "pain" (Yarkoni et al., 2011), it can be seen that several areas of the nociception matrix are commonly found activated in pain-related studies (Fig. 3.1, bottom row). These areas include the insular cortices, the putamen, the S2 and the pre/-postcentral gyri including S1. Overall, the overlap between the nociception matrix and the meta-analytic "pain mask" reinforces the validity of the concept of the nociception matrix.

The Neurosynth meta-analytic pain mask was also used by Wager and colleagues to define the brain regions from which they generated the neurologic pain signature (NPS). The NPS is essentially a vector that defines a weight for each voxel within the pain mask. The scalar product between this weight vector and the voxel activation vector yields a single number (the *NPS response*) that correlates with perceived pain intensity in awake subjects (cf. Sec. 1.2.4). Although the NPS has been shown to be modulated by remifentanil at a concentration similar to the lowest that we used in our study (Zunhammer et al., 2018), it is not known whether this way of condensing BOLD-fMRI data into a single number is also indicative of nociception during general anaesthesia. I have therefore determined the responses to the innocuous and noxious stimuli from our studies using the NPS weight vector kindly provided by Tor Wager (Fig. 3.2). It can be seen that the NPS reliably separated innocuous from moderate noxious stimuli in awake subjects and at mild propofol-induced sedation ($p<0.0001$ for each; Dunnett's *post hoc* tests to a 2×5 (stimulus intensities $\times$ propofol concentration level) repeated measures analysis of variance (RM-ANOVA)). After the loss of consciousness, no significant responses to either the moderate noxious or the intense noxious stimuli compared to the innocuous control stimuli can be detected for any level of propofol or remifentanil anaesthesia ($p>0.05$ for each; Dunnett's *post hoc* tests to RM-ANOVAs). However, the intense noxious stimuli consistently resulted in higher NPS responses than the innocuous or moderate noxious stimuli during all levels of propofol and remifentanil anaesthesia.

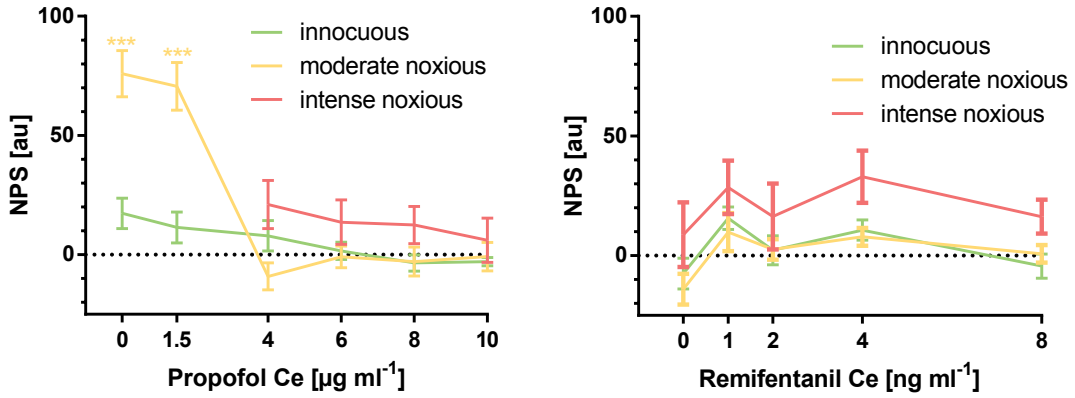

Figure 3.2: The neurologic pain signature during general anaesthesia. Shown are the responses of the neurologic pain signature (NPS) to innocuous (green), moderate noxious (yellow) and intense noxious (stimuli) during the propofol mono-anaesthesia (left) and the propofol-remifentanil anaesthesia runs (right). Asterisks indicate significant differences between noxious stimuli and innocuous control stimuli (***: $p < 0.0001$; Dunnett's *post hoc* tests to a 2×5 (stimulus intensities $\times$ propofol concentration level) RM-ANOVA). au, arbitrary units; Ce, effect-site concentration.

In contrast to our analysis from **study 2**, in which we quantified nociceptive cerebral activation by unweighted averaging of the BOLD-fMRI voxel responses in the brain regions of the nociception matrix, the NPS uses a weighted averaging of voxel responses within the pain mask. Thus, the statistically not significant discrimination between noxious and innocuous stimuli of the NPS response might be caused either (i) by the *a priori* exclusion of brain areas that are not associated with pain processing in awake subjects, but that are activated by noxious stimuli during general anaesthesia (i. e. those regions of the nociception matrix that are excluded by the pain mask; cf. Fig. 3.1 middle and bottom row), or (ii) because nociceptive cerebral connectivity and processing is largely altered by general anaesthetics in a way that is not anymore captured by the NPS weights or (iii) because the NPS captures the conscious integration of nociceptive activation that forms the perception of pain and that is therefore not present during unconsciousness. Nevertheless, the correspondence between the NPS responses and the amplitude of the spinal measure of nociception (the NFR), as well as the stronger NPS responses to intense noxious compared to moderate noxious or innocuous stimuli during general anaesthesia – although not statistically significant – are evidence that the NPS might be a measure of cerebral nociception even during unconsciousness. Additionally, it reinforces the notion that a cerebral neural signature of nociception during general anaesthesia can be established.

3.1.5. Differences between the pain matrix and the nociception matrix

Several brain regions that we identified in the nociception matrix are not usually part of the pain matrix of conscious subjects. The orbitofrontal cortex (OFC) that we have seen profoundly activated in our nociception matrix in the propofol/remifentanil anaesthesia data and also during higher levels of propofol mono-anaesthesia, is not usually implied in pain processing. However, it has been shown that the responses to noxious stimuli in the OFC and other areas that are associated with reward processing are predictive of the magnitude of opioid analgesia (Wanigasekera et al., 2012). Thus, the OFC, which is also densely populated by opioid receptors (Rabiner et al., 2011), may play a role in endogenous opioidergic modulation of nociceptive processing (Lee et al., 2014), which might still be its function during general anaesthesia. Interestingly, the OFC together with another region of the nociception matrix, the right angular gyrus, is part of the default mode resting state network (Raichle et al., 2001), which has been suggested to be disrupted during acute pain stimulation (Alshelh et al., 2018) as well as functionally reorganised in chronic pain conditions (Baliki et al., 2014).

In addition to cerebral activations by noxious stimuli, we also found profound deactivations during general anaesthesia, mainly in the calcarine sulcus and in the bilateral pre- and postcentral gyri. Deactivations are not often investigated in pain-related studies and their interpretation is difficult (Kong et al., 2010). However, they might indicate increased propofol-induced inhibition in these regions mediated by local GABAergic inhibitory neurons (Gómez et al., 2013), or reduced task-related activation due to decreased inputs from distant projection neurons (Gusnard and Raichle, 2001).

The calcarine sulcus of the occipital cortex is usually not reported in pain-evoked brain activations or deactivations and thus its role remains elusive. However, it has been previously shown to be deactivated (Iannetti et al., 2005) or activated (Kong et al., 2010) by painful stimuli, and its activation was found in one study to correlate negatively with pain intensity ratings (Coghill et al., 1999). Additionally, propofol has been shown to selectively decrease cerebral blood flow in the occipital cortex (Fiset et al., 1999). Thus, the propofol-induced blood flow decreases of this area might, at least in part, contribute to the stimulus-evoked profound reductions seen in our data.

3.2. Persistent nociception during general anaesthesia

In the main experimental part of this thesis, we have established that cerebral nociceptive processing persists even during a general anaesthesia that is deeper than used in current clinical practice. This was not obvious prior to our study, as there has not been any other study before which investigated nociceptive processing during deep general anaesthesia – mimicking both clinical anaesthetic depth and surgical noxious stimuli. This immediately raises the question of whether the nociceptive cerebral processing that we have detected has any relevant effects on patient outcomes, such as the development of chronic pain. As BOLD-fMRI is an indirect measure of neuronal activity, the activations detected by this method, especially during pharmacological modulation, do not imply any form of (higher) cognitive processing. Indeed, it is possible that only the different brain regions acting in concert give rise to purposeful physiological effects and that it is precisely this integrative action of the brain that is disrupted during drug-induced unconsciousness. We therefore investigated whether the level of intraoperative anti-nociception induced by analgesic drug doses comparable to those used in our experimental study was associated with persistent postoperative pain in a clinical study. As this was indeed the case, persistent intraoperative nociception seems to influence patient outcomes adversely. In this section, it will therefore be discussed by which neuronal, physiological and psychological mechanisms nociception actually or potentially contributes to adverse patient outcomes such as chronic pain.

3.2.1. Effects on patient outcomes

Preoperative and acute postoperative pain is associated with pain chronification (Kehlet et al., 2006), but it remains unclear whether intraoperative nociception also contributes to the development of chronic pain. To explore this hypothesis, we performed a clinical study in 110 patients undergoing major surgery and indeed found evidence that the levels of intraoperative analgesia are associated with persistent pain six months after the surgery (**study 3**). The main reason why this potential association remains largely uninvestigated is that there exists no validated measure to quantify intraoperative nociception (Borsook et al., 2010). However, the lack of experimental and observational evidence for adverse effects of intraoperative nociception cannot be taken as evidence against these effects. In contrast, it can be reasonably assumed that excessive intraoper-

ative nociception leads to adverse effects that range from the molecular to the network level of the CNS. Perioperative nociception is known to cause rapid neuronal sensitisation and altered gene expression (Latremoliere and Woolf, 2009; Borsook et al., 2010), leading to damages in the central nervous system (Costigan et al., 2009) as well as behavioural changes (Besson, 1999). The well investigated direct neuronal effects of sustained nociceptive stimulation that lead to a persistent, but usually fully reversible, facilitation and increased nociceptive sensitivity are called central sensitisation. Central sensitisation can result in the modulation of every aspect of pain perception (e. g. intensity, location, spatial extent), without actually reflecting a noxious input, but rather reflecting the functional efficacy of the central nociceptive pathways (Woolf, 2011). Thus, it might well be that central sensitisation contributes to the development of chronic pain. Accordingly, central sensitisation has been associated with clinical syndromes such as fibromyalgia, headache, neuropathic pain, postoperative pain and arthritis (Woolf, 2011).

