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The Neural Correlates of Pain and Sleep in Health and Disease

PhD Thesis submitted by

Raquel Adaia Sandoval Ortega

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Supervisor

Prof. Dr. Thomas Nevian

Department of Physiology

Faculty of Medicine of the University of Bern

Co-advisor

Prof. Dr. Anita Lüthi

Department of Fundamental Neurosciences

University of Lausanne

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Bern, Dean of the Faculty of Medicine

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Abstract

Chronic pain is endemic to the population, affecting on average one in every five individuals and incurring yearly costs of up to €300 billion in Europe alone. Despite the significant economic and societal impact of chronic pain, effective treatments remain elusive. Notably, nearly 90% of patients suffering from chronic pain report sleep disturbances. Interestingly, sleep disturbances exacerbate pain sensitivity, and good-quality sleep reduces pain ratings. The strong interplay between these two physiological processes, suggests that sleep has significant potential as a therapeutic approach for treating chronic pain. However, in order to use sleep as a therapy for chronic pain, it is necessary to identify and characterize the neural signatures of this interaction.

The goal of this thesis was to identify neural correlates of the interaction between sleep and pain, with the objective of providing neural signatures that would assist in the use of sleep as a therapeutic approach for pain management. To achieve this aim, we used simultaneous intracranial recordings of the anterior cingulate (ACC) and the primary somatosensory (S1) cortices in mice. The electroencephalogram (EEG) aided to identify global patterns of activity, and the electromyogram (ECG) was used to measure autonomic nervous system activation.

The evaluation of the effect of sleep on pain revealed that somatic information reached the ACC and S1 during sleep and that the characteristics of the stimulation, such as salience and valence, can be distinguished in sleep. Sleep strongly modulated the complex temporal dynamics in the delta, alpha and gamma bands observed in wake, although it kept features related to sensory processing and attention. Correlation of neural activity with heart rate measurements reduced the element of saliency, allowing to better separate noxious from non-noxious stimulation in both wake and sleep.

Studying the effect of pain on sleep, we found that chronic pain temporarily altered the sleep phenotype but induced long-lasting changes in the ACC, S1 and EEG. Upon induction of chronic pain, a progressive slow-down of global activity captured in the EEG, as well as gamma and theta increases in the ACC were observed. These changes partially recapitulated the thalamocortical dysrhythmia phenotype described in individuals with chronic pain. Furthermore, chronic pain impaired the age-related increase in synchrony of slow oscillations, and exacerbated the age-related decrease in heart rate across all arousal states.

In summary, this thesis contributes to the field of sleep and pain by presenting a comprehensive examination of the neurophysiological mechanisms underlying the interactions between these two physiological processes. The findings presented in this thesis provide a series of neurophysiological signatures that offer new insights into the complex relationship between sleep and pain.

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General introduction

"Biology is the study of complicated things that have the appearance of having been designed with purpose"

Richard Dawkins

1. Pain and Sleep interaction

"It was late and Anne had a busy day.
She was tired and the bed felt like a welcoming hug.
One hour went by and she wouldn't fall asleep.
It was too painful!
Anne had broken her leg during a ski trip the previous week."

1.1. Physiological manifests

Pain and sleep strongly influence each other (Koffel et al., 2016; Priebe et al., 2020; Sivertsen et al., 2015). Chronic pain has a wide range of etiologies and presents itself with multiple and diverse comorbidities (Finan et al., 2013). Sleep disturbances are the most prevalent comorbidity with nearly 90% of patients with chronic pain presenting sleep impairments (Breivik et al., 2005; Finan et al., 2013; Smith & Haythornthwaite, 2004) and heightened cognitive arousal (Byers et al., 2016). Intriguingly, while pain affects sleep quality and quantity, sleep also modulates the perception of pain (Afolalu et al., 2018; Smith & Haythornthwaite, 2004). For instance, one night sleep deprivation or fragmentation decreases the threshold for pain (Krause et al., 2019; Rosseland et al., 2018; Sivertsen et al., 2015; Staffe et al., 2019) and in the long term, poor sleep can increase the risk of developing chronic pain (Skarpsno et al., 2021).

Physicians define chronic pain as pain above 5 in the Numerical Rating Score[1] that persists for at least three to six months (Breivik et al., 2005; Hopp et al., 2014). This pathology ranges all ages and prevails in around 20% of the European and American population (Breivik et al., 2005; Hopp et al., 2014), carrying yearly costs of up to €300 billion in Europe alone (Hopp et al., 2014). Yet, there are no standardized nor effective treatments (Argoff, 2011; Finan et al., 2013; Haack et al., 2020).

According to clinical studies, adequate sleep can improve pain ratings (Faraut et al., 2015; Krause et al., 2019). Therefore, sleep may potentially be used as a treatment for pain. However, in order to effectively use sleep as a treatment for pain, it is crucial to understand the underlying neural correlates of the relationship between sleep and pain.

1.2. Measuring the interaction

Multiple brain imaging studies in a variety of species have been used to investigate the brain regions involved in the interaction between sleep and pain (Apkarian et al., 2005; Craig, 2002; Da Silva & Seminowicz, 2019; Nasseef et al., 2021; Wager et al., 2013; Wey et al., 2014;

[1] ***Numerical Rating Score (NRS).*** *Numeric scale between 0 and 10, 20 or 100 used to evaluate the level of pain of a patient. Zero represents "no pain at all" and the maximum value, "the worst pain ever possible"* (Haefeli & Elfering, 2006).

Woo et al., 2017). Additionally, electrophysiology as well as magnetoencephalography have demonstrated that brain regions responding to pain in wake also respond during sleep (Bastuji et al., 2008; Kakigi et al., 2003; Kitamura et al., 1996; Shaw et al., 2006; Wang et al., 2004; Wang et al., 2003). These studies collectively indicate that cortical reactivity to pain is preserved in sleep. However, the modulation of this cortical reactivity by sleep varies across studies. This discrepancy may be attributed to the use of different stimulation types, recording methods, and analysis methods, highlighting the importance of careful experimental design in studying the sleep-pain relationship.

Autonomic responses have been used to demonstrate that pain can be perceived during sleep, even in the absence of an arousal (Chouchou et al., 2011). Furthermore, motor reactions, such as finger lifting, in response to noxious stimuli during sleep have been used to confirm pain perception during REM (Mazza et al., 2012). Therefore, simultaneously monitoring physiological and overt behavioral responses to sensory stimuli can aid to contextualize and interpret cortical responses.

1.3. Mechanisms of interaction

1.3.1. Brain oscillations

Interventional studies, in which sleep manipulations were performed to investigate changes in pain correlates, found that sleep deprivation enhanced neural activity in the somatosensory cortex and decreased it in the thalamus, insula and Nucleus Accumbens (NAcc) upon noxious heat. Nonetheless, only the increase in the somatosensory cortex and the decrease in the thalamus predicted the decrease in the pain threshold (Krause et al., 2019). The authors of the study concluded that the increased activity in the somatosensory cortex and the decrease in areas related to top-down modulation of pain was driving the sleep-induced hyperalgesia. Other paradigms, like sleep fragmentation, showed that increased functional connectivity between the right executive control network and cortical areas, which are associated with pain awareness and attention (i.e. dorsolateral prefrontal cortex (dlPFC)), may account for part of the decreased pain thresholds upon sleep disruption (Letzen et al., 2020). Experimental sleep perturbation in healthy participants appears to disrupt the EEG spectral properties in the same way as chronic pain does, by reducing delta (1-4 Hz) and increasing alpha (11-16 Hz) power (Smith & Haythornthwaite, 2004). This similarity suggests that sleep disruption and chronic pain cause similar mesoscale changes.

1.3.2. Neuromodulators

Investigating the interaction between sleep and pain through the examination of changes in sleep following pain induction, Foo & Mason, 2003 postulated that chronic pain shifts the activity of the Raphe Magnus (RM) by increasing the firing of ON (pain facilitator) and OFF

(pain inhibitory) cells. This change in activity contributed to increased alertness, along with decreased inhibition of inhibitory cells in the dorsal horn of the spinal cord. Additionally, opioid receptors strongly modulate cells in the RM. Dysregulation of the opioid system follows both chronic pain (Higginbotham et al., 2022; Ji et al., 2021; Liu et al., 2019; Thompson et al., 2018; Wey et al., 2014) and sleep deprivation (Campbell et al., 2013; Eacret et al., 2020; Fadda et al., 1991; Smith et al., 2020), suggesting that the opioid system may serve as a potential mechanism linking sleep and pain. Interestingly, the monoaminergic system, which is important for sleep and wake promoting systems (Brown et al., 2012), is also dysregulated following sleep disruption (Menon et al., 2019; Thakkar & Mallick, 1993; Tsuchiya et al., 1969) as well as in chronic pain (Kędziora et al., 2023; Sahbaie et al., 2022; S. Yang & Chang, 2019). Antidepressants are often prescribed to treat chronic pain. The most commonly used antidepressants for chronic pain target the monoaminergic system (Häuser et al., 2009; Hayashida & Obata, 2019; Obata, 2017). Yet, the effects of monoamines in pain are complex and their dysregulation is not fully characterized (Argoff, 2011; Haack et al., 2020). For instance, fibromyalgia pain symptoms usually improve after use of serotonin reuptake inhibitors (SSRI) (Häuser et al., 2009; Koechlin et al., 2021; Walitt et al., 2015). However, serotonin exerts a dual effect on promoting and inhibiting pain, which seems to depend on receptor type, site of action and health status (Viguier et al., 2013). Norepinephrine (NE), like serotonin, is analgesic in the context of chronic pain (Martins et al., 2015). In healthy conditions, NE release following LC activation induces awakenings. In chronic pain, NE increases following enhanced LC activity, and likely facilitates awakenings. Dopamine has also been suggested to link sleep and pain, given that both sleep deprivation and chronic pain decrease activity of dopamine receptors. Moreover, boosting dopamine signaling via modafinil[2] intake or D2 receptor agonists normalizes pain-induced sleep disturbances (Finan & Smith, 2013; Haack et al., 2020). Other neurochemical systems also seem to bridge pain and sleep physiology. These are adenosine and nitric oxide signaling, orexinergic system, hypothalamus-pituitary-adrenal (HPA) axis, immune system, pituitary melatonin, and the endocannabinoid system (Haack et al., 2020).

1.3.3. Glymphatic system

The glymphatic system is the brain clearance mechanism and is primarily active during sleep. Chronic pain affects the glymphatic system, decreasing its ability to clear metabolites byproducts (Goldman et al., 2020; J. Kim et al., 2022; A. Wang et al., 2022; Yi et al., 2022).

[2] **Modafinil.** Medication used to treat sleepiness, most commonly in cases of narcolepsy. Modafinil increases dopamine in the brain parenchyma through dopamine reuptake inhibition. It additionally promotes the release of orexins from the lateral hypothalamus as well as of histamine from the tuberomammillary nucleus. Altogether contributes to increased arousal.

In summary, pain and sleep reciprocally interact at multiple levels and correlates of neural activity can be used to study this interaction. The use of drugs that modulate neural activity carry the intrinsic risk of aggravating either side of the pain-sleep equation. This can result from unintended activation of the target receptor in organs or cells that mediate deleterious side effects. Therefore, understanding the signatures of neural activity *in vivo* that define the sleep-pain interaction is a key step to define better-targeted therapeutic approaches.

2. Neuroelectrophysiology to study Sleep and Pain

"Your thoughts are waves. Be waves."