Apart from these direct neuronal effects, intraoperative nociception also triggers the surgical stress response, which is a general activation of sympathetic/endocrine systems through afferent nociceptive inputs to the hypothalamus (Finnerty et al., 2013). The resulting elicitation of endocrine, metabolic and inflammatory responses regulates a variety of essential physiological systems in order to prevent damage, but maladaptive responses may lead to both short- and long-term harm (McEwen, 2000). These deleterious effects include impairment of wound healing (Akca et al., 1999) and immune function (Salo, 1992) and altered autoregulation of visceral organs (Liu et al., 1995). Stress responses may also lead to effects on the brain (Rodrigues et al., 2009) in areas such as the insula, the PFC and the rACC (Liberzon et al., 2007) and may ultimately result in psychiatric disorders such as depression (Vermetten and Bremner, 2002).

In conclusion, so far there has been little research on the association between the intraoperative nociception/anti-nociception balance and postoperative deleterious effects, which is a consequence of the unavailability of reliable measures for the nociception/ anti-nociception balance during general anaesthesia. Nevertheless, as discussed above, a profound and plausible basis for deleterious outcome effects caused by intraoperative nociception exists. Accordingly, we could show in our clinical studies for the first time, to the best of our knowledge, that the intraoperative anti-nociceptive level is associated with persistent postoperative pain.

3.2.2. Chronic pain as a consequence of intraoperative nociception

Chronic pain is a major clinical and economic problem affecting a substantial proportion of the population (Breivik et al., 2006; Nahin, 2015; Fayaz et al., 2016). Pain chronification involves all levels of the nociceptive circuitry from the molecular up to the network level (Sandkühler, 2009; Prescott et al., 2014). In the spinal cord, synaptic plasticity in the form of LTP contributes to chronic pain (Sandkühler and Liu, 1998). In the brain, enhanced nociceptive neurotransmission mediated via synaptic plasticity was found in the thalamus (Zhao et al., 2006), the amygdala (Ikeda et al., 2007), the insula (Qiu et al., 2013), in the S1/S2 (Eto et al., 2011), in the PFC (Metz et al., 2009) and in the ACC (Bliss et al., 2016). On the cerebral level, changes within multiple brain regions are associated with chronic pain, including the anterior insula, the ACC, the BG, the thalamus, the PAG and the pre- and postcentral gyri (Cauda et al., 2014). Intriguingly, these affected regions overlap with the regions that we found responsive to intense noxious during general anaesthesia. On the network level, pain chronification was shown to be associated with a shift of the cerebral representation of pain from areas related to acute pain processing to areas implied in emotional processing (Hashmi et al., 2013).

Chronic pain is characterised by the detachment of pain perception from obvious nociceptive stimuli. It is commonly assumed that pain perception in this case is mediated through enhanced excitability of the nociceptive circuitry. However, leveraging contemporary theories of perception – the Bayesian perspective of predictive coding – it is conceivable that pain perception in the presence of innocuous stimuli is the result of a higher expectancy of pain (i.e. prior probability of pain) in conjunction with a previously learned association that pain perception involves the respective innocuous stimuli (i.e. likelihood, the probability of the innocuous stimulus given pain; Wiech et al., 2014; Hechler et al., 2016). As the resultant expected pain (i.e. the posterior probability of pain given the innocuous stimulus) does not match the actual sensory processing, it was hypothesised that chronic pain patients bring expectation and sensation into accordance by initiating actions that increase the sensory input, thereby actively creating the perception of pain – a mechanism called *active inference* (Hechler et al., 2016).

In conclusion, chronic pain is accompanied by changes in both the spinal and cerebral nociceptive circuitry ranging from the synaptic up to the cerebral network level. Additionally, maladaptive perceptual decision making might contribute to pain persistence.

3.2.3. Clinical surrogate measures of intraoperative nociception

In the experimental part of the thesis, we have successfully used a multimodal setup to assess nociception during general anaesthesia in an experimental setting. However, for clinical practice, a much simpler measure of nociception has to be developed that can be used to adjust anaesthetic and analgesic dosing in unconscious patients in order to improve the patient outcomes. An fMRI-based method is much too complex to be used in clinical practice and can only be used to validate such simpler measures of the nociception/anti-nociception balance. No such measure that can be reliably used in clinical practice exists to this day (Gruenewald and Ilies, 2013; von Dincklage, 2015).

Therefore, we have investigated as a supplementary work whether two nociceptive reflexes – the nociceptive flexion reflex (NFR) and the pupillary dilation reflex (PDR) – are valid measures of the nociception/anti-nociception balance, in the sense that they can indicate both anti-nociceptive under- as well as overdosing (**study 4**). While anti-nociceptive underdosing can be, at least to some extent, detected by the currently used clinical indicators of nociception (e. g. haemodynamic and body movement responses to noxious stimuli), no validated measure for detection of excessive anti-nociceptive dosing exists. We found that the PDR was associated with both anti-nociceptive under- and overdosing, while the NFR, at least in our setting, was only significantly associated with anti-nociceptive overdosing. As we have shown that the detection accuracy of the NFR can be greatly increased (**study 5**), one reason that might have contributed to the rather poor performance of the NFR in this study might be the application of the traditional, less accurate reflex detection procedure (Lichtner et al., 2015). In any case, both reflexes showed only a relatively low correlation with the surrogate measures of anti-nociceptive under- and overdosing.

In conclusion, although we could strengthen the notion that nociceptive reflexes are potential surrogate measures of the nociception/anti-nociception balance, their current stage of development does not commend their use in clinical routine. Importantly, as no better gold standard to assess nociception during general anaesthesia than clinical responses exists, the development of a comprehensive and reliable measure of nociception is required in order to (i) validate simpler surrogate measures and (ii) to investigate the clinical significance of persistent nociception on patient outcomes in the first place, which could then potentially be prevented by guided anti-nociceptive dosing.

3.3. Limitations

Several *caveats* have to be considered when interpreting the results from the studies on which this dissertation is based. Regarding the experimental studies on volunteer subjects using fMRI, EEG and spinal nociceptive reflexes, our sample size of ten subjects was relatively small. The sample size was based on previous studies that investigated nociception during general anaesthesia, but a formal sample size calculation could not be performed, as ours was the first study to investigate intense noxious stimuli at intensities comparable to noxious stimuli during deep general anaesthesia. Thus, we had to weigh the risk of performing measurements with potentially no information value against the not negligible risk for each volunteer participant, as each volunteer received an otherwise not necessary general anaesthesia in a highly complex setup with EEG, EMG, and electrical noxious stimulation inside the magnetic resonance imaging (MRI) scanner. It has to be kept in mind that the MRI scanner is a highly challenging site to perform a general anaesthesia due to the magnetic field prohibiting the use of many devices and the difficulties to reach the subject inside the scanner. However, the sample size was sufficiently large to investigate our main research hypothesis regarding the processing of intense noxious stimuli during different levels of general anaesthesia with statistical significance. Additionally, explicit reliability analyses of the random-effects model and fixed-effect analyses have shown strong evidence for the robustness of our reported results (Lichtner et al., 2018b).

Another limitation regarding the generalisability of the results from the experimental study is that we investigated only healthy young subjects who are not representative of the population. Thus, our results will have to be reproduced in follow-up studies in a clinical population in order to be generalised to all patients receiving general anaesthesia.

In our studies, we used transcutaneous electrical stimulation to deliver noxious stimuli to the subjects. In contrast to purely nociceptive stimuli such as painful heat, electrical stimuli also activate non-nociceptive nerve fibres and are thus not nociception-specific. However, real life noxious stimuli, such as those occurring during surgical interventions, are usually accompanied by non-nociceptive activations as well, potentially making electrical stimulation a more realistic model of those stimuli compared to pure noxious stimuli such as thermal stimuli.

In pharmacological fMRI studies such as ours, it has to be ensured that BOLD-fMRI activations are not confounded by pharmacological effects on the neurovascular-coupling, which is the physiologic basis of the BOLD-effect (Iannetti and Wise, 2007), especially as both propofol and remifentanil have been shown to influence the regional cerebral blood flow (Fiset et al., 1999; Wagner et al., 2001). In our study, we additionally recorded the EEG as a direct measure of cerebral nociceptive processing and the NFR as a measure of spinal nociception. The concordant dose-response characteristics in all measures of nociception during propofol/remifentanil general anaesthesia as well as the regional specificity of effects during different levels of propofol anaesthesia advocate for a common underlying effect rather than pharmacological effects on the BOLD-effect alone.

3.4. Towards a comprehensive measure of nociception in humans

Nociception is the neuronal processing of noxious stimuli that involves a plethora of neurons on several levels, ranging from the primary afferent neurons (the nociceptors) over the first interconnecting neurons in the spinal dorsal horn to the brainstem and to subcortical and cortical areas and even to downstream pathways emanating from cerebral areas. At each level, the processing and the transmission can be modulated by numerous influencing factors (e. g. sensitisation of peripheral neurons due to inflammation, anaesthetic drugs, differences in attention and mood). Thus, a truly complete **measure** of nociception would require to capture the neuronal activity at all of these levels.

As measuring neuronal activity *in vivo* is difficult, most of the currently available measures of nociception are not direct measures, but only surrogates of physiological responses to nociception. Such responses are conveyed at each level of nociceptive processing (e. g. nociceptive reflexes in the spinal cord, haemodynamic responses in the brainstem and in sympathetic ganglia, pain sensation in the brain). However, each of these responses are in turn susceptible to their own numerous influencing factors that can completely differ from those factors modulating the interneuronal transmission of noxious stimuli. Therefore, a truly comprehensive measure of nociception cannot be based solely on measures of physiological responses but requires measuring the actual neuronal activity. The most reliable and precise way of measuring neuronal activity is intracellular recording, which is clearly not feasible in humans. Therefore, non-invasive electrophysiological and neuroimaging techniques are the only available techniques.

In the experimental part of this thesis, we have developed and applied the first measure of nociceptive processing that is designed to measure neuronal activation instead of physiological responses and that included both spinal and cerebral measures. It can therefore be regarded as the currently most precise and comprehensive method of assessing spinal and cerebral nociception in humans that is technically and ethically feasible.

Using our setup, we have investigated the **mechanisms** of nociceptive processing during drug-induced general anaesthesia at different levels. We found that the hypnotic drug propofol and the analgesic drug remifentanil induced differential effects on different parts of the nociceptive pathway (Fig. 3.3). Propofol attenuated the processing of moderate noxious stimuli already at the spinal level but did not dose-dependently reduce spinal responses to intense noxious stimuli after the loss of consciousness. This can be explained by a threshold-increasing action of propofol at the spinal cord, whereby the intensity of the noxious stimuli that is required to elicit spinal and cerebral processing is increased by propofol. The intensity of the moderate noxious stimuli used in our experimental setup did not suffice to exceed that elevated threshold, while intense noxious stimuli were well beyond the threshold intensity and could therefore activate downstream recipients in the nociceptive pathway. On the cerebral level, increasing propofol concentration levels differentially changed the response amplitudes and the functional connectivity between nociceptive-processing cerebral regions. We hypothesise that during increasing anaesthetic depth, the processing of nociceptive information is directed away from temporal brain areas (ventral pathway) to more anterior brain areas (anterior pathway), which might contribute to the establishment and maintenance of a stable and deep unconsciousness (Fig. 3.3).

The opioid remifentanil induced a general but slight attenuation of spinal and cerebral responses to intense noxious stimuli. As a reduction on an upstream level of the nociceptive pathway (the spinal cord) can be expected to also reduce responses of downstream recipients (the brain), the attenuating action of remifentanil on the spinal level could contribute to the cerebral attenuation. However, we also found that remifentanil reduced the functional connectivity between nociceptive brain regions, which advocates for an additional direct cerebral effect of remifentanil on the reduction of brain connectivity. However, this might also be a consequence of an inhibiting effect of remifentanil on arousal-promoting nuclei in the brain and therefore not a direct effect of remifentanil on cortical regions.

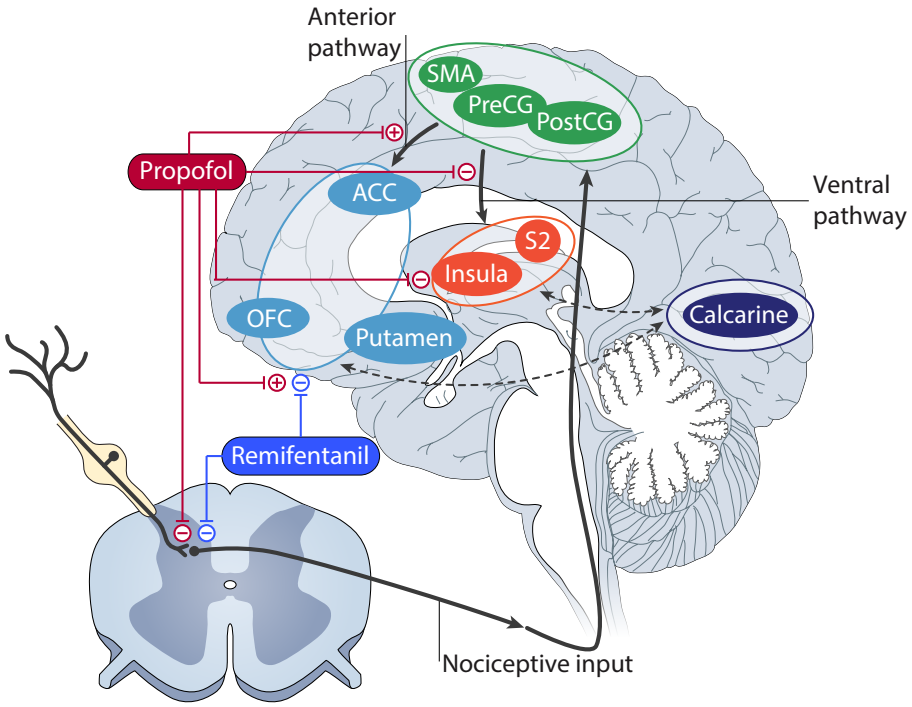

Figure 3.3: Putative mechanisms of propofol- and remifentanil-induced changes of nociceptive processing during general anaesthesia. Shown are the four groups of brain regions that we identified by their common within-group dose-response relationships and functional connectivities: The anterior group (blue), the central group (green), the temporal group (red) and the calcarine cortex (purple). Black arrows indicate the flow of nociceptive information. Plus and minus signs indicate an enhancing or attenuating effect induced by increasing doses of propofol (red) or remifentanil (blue). OFC, orbitofrontal cortex; ACC, anterior cingulate cortex; SMA, supplementary motor area; S2, secondary somatosensory cortex; PreCG, precentral gyrus; PostCG, postcentral gyrus.

Our findings of differential actions of hypnotic and analgesic drugs on the spinal cord and on different brain regions reiterate the aforementioned characteristics of the nociceptive system that all levels of nociceptive processing can be modulated differently by different influencing factors. In conclusion, we believe that our setup that combines fMRI, EEG and spinal reflex responses is a valid, reliable and the currently most precise measure for the measurement of nociceptive neuronal activity in humans.

Using our measurement setup to investigate mechanisms of nociceptive processing during general anaesthesia by assessment of nociceptive neuronal activation is of high scientific value. However, from a clinical standpoint, nociceptive neuronal activity alone has no clinical significance if it does not evoke any physiological responses that cause adverse **effects** in individuals. For instance, as during general anaesthesia the loss of consciousness precludes the perception of pain, nociceptive neuronal activation could be seen as

any other sensory stimulus modality (e. g. touch) unless it causes any further adverse effects. Thus, in the clinical context, only a measure of nociception that is able to indicate adverse patient outcomes caused by physiological responses to noxious stimuli has any relevance. In current clinical practice, body movement responses to noxious stimuli are the gold standard to indicate insufficient anti-nociception, which, as we have shown in our studies, are not indicative of persisting spinal and cerebral nociceptive processing at clinically relevant anaesthetic depths. As we could show in our clinical study that the level of intraoperative anti-nociception correlates with persistent postoperative pain, we believe that persisting intraoperative nociception that does not evoke acute clinically visible responses might foster the development of chronic pain through neuronal learning processes in the spinal cord in the brain such as central sensitisation and long-term potentiation. The nociceptive activation that might cause these direct neuronal effects cannot be indicated by current clinical measures of insufficient anti-nociception but might well be assessable using the setup developed and validated in this work. In conclusion, our setup allows to extend the diagnostic spread of nociceptive responses in humans and might allow to accurately assess nociceptive activation that causes adverse effects on patient outcomes (Fig. 3.4).

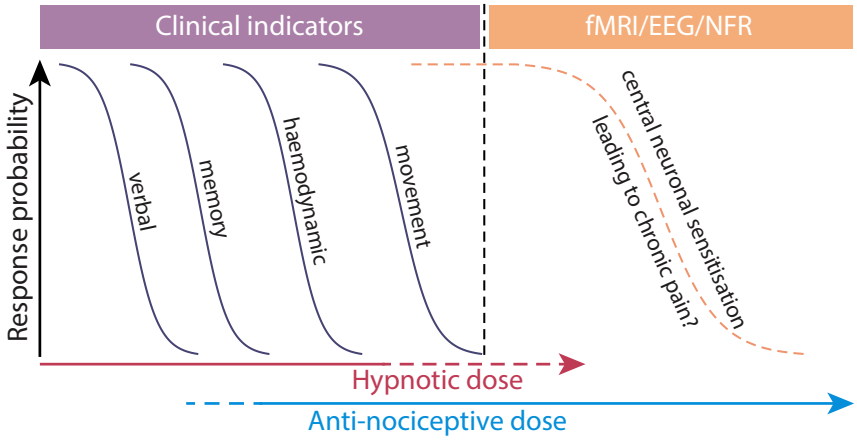

Figure 3.4: The effect of the anaesthetic depth on the probability of responses to noxious stimulation. Shown are the probabilities of well-known responses to noxious stimulation in dependence of the level of anaesthesia and anti-nociception (verbal, memory, haemodynamic and movement; solid purple lines) and their dependence on the hypnotic (red arrow) or anti-nociceptive (blue arrow) drug dose. Also displayed is the putative central neuronal sensitisation response (dashed orange line) to persisting nociception at levels that do not trigger any clinical responses and can therefore not be detected using current clinical indicators. However, these levels of persisting nociceptive processing might be detectable using the measurement setup developed in this work. fMRI, functional magnetic resonance imaging; EEG, electroencephalography; NFR, nociceptive flexion reflex.

3.5. Conclusion and outlook

In this work, we have demonstrated that a setup that combines fMRI, EEG and spinal nociceptive reflexes can be used to assess nociceptive processing during drug-induced unconsciousness. Using this setup, we could show for the first time that cerebral responses to noxious stimuli persist despite deep general anaesthesia that is considered clinically sufficient. Whether this level of persistent intra-anaesthetic nociception causes relevant effects on patient outcomes has not been investigated before. However, we have found evidence in our clinical study that low levels of anti-nociceptive dosing during general are indeed associated with high levels of persistent postoperative pain. This suggests that insufficiently suppressed nociception during general anaesthesia could give rise to the development of chronic pain.

To investigate this hypothesis – which has not been done so far due to the lack of validated assessment methods for nociception during unconsciousness – we plan to use our experimental setup to assess intraoperative nociception in patients immediately after actual surgery while they remain anaesthetised at the same level as during surgery and correlate these findings with immediate and persistent postoperative pain. The aim of that study is to investigate the possibility of generating a *neurologic signature of nociception*, by using machine learning techniques on intraoperative noxious stimulus-evoked fMRI, EEG and spinal reflex responses to develop a predictive model of immediate and persistent postoperative pain, similar to what has been done for acute pain perception (Wager et al., 2013). The development of such a signature would have multiple exquisite implications: (i) the possibility of generating this signature would provide a direct link between intraoperative nociceptive processing and postoperative pain, which has not yet been established, (ii) the signature itself would allow insights into the neural basis of that link via the weights that connect the single features (e. g. fMRI voxel activity, EEG and NFR amplitudes) with the prediction output of the signature, (iii) it would allow validating simpler surrogate measures of the signature response that can be actually applied in clinical practice and (iv) these surrogate measures would then allow to carefully titrate anti-nociceptive dosing during general anaesthesia, thereby potentially improving patient outcomes across the board. Additionally, assessing spinal intraoperative nociception via spinal fMRI could be used to unravel the spinal contribution to nociception

and its interplay with cerebral processing during general anaesthesia (Wheeler-Kingshott et al., 2014; Paquette et al., 2018).