2.1. The origin of neuronal oscillations

Electrical neural activity results from a hectic, but very coordinated, exchange of ions across the cell membrane of a neuron. The ion exchange between the intra- and extra-cellular spaces is highly dependent on the biophysical properties of neurons. These properties depend on the cell morphology (**Fig. 1**) together with the composition of ion channels, transporters as well as neurotransmitter receptors. Because neurons require a homeostatic balance of ions, any change in the intracellular composition of ions must be compensated elsewhere by either an influx or outflow of ions, creating an electric field around the neuron (**Fig. 1**). When multiple neurons are close together, such as in the brain, changes in the ion composition of the extracellular space generate voltage fluctuations that are termed *Local Field Potentials (LFP)* and reflect the activity of excitatory cells.

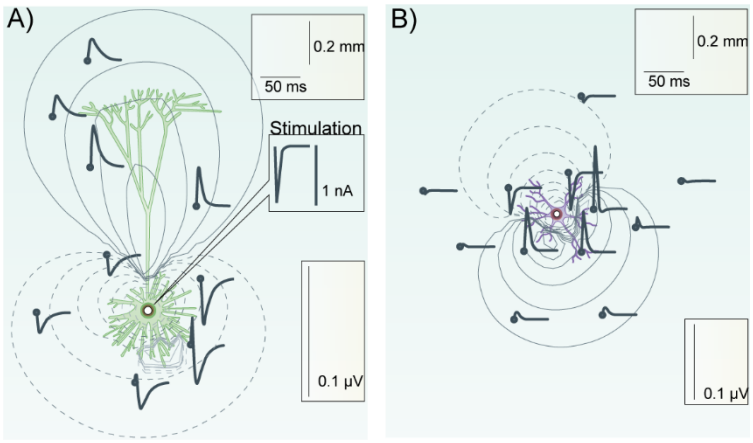


Figure 1. Modelled neuronal electrical fields. *A stimulation is delivered to the soma of the neurons (white dot). Fine lines represent the electrical fields around the soma (discontinuous lines) and the axon (continuous lines). Thick lines originating at a dot represent generated field potentials at the location of the dot.* ***A)*** *Example pyramidal cell.* ***B)*** *Example thalamic cell. Modified* from Einevoll et al., 2013.

These fluctuations in the LFP resemble oscillatory systems, which can be described in terms of *frequency* and *amplitude*. The frequency describes the number of cycles (or periods) of a

sinusoidal wave within a second (Hz) (**Fig. 2**). In neural systems, the frequency depends on the speed of change of extracellular ionic concentrations. The amplitude is the energy in the system at that frequency, which is proportional to the trough-crest distance. In neural recordings, the amplitude can be influenced by both technical and biological factors. Technical factors encompass:

1. Distance between the electrode and its reference
2. Location (i.e. intracranial vs. scalp and proximity to soma/dendrites)
3. Impedance of the electrode at the recording site

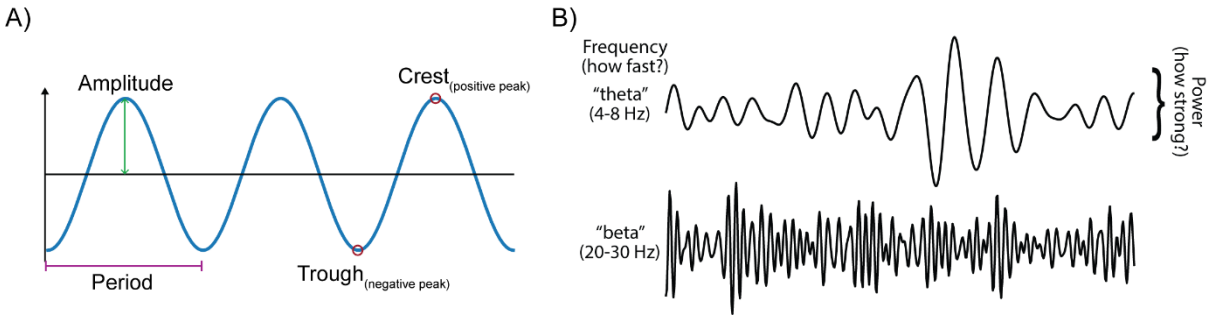


Figure 2. Parameters used to define oscillations. A) *Sinusoidal wave where the factors amplitude, period, crest and trough are marked.* ***B)*** *Examples of sinusoidal signals to show how the power and the frequency change the characteristics of the signals. Modified from* (Cohen, 2014).

Biological factors include:

1. Simultaneous firing of a group of neurons generating massive relative increases in voltage amplitudes due to high cations vs. anions ratios.
2. Anatomical organization of neurons. Neurons in the same orientation sum their electrical fields, generating greater amplitude oscillations (i.e. cortex). Neurons without a defined orientation cancel each other's electrical fields, resulting in low amplitude oscillations (i.e. thalamus).

2.2. Evoked Potentials (EP)

In response to external stimuli and motor or cognitive events, neurons synchronize generating voltage changes time-locked to a specific event that reflects summed pre-synaptic potentials. These events are termed Evoked Potentials (EP) if they are related to sensory perception or Event-related Potentials (ERP) when they derive from the execution of a motor or cognitive task.

Both EPs and ERPs are characterized by a series of consecutive positive and negative voltage deflections. These reflect activation of different groups of neurons (Muzyka & Estephan, 2019; Thorpe et al., 2021) and cognitive operations related to the stimulus or task (Sur & Sinha, 2009; Woodman, 2010). The fluctuations can initiate even before the delivery of the stimulation or initiation of the task and extend even after the behavioral response is made. In humans, the ERP fluctuations are divided into early components (within 100 msec after

stimulus) which largely depend on the physical stimulation, and late components, which reflect the cognitive aspects and are related to information processing (Thorpe et al., 2021).

EP are very reliable non-invasive readings that measure the proper functioning of the nervous system. In fact, in clinical settings, the integrity and function of the central nervous system is often tested with EPs. Furthermore, variations in the temporal dynamics of EPs, measured as latencies to the different peaks that compose the EP, are being used as biomarkers of neurodegenerative diseases (Lascano et al., 2017).