This clinical line of research aiming at investigating the external validity of our findings would ideally be supplemented by an animal line of research in rodents, in which the nociception-specificity of brain activation during general anaesthesia can be validated by simultaneous fMRI and electrophysiological measurements of nociception-specific neurons (Jonckers et al., 2015). And even simpler model organisms could be used for investigating the genetic and molecular constituents and mechanisms of nociception during general anaesthesia (Tracey et al., 2003; Karunanithi et al., 2018).

In conclusion, our studies have paved the way for the investigation and generation of assessment measures of nociception during unconsciousness, showing for the first time that intraoperative cerebral nociceptive processing persists and might be associated with postoperative pain. Future studies will have to closely investigate this potential association and the possibility of preventing adverse effects of patient outcomes through patient- and stimulus-adjusted anti-nociceptive dosing that is based on a surrogate measure of the intraoperative nociception/anti-nociception balance validated by a comprehensive neuroimaging-based measure of nociception.

List of abbreviations

ACC	Anterior cingulate cortex
BG	Basal ganglia
BOLD	Blood-oxygen-level-dependent
CNS	Central nervous system
DRG	Dorsal root ganglion
EEG	Electroencephalography
EMG	Electromyography
fMRI	Functional magnetic resonance imaging
GABA	γ-aminobutyric acid
$GABA_A$	γ-aminobutyric acid type A
LTP	Long-term potentiation
MRI	Magnetic resonance imaging
NFR	Nociceptive flexion reflex
NFRT	Nociceptive flexion reflex threshold
NMDA	N-Methyl-D-aspartic acid
NPS	Neurologic pain signature
NRS	Numerical rating scale
OFC	Orbitofrontal cortex
PAG	Periaqueductal grey
PCC	Posterior cingulate cortex
PDR	Pupillary dilation reflex
PDRT	Pupillary dilation reflex threshold
PFC	Prefrontal cortex
rACC	Rostral anterior cingulate cortex
RM-ANOVA	Repeated measures analysis of variance
RVM	Rostral ventromedial medulla
S1	Primary somatosensory cortex
S2	Secondary somatosensory cortex
SMA	Supplementary motor area
SSEP	Somatosensory evoked potential

References

Akca, O., Melischek, M., Scheck, T., Hellwagner, K., Arkiliç, C. F., Kurz, A., Kapral, S., Heinz, T., Lackner, F. X., and Sessler, D. I. (1999). Postoperative pain and subcutaneous oxygen tension. *Lancet*, 354(9172):41–42.

Alkire, M. T., Haier, R. J., Barker, S. J., Shah, N. K., Wu, J. C., and Kao, J. Y. (1995). Cerebral Metabolism during Propofol Anesthesia in Humans Studied with Positron Emission Tomography. *Anesthesiology*, 82(2):393–403.

Alkire, M. T., Hudetz, A. G., and Tononi, G. (2008). Consciousness and Anesthesia. *Science*, 322(5903):876–880.

Alshelh, Z., Marciszewski, K. K., Akhter, R., Di Pietro, F., Mills, E. P., Vickers, E. R., Peck, C. C., Murray, G. M., and Henderson, L. A. (2018). Disruption of default mode network dynamics in acute and chronic pain states. *NeuroImage Clin.*, 17:222–231.

American Society of Anesthesiologists (2002). Practice guidelines for sedation and analgesia by non-anesthesiologists. *Anesthesiology*, 96(4):1004–1017.

Antognini, J. F. and Carstens, E. (2002). In vivo characterization of clinical anaesthesia and its components. *Br. J. Anaesth.*, 89(1):156–166.

Apfel, C. C., Heidrich, F. M., Jukar-Rao, S., Jalota, L., Hornuss, C., Whelan, R. P., Zhang, K., and Cakmakkaya, O. S. (2012). Evidence-based analysis of risk factors for postoperative nausea and vomiting. *Br. J. Anaesth.*, 109(5):742–753.

Apkarian, A. V., Bushnell, M. C., Treede, R.-D., and Zubieta, J.-K. (2005). Human brain mechanisms of pain perception and regulation in health and disease. *Eur. J. Pain*, 9(4):463–484.

Arendt-Nielsen, L. and Chen, A. C. N. (2003). Lasers and other thermal stimulators for activation of skin nociceptors in humans. *Clin. Neurophysiol.*, 33(6):259–268.

Baliki, M. N. and Apkarian, A. V. (2015). Nociception, Pain, Negative Moods, and Behavior Selection. *Neuron*, 87(3):474–491.

Baliki, M. N., Mansour, A. R., Baria, A. T., and Apkarian, A. V. (2014). Functional Reorganization of the Default Mode Network across Chronic Pain Conditions. *PLoS One*, 9(9):e106133.

Basbaum, A. I., Bautista, D. M., Scherrer, G., and Julius, D. (2009). Cellular and Molecular Mechanisms of Pain. *Cell*, 139(2):267–284.

Besson, J. M. (1999). The neurobiology of pain. *Lancet*, 353(9164):1610–1615.

Bliss, T. V. P., Collingridge, G. L., Kaang, B.-K., and Zhuo, M. (2016). Synaptic plasticity in the anterior cingulate cortex in acute and chronic pain. *Nat. Rev. Neurosci.*, 17(8):485–496.

Bonhomme, V., Fiset, P., Meuret, P., Backman, S., Plourde, G., Paus, T., Bushnell, M. C., and Evans, A. C. (2001). Propofol Anesthesia and Cerebral Blood Flow Changes Elicited by Vibrotactile Stimulation: A Positron Emission Tomography Study. *J. Neurophysiol.*, 85(3):1299–1308.

Borsook, D., George, E., Kussman, B., and Becerra, L. (2010). Anesthesia and perioperative stress: Consequences on neural networks and postoperative behaviors. *Prog. Neurobiol.*, 92(4):601–612.

Boveroux, P., Vanhaudenhuyse, A., Bruno, M.-A., Noirhomme, Q., Lauwick, S., Luxen, A., Degueldre, C., Plenevaux, A., Schnakers, C., Phillips, C., Brichant, J.-F., Bonhomme, V., Maquet, P., Greicius, M. D., Laureys, S., and Boly, M. (2010). Breakdown of within- and between-network Resting State Functional Magnetic Resonance Imaging Connectivity during Propofol-induced Loss of Consciousness. *Anesthesiology*, 113(5):1038–1053.

Breivik, H., Collett, B., Ventafridda, V., Cohen, R., and Gallacher, D. (2006). Survey of chronic pain in Europe: Prevalence, impact on daily life, and treatment. *Eur. J. Pain*, 10(4):287–287.

Brown, E. N., Lydic, R., and Schiff, N. D. (2010). General Anesthesia, Sleep, and Coma. *N. Engl. J. Med.*, 363(27):2638–2650.

Brown, E. N., Purdon, P. L., and Van Dort, C. J. (2011). General Anesthesia and Altered States of Arousal: A Systems Neuroscience Analysis. *Annu. Rev. Neurosci.*, 34(1):601–628.

Bushnell, M. C., Čeko, M., and Low, L. A. (2013). Cognitive and emotional control of pain and its disruption in chronic pain. *Nat. Rev. Neurosci.*, 14(7):502–511.

Cauda, F., Palermo, S., Costa, T., Torta, R., Duca, S., Vercelli, U., Geminiani, G., and Torta, D. M. E. (2014). Gray matter alterations in chronic pain: A network-oriented meta-analytic approach. *NeuroImage Clin.*, 4:676–686.

Cheng, Y., Chen, C., Lin, C.-P., Chou, K.-H., and Decety, J. (2010). Love hurts: An fMRI study. *NeuroImage*, 51(2):923–929.

Coghill, R. C., Sang, C. N., Maisog, J. M., and Iadarola, M. J. (1999). Pain Intensity Processing Within the Human Brain: A Bilateral, Distributed Mechanism. *J. Neurophysiol.*, 82(4):1934–1943.

Colon, E., Bittner, E. A., Kussman, B., McCann, M. E., Soriano, S., and Borsook, D. (2017). Anesthesia, brain changes, and behavior: Insights from neural systems biology. *Prog. Neurobiol.*, 153:121–160.

Costigan, M., Scholz, J., and Woolf, C. J. (2009). Neuropathic Pain: A Maladaptive Response of the Nervous System to Damage. *Annu. Rev. Neurosci.*, 32(1):1–32.

Cowen, R., Stasiowska, M. K., Laycock, H., and Bantel, C. (2015). Assessing pain objectively: The use of physiological markers. *Anaesthesia*, 70(7):828–847.

Davis, K. D. (2011). Neuroimaging of pain: What does it tell us? *Curr. Opin. Support. Palliat. Care*, 5(2):116.

Davis, K. D., Flor, H., Greely, H. T., Iannetti, G. D., Mackey, S., Ploner, M., Pustilnik, A., Tracey, I., Treede, R.-D., and Wager, T. D. (2017). Brain imaging tests for chronic pain: Medical, legal and ethical issues and recommendations. *Nat. Rev. Neurol.*, 13(10):624–638.

Disbrow, E., Buonocore, M., Antognini, J., Carstens, E., and Rowley, H. A. (1998). Somatosensory cortex: A comparison of the response to noxious thermal, mechanical, and electrical stimuli using functional magnetic resonance imaging. *Hum. Brain Mapp.*, 6(3):150–159.