2.3. The meaning of frequency bands

Voltage recordings from neural data are the result of the linear weighted sum of multiple oscillatory systems. Therefore, the signals can be split up onto the different frequency oscillations that compose them. The result of this spectral segregation are spectro-temporal matrices, called *spectrograms*. Spectrograms allow us to follow the temporal changes in the frequency strength, measured as amplitude or power.

Neighboring frequencies tend to follow very similar dynamics, therefore, these have been grouped into so-called *frequency ranges*. These are delta (1-4 Hz), theta (4-8 Hz), alpha (8-20 Hz), sigma (11-16), beta (20-30 Hz) and gamma (> 30 Hz).

Electrophysiological experiments in humans and animals have shown that cognitive, attentional and sensory processes, among others, engage a specific combination of the aforementioned frequency bands.

For instance, sensory processing of any sensory modality has been found to induce gamma oscillations, setting gamma as a correlate of sensory processing. Attention, on the other hand, changes the power of alpha and gamma (Bacigalupo & Luck, 2019; Başar et al., 2001; Jensen et al., 2007; Karakaş et al., 2001; Klimesch, 2012; Wöstmann et al., 2021). The spectral dynamics of attention are particularly interesting. Enhanced attention towards the source of the stimulation suppresses attention on contralateral parietal areas (Bacigalupo & Luck, 2019; Ikkai et al., 2016; Klimesch, 2012; Magosso et al., 2019; Wöstmann et al., 2021) at the same time that increases alpha in ipsilateral parietal areas as a mechanism to suppress sensory input from the unattended location (Händel et al., 2011). Gamma, on the other hand, is increased (Jensen et al., 2007).

Frequency bands not only define cognitive processes, but these also help characterize brain states. For instance, while NREM sleep is defined by high delta power, REM is distinguished by theta, and wake is characterized by prominent gamma and theta activity (Adamantidis et al., 2019; Destexhe, 2009; Eban-Rothschild et al., 2017; Horner & Peever, 2017). Other states such as walking have been found to increase theta and closing the eyes increases alpha (Brown et al., 2012).

Because mental states and processes change the power distribution of frequency bands, these frequency bands are often used as a proxy to confirm that a given mental process or state has been evoked.

3. Pain

" 'OOOOUUUCHHHH!!!' shouted Maria.
*'M****** f***** little pieces of s***. These are going out the window!'*
Maria thought in tears after stepping on a Lego mini rocket."

3.1. The socioeconomic cost of pain and its comorbidities

Chronic pain-related costs surmount to an appalling $560-630 billion in the US and €300 billion in Europe. These costs represent 3 to 10% of the GDP and principally stem from ineffective treatment, indirect costs of overwhelmed caregivers (i.e. decrease in psychological health) (Dueñas et al., 2016), and loss of workforce (Hopp et al., 2014). In average, 20% of the population (2-40% in US, 12-30% in Europe) suffer from chronic pain (Breivik et al., 2005; Hopp et al., 2014; Yong et al., 2022) and often develop comorbidities such as sleep disturbances and depression, increasing the odds of incapacitation (Dueñas et al., 2016).

Breivik et al.'s 2005 comprehensive survey showed that the quality of life in patients with chronic pain is severely reduced. Surveyed patients were no longer able to normally maintain relations with family and friends (27%), have sexual relations (43%), walk (47%), drive (47%), attend social activities (48%), do household chores (56%), work outside home (63%) nor exercise (73%). Moreover, chronic pain undermines the working life of affected people (Breivik et al., 2005; Dueñas et al., 2016; Radat et al., 2013). From the surveyed participants only 31% continued working full time. Due to their pain, 25% of participants decreased their employment rate and 19% lost their job. Furthermore, those respondents still in the workforce (43%) lost an average of 7.8 days of work in the six months previous to the survey (1-9 days lost (23%), 10-15 days lost (9%), ≥10 days lost (13%)). Moreover, the greater the pain, the greater the loss of workdays (Dueñas et al., 2016).

Shockingly, despite the devastating socioeconomic effects of pain, there are no effective treatments (Breivik et al., 2005; Dueñas et al., 2016; Hopp et al., 2014). In cases like in the US, the lack of effective treatment for chronic pain coupled with difficult access to health care led to an alarming increase in opioid consumption that culminated in the current daunting opioid crisis.

One of the complexities of chronic pain is the large variety of comorbidities it frequently presents (Nicholson & Verma, 2004; Pereira et al., 2017; Radat et al., 2013; Vartiainen et al., 2022; Wilson et al., 2002; Yalcin & Barrot, 2014). The most common ones are sleep disorders (60-90%) and psychiatric symptoms such as depression (21-100%) and anxiety (20-39%) (Breivik et al., 2005; Nicholson & Verma, 2004; Radat et al., 2013). In the case of *neuropathic pain* – a type of chronic pain caused by a lesion or dysfunction of the nervous system – poor sleep, depression, and anxiety define the time course of the disease as well as the chances of recovery (Nicholson & Verma, 2004). The fact that all these pathologies reinforce each other encourages the use of holistic approaches to treat chronic pain (Nicholson & Verma, 2004). De Ridder and Vanneste proposed that chronic pain comorbidities stem from aberrant interactions within the triple network model (default mode network, saliency network and

executive network). This is because many brain areas of the "pain matrix" overlap with the aforementioned networks. Additionally, all ascending and descending pain pathways also overlap with the triple network model (De Ridder et al., 2022).

Box 1. Measuring pain in animal models.

The pain experience is subjective and composed of physical and emotional factors. The physiological state of an individual as well as the culture and society influence the pain threshold causing a great variability between individuals. Yet, only verbalization of the pain experience allows assessment and treatment.