D'Mello, R. and Dickenson, A. H. (2008). Spinal cord mechanisms of pain. *Br. J. Anaesth.*, 101(1):8–16.

Eippert, F., Bingel, U., Schoell, E. D., Yacubian, J., Klinger, R., Lorenz, J., and Büchel, C. (2009). Activation of the Opioidergic Descending Pain Control System Underlies Placebo Analgesia. *Neuron*, 63(4):533–543.

Ellermeier, W. and Westphal, W. (1995). Gender differences in pain ratings and pupil reactions to painful pressure stimuli. *Pain*, 61(3):435–439.

Eto, K., Wake, H., Watanabe, M., Ishibashi, H., Noda, M., Yanagawa, Y., and Nabekura, J. (2011). Inter-regional Contribution of Enhanced Activity of the Primary Somatosensory Cortex to the Anterior Cingulate Cortex Accelerates Chronic Pain Behavior. *J. Neurosci.*, 31(21):7631–7636.

Fayaz, A., Croft, P., Langford, R. M., Donaldson, L. J., and Jones, G. T. (2016). Prevalence of chronic pain in the UK: A systematic review and meta-analysis of population studies. *BMJ Open*, 6(6):e010364.

Finnerty, C. C., Mabvuure, N. T., Ali, A., Kozar, R. A., and Herndon, D. N. (2013). The Surgically Induced Stress Response. *JPEN J. Parenter. Enteral Nutr.*, 37(5 suppl):21S–29S.

Fiset, P., Paus, T., Daloze, T., Plourde, G., Meuret, P., Bonhomme, V., Hajj-Ali, N., Backman, S. B., and Evans, A. C. (1999). Brain Mechanisms of Propofol-Induced Loss of Consciousness in Humans: A Positron Emission Tomographic Study. *J. Neurosci.*, 19(13):5506–5513.

France, C. R., Rhudy, J. L., and McGlone, S. (2009). Using normalized EMG to define the nociceptive flexion reflex (NFR) threshold: Further evaluation of standardized NFR scoring criteria. *Pain*, 145(1–2):211–218.

Franks, N. P. (2008). General anaesthesia: From molecular targets to neuronal pathways of sleep and arousal. *Nat. Rev. Neurosci.*, 9(5):370–386.

Frölich, M. A., Price, D. D., Robinson, M. E., Shuster, J. J., Theriaque, D. W., and Heft, M. W. (2005). The Effect of Propofol on Thermal Pain Perception. *Anesth. Analg.*, 100(2):481.

Garland, E. L. (2012). Pain Processing in the Human Nervous System: A Selective Review of Nociceptive and Biobehavioral Pathways. *Prim. Care*, 39(3):561–571.

Gebhart, G. F. (2004). Descending modulation of pain. *Neurosci. Biobehav. Rev.*, 27(8):729–737.

Gómez, F., Phillips, C., Soddu, A., Boly, M., Boveroux, P., Vanhaudenhuyse, A., Bruno, M.-A., Gosseries, O., Bonhomme, V., Laureys, S., and Noirhomme, Q. (2013). Changes in Effective Connectivity by Propofol Sedation. *PLoS One*, 8(8):e71370.

Gruenewald, M. and Ilies, C. (2013). Monitoring the nociception–anti-nociception balance. *Best Pract. Res. Clin. Anaesthesiol.*, 27(2):235–247.

Guglielminotti, J., Grillot, N., Paule, M., Mentré, F., Servin, F., Montravers, P., and Longrois, D. (2015). Prediction of Movement to Surgical Stimulation by the Pupillary Dilatation Reflex Amplitude Evoked by a Standardized Noxious Test. *Anesthesiology*, 122(5):985–993.

Guignard, B. (2006). Monitoring analgesia. *Best Pract. Res. Clin. Anaesthesiol.*, 20(1):161–180.

Guignard, B., Bossard, A. E., Coste, C., Sessler, D. I., Lebrault, C., Alfonsi, P., Fletcher, D., and Chauvin, M. (2000). Acute Opioid Tolerance: Intraoperative Remifentanil Increases Postoperative Pain and Morphine Requirement. *Anesthesiology*, 93(2):409–417.

Gusnard, D. A. and Raichle, M. E. (2001). Searching for a baseline: Functional imaging and the resting human brain. *Nat. Rev. Neurosci.*, 2(10):685–694.

Hashmi, J. A., Baliki, M. N., Huang, L., Baria, A. T., Torbey, S., Hermann, K. M., Schnitzer, T. J., and Apkarian, A. V. (2013). Shape shifting pain: Chronification of back pain shifts brain representation from nociceptive to emotional circuits. *Brain*, 136(9):2751–2768.

Hechler, T., Endres, D., and Thorwart, A. (2016). Why Harmless Sensations Might Hurt in Individuals with Chronic Pain: About Heightened Prediction and Perception of Pain in the Mind. *Front. Psychol.*, 7.

Heinricher, M. M., Morgan, M. M., Tortorici, V., and Fields, H. L. (1994). Disinhibition of off-cells and antinociception produced by an opioid action within the rostral ventromedial medulla. *Neuroscience*, 63(1):279–288.

Hofbauer, R. K., Fiset, P., Plourde, G., Backman, S. B., and Bushnell, M. C. (2004). Dose-dependent Effects of Propofol on the Central Processing of Thermal Pain. *Anesthesiology*, 100(2):386–394.

Hosobuchi, Y., Adams, J. E., and Linchitz, R. (1977). Pain relief by electrical stimulation of the central gray matter in humans and its reversal by naloxone. *Science*, 197(4299):183–186.

Hudetz, A. G. (2012). General Anesthesia and Human Brain Connectivity. *Brain Connect.*, 2(6):291–302.

Hudetz, A. G. and Mashour, G. A. (2016). Disconnecting Consciousness: Is There a Common Anesthetic End Point? *Anesth. Analg.*, 123(5):1228–1240.

Iannetti, G. D. and Wise, R. G. (2007). BOLD functional MRI in disease and pharmacological studies: Room for improvement? *Magn. Reson. Imaging*, 25(6):978–988.

Iannetti, G. D., Zambreanu, L., Wise, R. G., Buchanan, T. J., Huggins, J. P., Smart, T. S., Vennart, W., and Tracey, I. (2005). Pharmacological modulation of pain-related brain activity during normal and central sensitization states in humans. *Proc. Natl. Acad. Sci. U.S.A.*, 102(50):18195–18200.

Ikeda, R., Takahashi, Y., Inoue, K., and Kato, F. (2007). NMDA receptor-independent synaptic plasticity in the central amygdala in the rat model of neuropathic pain. *Pain*, 127(1):161.

Jakuscheit, A., Weth, J., Lichtner, G., Jurth, C., Rehberg, B., and von Dincklage, F. (2017). Intraoperative monitoring of analgesia using nociceptive reflexes correlates with delayed extubation and immediate postoperative pain: A prospective observational study. *Eur. J. Anaesthesiol.*, 34(5):297.

Jonckers, E., Shah, D., Hamaide, J., Verhoye, M., and Van der Linden, A. (2015). The power of using functional fMRI on small rodents to study brain pharmacology and disease. *Front. Pharmacol.*, 6.

Jordan, D., Ilg, R., Riedl, V., Schorer, A., Grimberg, S., Neufang, S., Omerovic, A., Berger, S., Untergehrer, G., Preibisch, C., Schulz, E., Schuster, T., Schröter, M., Spoormaker, V., Zimmer, C., Hemmer, B., Wohlschläger, A., Kochs, E. F., and Schneider, G. (2013). Simultaneous Electroencephalographic and Functional Magnetic Resonance Imaging Indicate Impaired Cortical Top–Down Processing in Association with Anesthetic-induced Unconsciousness. *Anesthesiology*, 119(5):1031–1042.

Julius, D. and Basbaum, A. I. (2001). Molecular mechanisms of nociception. *Nature*, 413(6852):203–210.

Karunanithi, S., Troup, M., and van Swinderen, B. (2018). Using Drosophila to Understand General Anesthesia: From Synapses to Behavior. In Eckenhoff, R. G. and

Dmochowski, I. J., editors, *Methods in Enzymology*, volume 602 of *Chemical and Biochemical Approaches for the Study of Anesthetic Function, Part A*, pages 153–176. Academic Press.

Kehlet, H., Jensen, T. S., and Woolf, C. J. (2006). Persistent postsurgical pain: Risk factors and prevention. *Lancet*, 367(9522):1618–1625.

Kemna, L. J. and Posse, S. (2001). Effect of Respiratory CO2 Changes on the Temporal Dynamics of the Hemodynamic Response in Functional MR Imaging. *NeuroImage*, 14(3):642–649.

Kennedy, D. and Norman, C. (2005). What don't we know? *Science*, 309(5731):75.

Kern, S. E., Xie, G., White, J. L., and Egan, T. D. (2004). A Response Surface Analysis of Propofol–Remifentanil Pharmacodynamic Interaction in Volunteers. *Anesthesiology*, 100(6):1373–1381.

Kim, S. H., Stoicea, N., Soghomonyan, S., and Bergese, S. D. (2014). Intraoperative use of remifentanil and opioid induced hyperalgesia/acute opioid tolerance: Systematic review. *Front. Pharmacol.*, 5.

Kong, J., Loggia, M. L., Zyloney, C., Tu, P., LaViolette, P., and Gollub, R. L. (2010). Exploring the brain in pain: Activations, deactivations and their relation:. *Pain*, 148(2):257–267.

Kragel, P. A., Koban, L., Barrett, L. F., and Wager, T. D. (2018). Representation, Pattern Information, and Brain Signatures: From Neurons to Neuroimaging. *Neuron*, 99(2):257–273.

Kuner, R. and Flor, H. (2017). Structural plasticity and reorganisation in chronic pain. *Nat. Rev. Neurosci.*, 18(1):20–30.

Långsjö, J. W., Salmi, E., Kaisti, K. K., Aalto, S., Hinkka, S., Aantaa, R., Oikonen, V., Viljanen, T., Kurki, T., Silvanto, M., and Scheinin, H. (2004). Effects of Subanesthetic Ketamine on Regional Cerebral Glucose Metabolism in Humans. *Anesthesiology*, 100(5):1065–1071.

Larson, M. D. (2008). Mechanism of opioid-induced pupillary effects. *Clin. Neurophysiol.*, 119(6):1358–1364.

Latremoliere, A. and Woolf, C. J. (2009). Central Sensitization: A Generator of Pain Hypersensitivity by Central Neural Plasticity. *J. Pain*, 10(9):895–926.

Lee, M. C., Wanigasekera, V., and Tracey, I. (2014). Imaging opioid analgesia in the human brain and its potential relevance for understanding opioid use in chronic pain. *Neuropharmacology*, 84:123–130.

Lee, U., Ku, S., Noh, G., Baek, S., Choi, B., and Mashour, G. A. (2013). Disruption of Frontal–Parietal Communication by Ketamine, Propofol, and Sevoflurane. *Anesthesiology*, 118(6):1264–1275.

Legrain, V., Iannetti, G. D., Plaghki, L., and Mouraux, A. (2011). The pain matrix reloaded: A salience detection system for the body. *Prog. Neurobiol.*, 93(1):111–124.

Liberzon, I., King, A. P., Britton, J. C., Phan, K. L., Abelson, J. L., and Taylor, S. F. (2007). Paralimbic and Medial Prefrontal Cortical Involvement in Neuroendocrine Responses to Traumatic Stimuli. *Am. J. Psychiatry*, 164(8):1250–1258.

Lichtner, G., Auksztulewicz, R., Kirilina, E., Velten, H., Mavrodis, D., Scheel, M., Blankenburg, F., and von Dincklage, F. (2018a). Effects of propofol anesthesia on the processing of noxious stimuli in the spinal cord and the brain. *NeuroImage*, 172:642–653.

Lichtner, G., Auksztulewicz, R., Velten, H., Mavrodis, D., Scheel, M., Blankenburg, F., and von Dincklage, F. (2018b). Nociceptive activation in spinal cord and brain persists during deep general anaesthesia. *Br. J. Anaesth.*, 121(1):291–302.

Lichtner, G., Golebiewski, A., Schneider, M. H., and von Dincklage, F. (2015). Introduction and validation of a less painful algorithm to estimate the nociceptive flexion reflex threshold. *Brain Res.*, 1608:147–156.

Lichtner, G., Hösl, T. M., Jakuscheit, A., Jurth, C., and von Dincklage, F. (2016). Optimizing Nociceptive Flexion Reflex (NFR) Scoring Criteria by Adjusting for Noise and Reflex Properties and Sampling Rate. *Clin. J. Pain*, 32(9):773–783.

Liu, S., Carpenter, R. L., and Neal, J. M. (1995). Epidural Anesthesia and Analgesia. Their Role in Postoperative Outcome. *Anesthesiology*, 82(6):1474–1506.

Liu, X., Lauer, K. K., Ward, B. D., Rao, S. M., Li, S.-J., and Hudetz, A. G. (2012). Propofol disrupts functional interactions between sensory and high-order processing of auditory verbal memory. *Hum. Brain Mapp.*, 33(10):2487–2498.

Loeser, J. D. and Treede, R.-D. (2008). The Kyoto protocol of IASP Basic Pain Terminology. *Pain*, 137(3):473–477.

MacDonald, A. A., Naci, L., MacDonald, P. A., and Owen, A. M. (2015). Anesthesia and neuroimaging: Investigating the neural correlates of unconsciousness. *Trends Cogn. Sci.*, 19(2):100–107.

Macintyre, P. E., Loadsman, J. A., and Scott, D. A. (2011). Opioids, ventilation and acute pain management. *Anaesth. Intensive Care*, 39(4):545–558.

Mason, P. (2012). Medullary circuits for nociceptive modulation. *Curr. Opin. Neurobiol.*, 22(4):640–645.

Matute, E., Rivera-Arconada, I., and López-García, J. A. (2004). Effects of propofol and sevoflurane on the excitability of rat spinal motoneurones and nociceptive reflexes in vitro. *Br. J. Anaesth.*, 93(3):422–427.

McEwen, B. S. (2000). The neurobiology of stress: From serendipity to clinical relevance11Published on the World Wide Web on 22 November 2000. *Brain Res.*, 886(1):172–189.

McMahon, S. B., Koltzenburg, M., Tracey, I., and Turk, D. (2013). *Wall & Melzack's Textbook of Pain*. Elsevier Health Sciences.

Merrill, D. R., Bikson, M., and Jefferys, J. G. R. (2005). Electrical stimulation of excitable tissue: Design of efficacious and safe protocols. *J. Neurosci. Methods*, 141(2):171–198.

Mertens, M. J., Olofsen, E., Engbers, F. H. M., Burm, A. G. L., Bovill, J. G., and Vuyk, J. (2003). Propofol Reduces Perioperative Remifentanil Requirements in a Synergistic MannerResponse Surface Modeling of Perioperative Remifentanil–Propofol Interactions. *Anesthesiology*, 99(2):347–359.

Metz, A. E., Yau, H.-J., Centeno, M. V., Apkarian, A. V., and Martina, M. (2009). Morphological and functional reorganization of rat medial prefrontal cortex in neuropathic pain. *Proc. Natl. Acad. Sci. U.S.A.*, 106(7):2423–2428.

Millan, M. J. (2002). Descending control of pain. *Prog. Neurobiol.*, 66(6):355–474.

Moayedi, M., Salomons, T. V., and Atlas, L. Y. (2018). Pain Neuroimaging in Humans: A Primer for Beginners and Non-Imagers. *J. Pain*, 19(9):961.e1–961.e21.

Nagashima, K., Zorumski, C. F., and Izumi, Y. (2005). Propofol Inhibits Long-term Potentiation but Not Long-term Depression in Rat Hippocampal Slices. *Anesthesiology*, 103(2):318–326.

Nahin, R. L. (2015). Estimates of Pain Prevalence and Severity in Adults: United States, 2012. *J. Pain*, 16(8):769–780.

Ní Mhuircheartaigh, R., Rosenorn-Lanng, D., Wise, R., Jbabdi, S., Rogers, R., and Tracey, I. (2010). Cortical and Subcortical Connectivity Changes during Decreasing Levels of Consciousness in Humans: A Functional Magnetic Resonance Imaging Study using Propofol. *J. Neurosci.*, 30(27):9095–9102.

Ní Mhuircheartaigh, R., Warnaby, C., Rogers, R., Jbabdi, S., and Tracey, I. (2013). Slow-Wave Activity Saturation and Thalamocortical Isolation During Propofol Anesthesia in Humans. *Sci. Transl. Med.*, 5(208):208ra148.

Nikolajsen, L., Brandsborg, B., Lucht, U., Jensen, T. S., and Kehlet, H. (2006). Chronic pain following total hip arthroplasty: A nationwide questionnaire study. *Acta Anaesthesiol. Scand.*, 50(4):495–500.

Oertel, B., Preibisch, C., Wallenhorst, T., Hummel, T., Geisslinger, G., Lanfermann, H., and Lötsch, J. (2008). Differential Opioid Action on Sensory and Affective Cerebral Pain Processing. *Clin. Pharmacol. Ther.*, 83(4):577–588.

Paquette, T., Jeffrey-Gauthier, R., Leblond, H., and PichÉ, M. (2018). Functional Neuroimaging of Nociceptive and Pain-Related Activity in the Spinal Cord and Brain: Insights From Neurovascular Coupling Studies. *Anat. Rec.*, 301(9):1585–1595.

Petrovic, P., Kalso, E., Petersson, K. M., and Ingvar, M. (2002). Placebo and Opioid Analgesia– Imaging a Shared Neuronal Network. *Science*, 295(5560):1737–1740.

Pilge, S., Jordan, D., Kreuzer, M., Kochs, E. F., and Schneider, G. (2014). Burst suppression-MAC and burst suppression-CP50 as measures of cerebral effects of anaesthetics. *Br. J. Anaesth.*, 112(6):1067–1074.

Porreca, F., Ossipov, M. H., and Gebhart, G. F. (2002). Chronic pain and medullary descending facilitation. *Trends Neurosci.*, 25(6):319–325.

Prescott, S. A., Ma, Q., and De Koninck, Y. (2014). Normal and abnormal coding of somatosensory stimuli causing pain. *Nat. Neurosci.*, 17(2):183–191.

Preusser, S., Thiel, S. D., Rook, C., Roggenhofer, E., Kosatschek, A., Draganski, B., Blankenburg, F., Driver, J., Villringer, A., and Pleger, B. (2015). The perception of touch and the ventral somatosensory pathway. *Brain*, 138(3):540–548.

Purdon, P. L., Sampson, A., Pavone, K. J., and Brown, E. N. (2015). Clinical Electroencephalography for Anesthesiologists: Part I: Background and Basic Signatures. *Anesthesiology*, 123(4):937–960.

Qiu, S., Chen, T., Koga, K., Guo, Y.-y., Xu, H., Song, Q., Wang, J.-j., Descalzi, G., Kaang, B.-K., Luo, J.-h., Zhuo, M., and Zhao, M.-g. (2013). An Increase in Synaptic NMDA Receptors in the Insular Cortex Contributes to Neuropathic Pain. *Sci. Signal.*, 6(275):ra34.

Rabiner, E. A., Beaver, J., Makwana, A., Searle, G., Long, C., Nathan, P. J., Newbould, R. D., Howard, J., Miller, S. R., Bush, M. A., Hill, S., Reiley, R., Passchier, J., Gunn, R. N., Matthews, P. M., and Bullmore, E. T. (2011). Molecular and functional neuroimaging of human opioid receptor pharmacology. *Mol. Psychiatry*, 16(8):785.

Raichle, M. E., MacLeod, A. M., Snyder, A. Z., Powers, W. J., Gusnard, D. A., and Shulman, G. L. (2001). A default mode of brain function. *Proc. Natl. Acad. Sci. U.S.A.*, 98(2):676–682.

Raij, T. T., Numminen, J., Närvänen, S., Hiltunen, J., and Hari, R. (2005). Brain correlates of subjective reality of physically and psychologically induced pain. *Proc. Natl. Acad. Sci. U.S.A.*, 102(6):2147–2151.