In animal research, verbal communication is not possible and high variability in the pain threshold should be assumed. Thus, pain assessment in animals lacks certainty. Despite noxious stimuli may increase vocalization, induce withdrawal of the stimulated body part or increase agitation, there is no certitude these behaviors are related to pain. Therefore, they are referred as *pain-like behaviors*. The only certainty is that noxious stimuli activate nociceptive sensory neurons that transmit the evoked electrical signals to the central nervous system. For this reason, instead of using the term pain, *nociception* is preferred (Deuis et al., 2017).

There is a large collection of tests available to assess the animals' level of pain. These fall into two categories (Deuis et al., 2017):

1) Evaluation of the sensory component (i.e. pain threshold)
2) Evaluation of the affective-motivational component (i.e. anhedonia)

However, uncertainty remains regarding whether the observed behavioral phenotype in these tests truly relates to pain. Hence, efforts are being directed into better understanding how animals communicate pain (Sadler et al., 2022).

Novel tools for the assessment of pain focus on the development of pain scales based on naturalistic passive behaviors. Some examples are:

1) Facial expressions (Langford et al., 2010)
2) Clusters of behaviors (Turner et al., 2019)
3) Pose estimation (Abdus-Saboor et al., 2019; Fried et al., 2020; Jones et al., 2020)

3.2. The neural substrates of pain

The International Association for the Study of Pain (IASP) defines pain as *"an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage"*. In addition, the IASP adds six supplementary points to refine the description of pain:

1) Pain is a personal experience influenced by biological, psychological, and social factors.
2) Pain and nociception are not synonyms. Nociception only refers to activation of sensory neurons.
3) The concept of pain is learned through experience.
4) Individual reports of pain experiences should be respected.

5) Adverse effects on function as well as social and psychological well-being may derive from pain, even though pain usually serves an adaptive role.
6) The inability to communicate the experience of pain does not discard potential occurrence of pain in a human or a nonhuman animal.

Considering the multifaceted experience of pain – involving sensory, cognitive and affective-motivational aspects – it is important to mention that the term *nociception* is preferred in animal research (**see Box 1**).

3.2.1. From periphery to central nervous system

Pain processing involves four steps: transduction, transmission, modulation and perception (De Ridder et al., 2022).

1. **Transduction.** This consists in the activation of sensory receptors in free nerve endings by a noxious stimulation. These receptors will transduce a mechanical or chemical signal into electrical signals that downstream neurons can interpret.

2. **Transmission.** Electrical signals generated in the free nerve endings are transferred to the dorsal horn via the dorsal root ganglia. The anterolateral system initiates at this point and consists of the spinoreticular tract (SRT), the spinotectal tract (STeT) and the *spinothalamic tract* (STT) or spinal lemniscus (**Fig. 3**). The SRT mediates stimulus-related alertness and arousal, and the STeT, stimulus-directed orientation of eyes and head (De Ridder et al., 2022). The STT transmits innocuous (a.k.a. non-noxious) and noxious potentials related to pain, touch, itch, and temperature to the thalamus. It is further subdivided in the anterior and the lateral STT. The anterior STT (a.k.a. conventional pain pathway or *lateral pain pathway*) conveys the discriminatory components (i.e. intensity, location and characteristics) of peripheral stimuli and targets the lateral thalamus (VPL, VPI), which then project to the somatosensory cortex. The lateral STT (a.k.a. lamina I spinothalamic pathway or *medial pain pathway*) relays to medial thalamic nuclei (mediodorsal and ventromedial posterior). These in turn, project to the dorsal anterior cingulate cortex (dACC) and the anterior insula (AI), encoding the affective-motivational aspects of pain, or in other words, the unpleasantness and suffering of the pain experience. Thus, given the anatomical and functional separation of the lateral and the medial pain pathways, the sensory component (pain) and the emotional component (suffering) are not necessarily experienced simultaneously (De Ridder et al., 2022).

3. **Modulation.** An interplay between the two ascending pathways (lateral and medial pain pathways) and a third descending pathway (De Ridder et al., 2022; Vanneste & De Ridder, 2021). The latter starts in the dlPFC and runs through the pregenual anterior cingulate cortex (pgACC) to the thalamoreticular nucleus (TRN), periaqueductal gray (PAG) and rostroventral medulla oblongata to the spinal cord

where it modulates the spinal gate. The hypothalamus is also implicated and norepinephrine (NE), serotonin (5HT), dopamine (DA) and endogenous opioids are the main neurotransmitters (De Ridder et al., 2022; Vanneste & De Ridder, 2021). The descending pain pathway modulates pain via stress-mediated pain inhibition and placebo analgesia (Vanneste & De Ridder, 2021). However, it is worth noting that the descending pathway can also facilitate pain.