Rainville, P., Duncan, G. H., Price, D. D., Carrier, B., and Bushnell, M. C. (1997). Pain Affect Encoded in Human Anterior Cingulate But Not Somatosensory Cortex. *Science*, 277(5328):968–971.

Rantanen, M., Yppärilä-Wolters, H., van Gils, M., Yli-Hankala, A., Huiku, M., Kymäläinen, M., and Korhonen, I. (2007). Tetanic stimulus of ulnar nerve as a predictor of heart rate response to skin incision in propofol–remifentanil anaesthesia. *Br. J. Anaesth.*, 99(4):509–513.

Reddan, M. C. and Wager, T. D. (2018). Modeling Pain Using fMRI: From Regions to Biomarkers. *Neurosci. Bull.*, 34(1):208–215.

Reddy, K. S. K., Naidu, M. U. R., Rani, P. U., and Rao, T. R. K. (2012). Human experimental pain models: A review of standardized methods in drug development. *J. Res. Med. Sci.*, 17(6):587–595.

Rhudy, J. L. and France, C. R. (2007). Defining the nociceptive flexion reflex (NFR) threshold in human participants: A comparison of different scoring criteria. *Pain*, 128(3):244–253.

Rodrigues, S. M., LeDoux, J. E., and Sapolsky, R. M. (2009). The Influence of Stress Hormones on Fear Circuitry. *Annu. Rev. Neurosci.*, 32(1):289–313.

Rudolph, U. and Antkowiak, B. (2004). Molecular and neuronal substrates for general anaesthetics. *Nat. Rev. Neurosci.*, 5(9):709–720.

Salo, M. (1992). Effects of anaesthesia and surgery on the immune response. *Acta Anaesthesiol. Scand.*, 36(3):201–220.

Salomons, T. V., Iannetti, G. D., Liang, M., and Wood, J. N. (2016). The "Pain Matrix" in Pain-Free Individuals. *JAMA Neurol.*, 73(6):755.

Sandkühler, J. (2009). Models and Mechanisms of Hyperalgesia and Allodynia. *Physiol. Rev.*, 89(2):707–758.

Sandkühler, J. and Liu, X. (1998). Induction of long-term potentiation at spinal synapses by noxious stimulation or nerve injury. *Eur. J. Neurosci.*, 10(7):2476–2480.

Sandrini, G., Serrao, M., Rossi, P., Romaniello, A., Cruccu, G., and Willer, J. C. (2005). The lower limb flexion reflex in humans. *Prog. Neurobiol.*, 77(6):353–395.

Saper, C. B., Scammell, T. E., and Lu, J. (2005). Hypothalamic regulation of sleep and circadian rhythms. *Nature*, 437(7063):1257–1263.

Satpute, A. B., Kragel, P. A., Barrett, L. F., Wager, T. D., and Bianciardi, M. (2018). Deconstructing arousal into wakeful, autonomic and affective varieties. *Neurosci. Lett.*

Shafer, S. L. and Stanski, D. R. (2008). Defining depth of anesthesia. In *Modern Anesthetics*, volume 182 of *Handbook of Experimental Pharmacology*, pages 409–423. Springer-Verlag, Berlin Heidelberg.

Shyu, B.-C., Sikes, R. W., Vogt, L. J., and Vogt, B. A. (2010). Nociceptive Processing by Anterior Cingulate Pyramidal Neurons. *J. Neurophysiol.*, 103(6):3287–3301.

Stein, C. (2016). Opioid Receptors. *Annu. Rev. Med.*, 67(1):433–451.

Sury, M. R. J., Palmer, J. H. M. G., Cook, T. M., and Pandit, J. J. (2014). The State of UK anaesthesia: A survey of National Health Service activity in 2013. *Br. J. Anaesth.*, 113(4):575–584.

Tracey, I. and Mantyh, P. W. (2007). The Cerebral Signature for Pain Perception and Its Modulation. *Neuron*, 55(3):377–391.

Tracey, W. D., Wilson, R. I., Laurent, G., and Benzer, S. (2003). Painless, a Drosophila Gene Essential for Nociception. *Cell*, 113(2):261–273.

Uhrig, L., Dehaene, S., and Jarraya, B. (2014). Cerebral mechanisms of general anesthesia. *Ann. Fr. Anesth. Reanim.*, 33(2):72–82.

Untergehrer, G., Jordan, D., Kochs, E. F., Ilg, R., and Schneider, G. (2014). Fronto-Parietal Connectivity Is a Non-Static Phenomenon with Characteristic Changes during Unconsciousness. *PLoS One*, 9(1):e87498.

Vanegas, H. and Schaible, H.-G. (2004). Descending control of persistent pain: Inhibitory or facilitatory? *Brain Res. Rev.*, 46(3):295–309.

Velly, L. J., Rey, M. F., Bruder, N. J., Gouvitsos, F. A., Witjas, T., Regis, J. M., Peragut, J. C., and Gouin, F. M. (2007). Differential Dynamic of Action on Cortical and Subcortical Structures of Anesthetic Agents during Induction of Anesthesia. *Anesthesiology*, 107(2):202–212.

Vermetten, E. and Bremner, J. D. (2002). Circuits and systems in stress. II. Applications to neurobiology and treatment in posttraumatic stress disorder. *Depress. Anxiety*, 16(1):14–38.

von Dincklage, F. (2015). Monitoring von Schmerz, Nozizeption und Analgesie unter Allgemeinanästhesie. *Anaesthesist*, 64(10):758–764.

von Dincklage, F., Correll, C., Schneider, M. H. N., Rehberg, B., and Baars, J. H. (2012). Utility of Nociceptive Flexion Reflex Threshold, Bispectral Index, Composite Variability Index and Noxious Stimulation Response Index as measures for nociception during general anaesthesia. *Anaesthesia*, 67(8):899–905.

von Dincklage, F., Hackbarth, M., Mager, R., Rehberg, B., and Baars, J. H. (2010a). Monitoring of the responsiveness to noxious stimuli during anaesthesia with propofol and remifentanil by using RIII reflex threshold and bispectral index. *Br. J. Anaesth.*, 104(2):201–208.

von Dincklage, F., Jakuscheit, A., Weth, J., Lichtner, G., Jurth, C., and Rehberg-Klug, B. (2018). Higher doses of intraoperative analgesia are associated with lower levels of persistent pain and less analgesic consumption six months after total hip arthroplasty. *Eur. J. Pain*, 22(4):691–699.

von Dincklage, F., Velten, H., Rehberg, B., and Baars, J. H. (2010b). Monitoring of the responsiveness to noxious stimuli during sevoflurane mono-anaesthesia by using RIII reflex threshold and bispectral index. *Br. J. Anaesth.*, 104(6):740–745.

Wager, T. D., Atlas, L. Y., Botvinick, M. M., Chang, L. J., Coghill, R. C., Davis, K. D., Iannetti, G. D., Poldrack, R. A., Shackman, A. J., and Yarkoni, T. (2016). Pain in the ACC? *Proc. Natl. Acad. Sci. U.S.A.*, 113(18):E2474–E2475.

Wager, T. D., Atlas, L. Y., Lindquist, M. A., Roy, M., Woo, C.-W., and Kross, E. (2013). An fMRI-Based Neurologic Signature of Physical Pain. *N. Engl. J. Med.*, 368(15):1388–1397.

Wagner, K. J., Sprenger, T., Kochs, E. F., Tölle, T. R., Valet, M., and Willoch, F. (2007). Imaging Human Cerebral Pain Modulation by Dose-dependent Opioid Analgesia A Positron Emission Tomography Activation Study Using Remifentanil. *Anesthesiology*, 106(3):548–556.

Wagner, K. J., Willoch, F., Kochs, E. F., Siessmeier, T., Tölle, T. R., Schwaiger, M., and Bartenstein, P. (2001). Dose-dependent Regional Cerebral Blood Flow Changes during Remifentanil Infusion in Humans: A Positron Emission Tomography Study. *Anesthesiology*, 94(5):732–739.

Wanigasekera, V., Lee, M. C., Rogers, R., Kong, Y., Leknes, S., Andersson, J., and Tracey, I. (2012). Baseline reward circuitry activity and trait reward responsiveness predict expression of opioid analgesia in healthy subjects. *Proc. Natl. Acad. Sci. U.S.A.*, 109(43):17705–17710.

Warnaby, C. E., Seretny, M., Ní Mhuircheartaigh, R., Rogers, R., Jbabdi, S., Sleigh, J., and Tracey, I. (2016). Anesthesia-induced Suppression of Human Dorsal Anterior Insula Responsivity at Loss of Volitional Behavioral Response. *Anesthesiology*, 124(4):766–778.

Weiser, T. G., Haynes, A. B., Molina, G., Lipsitz, S. R., Esquivel, M. M., Uribe-Leitz, T., Fu, R., Azad, T., Chao, T. E., Berry, W. R., and Gawande, A. A. (2015). Estimate of the global volume of surgery in 2012: An assessment supporting improved health outcomes. *Lancet*, 385:S11.

West, S. J., Bannister, K., Dickenson, A. H., and Bennett, D. L. (2015). Circuitry and plasticity of the dorsal horn – Toward a better understanding of neuropathic pain. *Neuroscience*, 300:254–275.

Wheeler-Kingshott, C. A., Stroman, P. W., Schwab, J. M., Bacon, M., Bosma, R., Brooks, J., Cadotte, D. W., Carlstedt, T., Ciccarelli, O., Cohen-Adad, J., Curt, A., Evangelou, N., Fehlings, M. G., Filippi, M., Kelley, B. J., Kollias, S., Mackay, A., Porro, C. A., Smith, S., Strittmatter, S. M., Summers, P., Thompson, A. J., and Tracey, I. (2014). The current state-of-the-art of spinal cord imaging: Applications. *NeuroImage*, 84:1082–1093.

Wiech, K. (2016). Deconstructing the sensation of pain: The influence of cognitive processes on pain perception. *Science*, 354(6312):584–587.