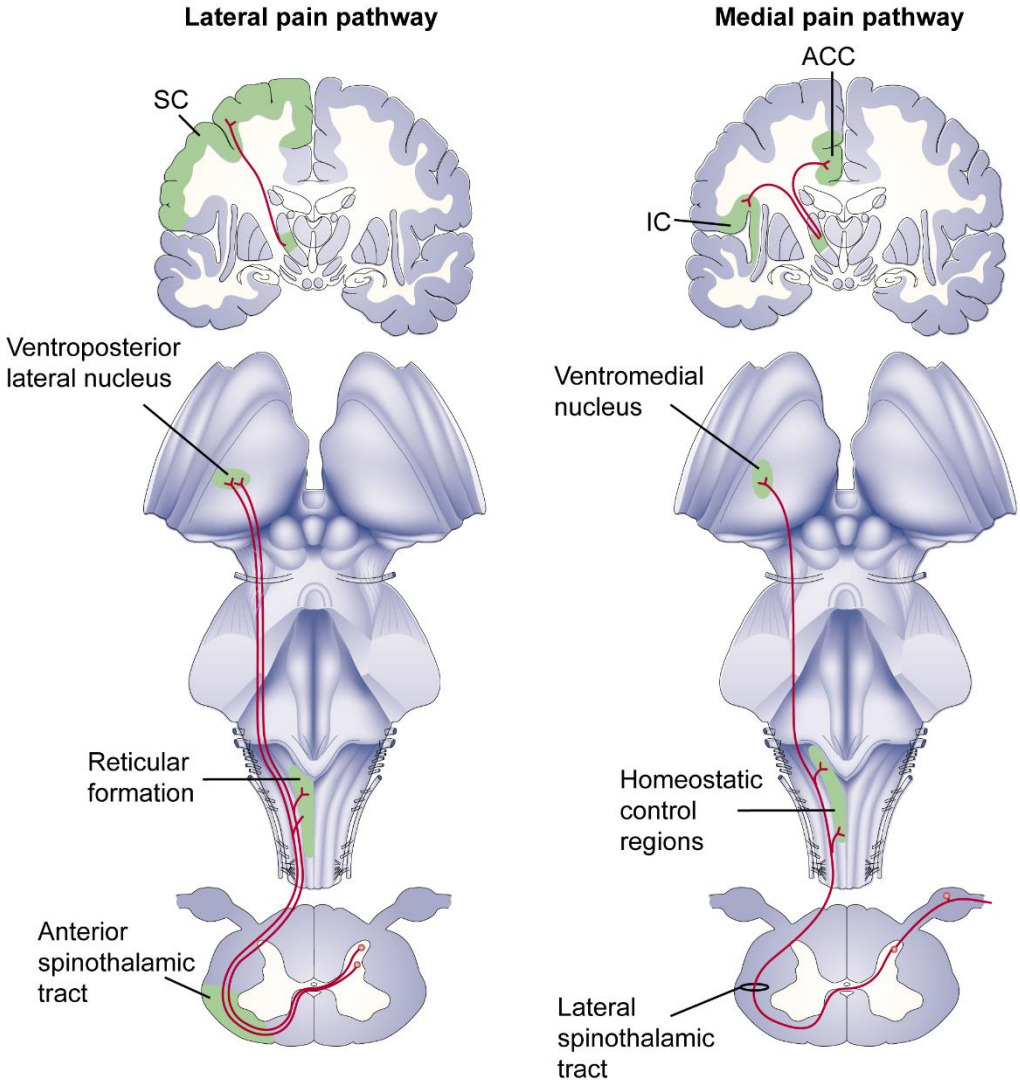


Figure 3. Spinothalamic tract. *Lateral and anterior subdivision of the spinothalamic tract representing the medial and the lateral pain pathways, respectively. ACC, anterior cingulate cortex; IC, insular cortex; SC; somatosensory cortex. Modified from Craig, 2002.*

4. **Perception.** This ensues when the stimulus is brought into consciousness through the creation of a percept, or in other words, an internal representation of the outer and inner world (De Ridder et al., 2022).

3.2.2. The pain matrix

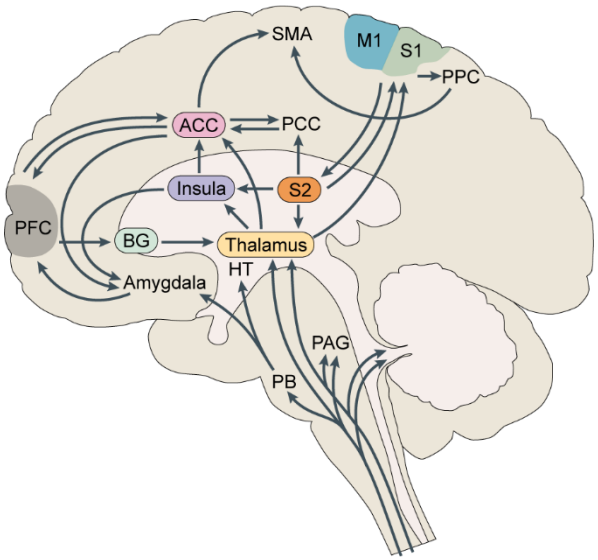


Figure 4. Pain matrix. *Brain areas commonly activated by ascending noxious information and their connectivity. BG, basal ganglia; HT, hypothalamus; M1, primary motor cortex; PAG, periaqueductal gray; PB, parabrachial nucleus; PCC, posterior cingulate cortex; PFC, prefrontal cortex; PPC, posterior parietal cortex; SMA, supplementary motor areas; S1, primary somatosensory cortex; S2, secondary somatosensory cortex (Davis et al., 2017).*

Neuroimaging studies (EEG, magnetoencephalography (MEG), positron emission tomography (PET), functional magnetic resonance imaging (fMRI)) consistently have shown activation of a wide network of cortical and subcortical structures upon noxious stimuli. This network has been named the *"pain matrix"* and mainly consists of the primary (S1) and secondary (S2) somatosensory cortices, the anterior cingulate cortex (ACC) and the anterior insula (AI) (Garcia-Larrea & Peyron, 2013; Legrain et al., 2011) (**Fig. 4**). Other areas activated less consistently, and not depicted in **Fig. 4**, include the striatum, hippocampus, cerebellum and temporoparietal junction. Their activation, however, seems to be dependent on the context of pain (Garcia-Larrea & Peyron, 2013). Activation of the "pain matrix" has been considered as the origin of the percept of pain (Legrain et al., 2011) reflecting the sensory, affective, and cognitive dimensions of the experience of pain (Garcia-Larrea & Peyron, 2013). However, the overlap of many of these areas with networks such as the saliency network, which encodes the behavioral relevance of a stimulation (De Ridder et al., 2022; Garcia-Larrea & Peyron, 2013), has put in doubt whether pain-mediated activation of the "pain matrix" uniquely processes pain instead of other types of information (i.e. saliency) (Legrain et al., 2011).

The pain matrix seems to be conserved in neuropathic pain. Yet, some changes in neural activity emerge. These include tonic hypoactivity in the thalamus, decreased responsiveness in prefrontal cortices related to neuropathic allodynia, and changes in the operculoinsular interhemispheric balances, favoring ipsilateral responses to pain (Garcia-Larrea & Peyron, 2013).

Interestingly, activation of areas of the "pain matrix" remain intact in individuals lacking Nav1.7 (Salomons et al., 2016). Nav1.7 is a sodium channel essential for pain sensitivity and its absence abolishes the ability to detect pain. Even though the statistical power of this study is low – it only consisted on two Nav1.7 deficient subjects –, it supports the argument that pain-related areas may be encoding more than just pain.

3.3. From acute to chronic pain

Although pain is mostly an adaptive response to escape potential damage and, consequently, its duration is limited, pain occasionally becomes maladaptive. In these cases, intense pain persists for extended duration and is referred to as chronic pain. *Acute pain* typically lasts less than 4 weeks, becomes *subacute* when it extends up to 12 weeks, and is defined as *chronic* when it lasts longer than 3 months, persisting beyond the time needed for the tissue healing process (Yong et al., 2022) (**Fig. 5**).

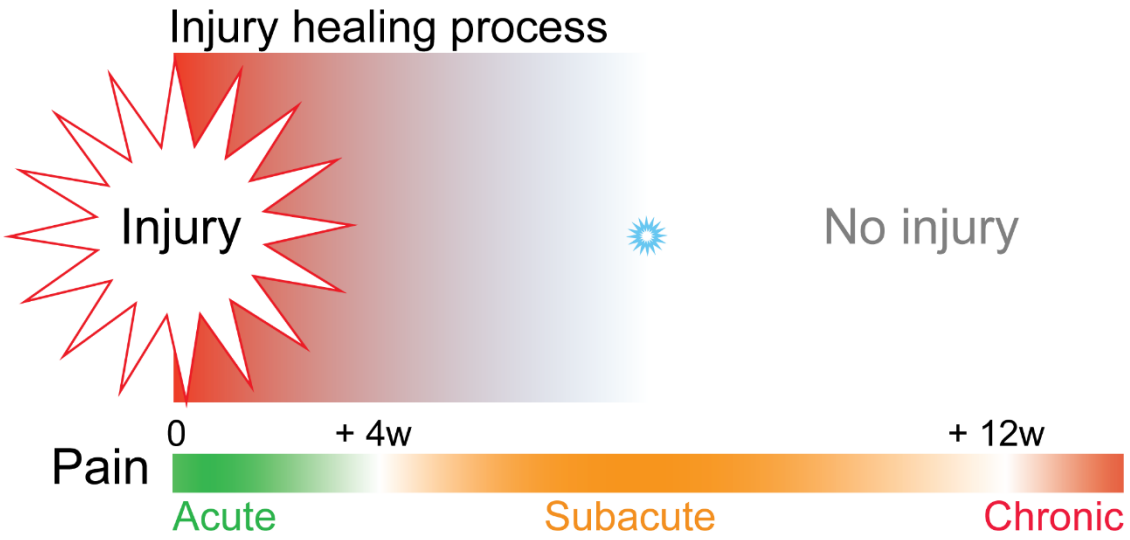


Figure 5. Temporal evolution from acute to chronic pain upon tissue injury. *Acute pain lasts 4 weeks, then it becomes subacute. During this time the injury can heal but the pain can persist. Once the injury has been healed but pain persists for longer than 12 weeks (3 months), the pain is classified as chronic pain.*

3.3.1. Electrophysiological signatures

Persistence of pain in the absence of trauma indicates that faulty neural activity may be a more plausible mediator of chronic pain rather than persistent noxious sensory input. In fact, it has been proposed that the imbalance resides between the areas that process pain input (dACC and somatosensory cortex) and the areas that mediate pain suppression (pgACC). The resulting electrophysiological phenotype of such imbalance is a slowdown of resting-state alpha activity that feeds theta power and is accompanied by an *edge effect* – beta-gamma increases that cause persistent theta-gamma cross-frequency coupling – (Llinás et al., 1999; Vanneste et al., 2018; Vanneste & De Ridder, 2021). These changes in oscillations have been named *thalamocortical dysrhythmia (TCD)* (**Fig. 6**) and reliably appear in patients with chronic pain (Di Pietro et al., 2018; Fallon et al., 2018; Llinás et al., 1999; Vanneste et al., 2018). Behaviorally, increases in theta seem to drive negative psychiatric symptoms (i.e. depression) while increases in gamma promote positive symptoms (i.e. enhanced pain perception) (Vanneste et al., 2018).

Another characteristic in chronic pain is the increase in connectivity strength between the default mode network and pain-related brain areas such as the somatosensory cortex, which

may explain why pain becomes an integral part of the patient's life (De Ridder et al., 2022). The default mode network has been linked to the parasympathetic nervous system and energy storage. Intriguingly, while acute pain increases the metabolic rate by 60%, chronic pain, only by 15%, suggesting a greater involvement of energy saving brain-related areas, such as the default mode network (De Ridder et al., 2022).