Wiech, K., Vandekerckhove, J., Zaman, J., Tuerlinckx, F., Vlaeyen, J. W. S., and Tracey, I. (2014). Influence of prior information on pain involves biased perceptual decision-making. *Curr. Biol.*, 24(15):R679–R681.

Willer, J. C. (1977). Comparative study of perceived pain and nociceptive flexion reflex in man. *Pain*, 3(1):69–80.

Wise, R. G., Rogers, R., Painter, D., Bantick, S., Ploghaus, A., Williams, P., Rapeport, G., and Tracey, I. (2002). Combining fMRI with a Pharmacokinetic Model to Determine Which Brain Areas Activated by Painful Stimulation Are Specifically Modulated by Remifentanil. *NeuroImage*, 16(4):999–1014.

Wise, R. G., Williams, P., and Tracey, I. (2004). Using fMRI to Quantify the Time Dependence of Remifentanil Analgesia in the Human Brain. *Neuropsychopharmacology*, 29(3):626–635.

Woo, C.-W., Schmidt, L., Krishnan, A., Jepma, M., Roy, M., Lindquist, M. A., Atlas, L. Y., and Wager, T. D. (2017). Quantifying cerebral contributions to pain beyond nociception. *Nat. Commun.*, 8:14211.

Woolf, C. J. (2011). Central sensitization: Implications for the diagnosis and treatment of pain. *Pain*, 152(3, Supplement):S2–S15.

Yarkoni, T., Poldrack, R. A., Nichols, T. E., Van Essen, D. C., and Wager, T. D. (2011). Large-scale automated synthesis of human functional neuroimaging data. *Nat. Methods*, 8(8):665–670.

Yeo, S. S., Chang, P. H., and Jang, S. H. (2013). The Ascending Reticular Activating System from Pontine Reticular Formation to the Thalamus in the Human Brain. *Front. Hum. Neurosci.*, 7.

Zhao, P., Waxman, S. G., and Hains, B. C. (2006). Sodium Channel Expression in the Ventral Posterolateral Nucleus of the Thalamus after Peripheral Nerve Injury. *Mol. Pain*, 2:27.

Zunhammer, M., Bingel, U., and Wager, T. D. (2018). Placebo Effects on the Neurologic Pain Signature: A Meta-analysis of Individual Participant Functional Magnetic Resonance Imaging Data. *JAMA Neurol.*

Study 1

For copyright reasons the original publication is not included in the online version of the thesis. Please access the publication via the DOI provided below.

Lichtner, G.*, Auksztulewicz, R.*, Kirilina, E., Velten, H., Mavrodis, D., Scheel, M., Blankenburg, F.†, and von Dincklage, F.† (2018). Effects of propofol anesthesia on the processing of noxious stimuli in the spinal cord and the brain. *NeuroImage*, 172:642–653.

*, †: contributed equally

DOI: https://doi.org/10.1016/j.neuroimage.2018.02.003

Study 2

For copyright reasons the original publication is not included in the online version of the thesis. Please access the publication via the DOI provided below.

Lichtner, G., Auksztulewicz, R., Velten, H., Mavrodis, D., Scheel, M., Blankenburg, F., and von Dincklage, F. (2018). Nociceptive activation in spinal cord and brain persists during deep general anaesthesia. *Br. J. Anaesth.*, 121(1):291—302.

DOI: https://doi.org/10.1016/j.bja.2018.03.031

Study 3

For copyright reasons the original publication is not included in the online version of the thesis. Please access the publication via the DOI provided below.

von Dincklage, F., Jakuscheit, A., Weth, J., **Lichtner, G.**, Jurth, C., and Rehberg-Klug, B. (2018). Higher doses of intraoperative analgesia are associated with lower levels of persistent pain and less analgesic consumption six months after total hip arthroplasty. *Eur. J. Pain*, 22(4):691–699.

DOI: https://doi.org/10.1002/ejp.1145

Study 4

For copyright reasons the original publication is not included in the online version of the thesis. Please access the publication via the DOI provided below.

Jakuscheit, A., Weth, J., **Lichtner, G.**, Jurth, C., Rehberg, B., and von Dincklage, F. (2017). Intraoperative monitoring of analgesia using nociceptive reflexes correlates with delayed extubation and immediate postoperative pain: A prospective observational study. *Eur. J. Anaesthesiol.*, 34(5):297.

DOI: https://doi.org/10.1097/EJA.0000000000000597

Study 5

For copyright reasons the original publication is not included in the online version of the thesis. Please access the publication via the DOI provided below.

Lichtner, G., Hösl, T. M., Jakuscheit, A., Jurth, C., and von Dincklage, F. (2016). Optimizing Nociceptive Flexion Reflex (NFR) Scoring Criteria by Adjusting for Noise and Reflex Properties and Sampling Rate. *Clin. J. Pain*, 32(9):773–783.

DOI: https://doi.org/10.1097/AJP.0000000000000323

Acknowledgements

I want to thank my supervisors PD Dr Falk von Dincklage and Prof. Dr Felix Blankenburg for the possibility to conduct this research and for the excellent training and supervision that I have received in addition to all the helpful and constructive discussions.

I also want to thank my colleagues Axel Jakuscheit, Carlo Jurth and Johannes Weth for creating an always enjoyable atmosphere during and after work. I would also like to thank Dr Ryszard Auksztulewicz and Dr Evgeniya Kirilina for their support during data analysis and interpretation.

I am very grateful to Dr Niclas Gimber and Dr David Owald for fruitful discussions on my work, on the scientific system and on everything else.

I would further like to acknowledge the excellent scientific training that I have received from Prof. Dr Frank Noé and Prof. Dr Volker Haucke during my previous work, which provided me with the scientific know-how that allowed me to pursue this work.

Furthermore, I want to thank PD Dr Jan Baars for his support during my work and my colleagues Dr Helena Velten, Teresa Dörig and Anna Golebiewski for their commitment.

Finally, I want to thank my family and friends for their support.

Appendices

A. Lebenslauf

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

For reasons of confidentiality my curriculum vitae has been removed from the electronic version of this dissertation.

B. Eidesstattliche Erklärung

Hiermit erkläre ich an Eides statt, dass ich die vorliegende Arbeit eigenständig und ohne unerlaubte Hilfe verfasst habe und dass ich Ideen und Gedanken aus Arbeiten anderer entsprechend gekennzeichnet habe.

Ort, Datum Unterschrift

C. Erklärung über Eigenanteil an Veröffentlichungen

Erklärung gemäß § 7 Abs. 3 Satz 4 der Promotionsordnung über den Eigenanteil an den veröffentlichten oder zur Veröffentlichung vorgesehenen eingereichten wissenschaftlichen Schriften im Rahmen meiner publikationsbasierten Arbeit.

I. Name, Vorname: Lichtner, Gregor
Institut: Fachbereich Erziehungswissenschaft und Psychologie, Arbeitsbereich Neurocomputation and Neuroimaging
Promotionsfach: Psychologie
Titel: Master of Science (MSc)

II. **Nummerierte Aufstellung der eingereichten Schriften (Titel, Autoren, wo und wann veröffentlicht bzw. eingereicht):**

[1] **Lichtner, G.***, Auksztulewicz, R.*, Kirilina, E., Velten, H., Mavrodis, D., Scheel, M., Blankenburg, F.†, and von Dincklage, F.† (2018). Effects of propofol anesthesia on the processing of noxious stimuli in the spinal cord and the brain. *NeuroImage*, 172:642–653.

[2] **Lichtner, G.**, Auksztulewicz, R., Velten, H., Mavrodis, D., Scheel, M., Blankenburg, F., and von Dincklage, F. (2018). Nociceptive activation in spinal cord and brain persists during deep general anaesthesia. *Br. J. Anaesth.*, 121(1):291—302.

[3] von Dincklage, F., Jakuscheit, A., Weth, J., **Lichtner, G.**, Jurth, C., and Rehberg-Klug, B. (2018). Higher doses of intraoperative analgesia are associated with lower levels of persistent pain and less analgesic consumption six months after total hip arthroplasty. *Eur. J. Pain*, 22(4):691–699.

[4] Jakuscheit, A., Weth, J., **Lichtner, G.**, Jurth, C., Rehberg, B., and von Dincklage, F. (2017). Intraoperative monitoring of analgesia using nociceptive reflexes correlates with delayed extubation and immediate postoperative pain: A prospective observational study. *Eur. J. Anaesthesiol.*, 34(5):297.

[5] **Lichtner, G.**, Hösl, T. M., Jakuscheit, A., Jurth, C., and von Dincklage, F. (2016). Optimizing Nociceptive Flexion Reflex (NFR) Scoring Criteria by Adjusting for Noise and Reflex Properties and Sampling Rate. *Clin. J. Pain*, 32(9):773–783.

*, †: contributed equally

III. **Darlegung des eigenen Anteils an diesen Schriften:**

Die Bewertung des Eigenanteils richtet sich nach der Skala: vollständig – überwiegend – mehrheitlich – in Teilen und enthält nur für den jeweiligen Artikel relevante Arbeitsbereiche.

zu II. 1: Entwicklung des Messaufbaus (in Teilen), Datenerhebung (in Teilen), Datenauswertung (vollständig), Ergebnisdiskussion (überwiegend), Erstellen des Manuskripts (überwiegend)

zu II. 2: Entwicklung des Messaufbaus (in Teilen), Datenerhebung (in Teilen), Datenauswertung (vollständig), Ergebnisdiskussion (überwiegend), Erstellen des Manuskripts (überwiegend)

zu II. 3: Datenauswertung (in Teilen), Ergebnisdiskussion (in Teilen), Erstellen des Manuskripts (in Teilen)

zu II. 4: Datenauswertung (in Teilen), Ergebnisdiskussion (in Teilen), Erstellen des Manuskripts (in Teilen)

zu II. 5: Konzeption (vollständig), Methodenentwicklung (vollständig), Versuchsdesign (vollständig), Programmierung (vollständig), Datenerhebung (vollständig), Datenauswertung (vollständig), Ergebnisdiskussion (überwiegend), Erstellen des Manuskripts (überwiegend)