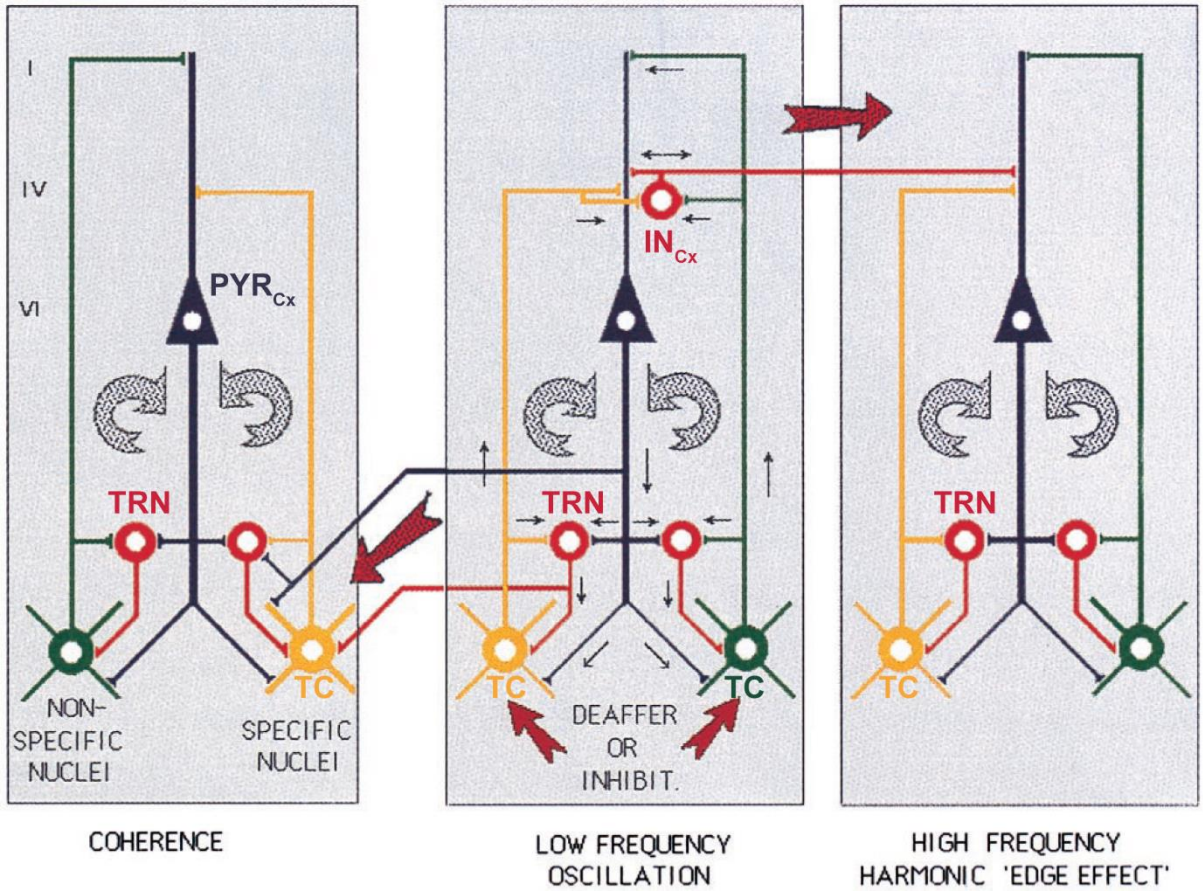


Figure 6. Model of thalamocortical dysrhythmia. *Normal functioning thalamocortical circuits (left). Increased hypepolarization in TC neurons (i.e. deafferentiation or too much inhibition) inactivate T-type calcium channels producing oscillations in the theta range (center). This type of acitivty induces cortico-cortical inhibitory activity (IN$_{Cx}$) that entrains gamma activity in neighboring cortical columns (right). Cx, cortex; IN, interneuron; PYR, pyramidal neuron; TC, thalamocortical; TRN, thalamic reticular nucleus. (modified from Llinás et al., 1999).*

3.3.2. From central sensitization to centralized pain

Central sensitization in the spinal cord can become the precursor of chronic pain. During central sensitization, neuronal excitability is enhanced, causing facilitation, augmentation, potentiation, and amplification of responses to noxious and non-noxious stimuli.

Central sensitization can remit, but in those cases where it persists, painful sensations arise even in the absence of stimuli (Latremoliere & Woolf, 2009). In some instances, however, pain persists in the absence of bottom-up sensory inputs (central sensitization) and will be generated by bottom-up processes (Hauck et al., 2015), becoming centralized pain.

The shift from central sensitization to centralized pain seems to be mediated by altered astrocyte-neuron coupling and can be observed in infraslow oscillations (< 0.1 Hz) within ascending pain pathways (De Ridder et al., 2021). Mechanisms that aid in this transition include epigenetics (Buchheit et al., 2012; Topham et al., 2020), microglia (Hiraga et al., 2022; Salberg et al., 2020), activation of the hypothalamic-pituitary-adrenal (HPA) axis (Eller-Smith et al., 2018; Salberg et al., 2020) and the microbiome (Guo et al., 2019).

3.3.3. Molecular signatures

Ultimately, lasting changes in oscillations result from lasting changes in the flow of ions. In turn, lasting changes in ion fluctuations stem from changes in the composition of ion channels, transporters and neurotransmitter receptors. Therefore, lasting changes in oscillations are due to molecular changes involving ion channels, transporters and neurotransmitter receptors.

Molecular changes related to central sensitization or centralized pain have been found in multiple brain areas related to affective and reward processing. In the ACC, cortical disinhibition (Blom et al., 2014) via a dysfunction of HCN channels (Santello & Nevian, 2015) has been described as well as increased phosphorylation of GluR1 (Xu et al., 2008). Upregulation of GluN2B-containing NMDAR has been found in the ACC (Hogrefe et al., 2022; Wu et al., 2005; Yang et al., 2015), insular cortex (Qiu et al., 2013) and PFC (Metz et al., 2009). Expression of κ-opioid receptors is reduced in the NAcc (Chang et al., 2014), where reduced expression of VGLUT-1 positive terminals suggests decreased glutamate release (Qi et al., 2018). In the amygdala, hyperactivation of the ERK pathway and increased mRNA expression of glucocorticoid receptors as well as of the metabotropic glutamate receptors mGluR1 and mGluR5 have been reported (Neugebauer, 2020).

Sensory related areas also sustain molecular changes in chronic pain. Decreases in GABAR in the thalamus (Zhang et al., 2017) play a role in the remodeling of thalamic afferents (Nagumo et al., 2020) and seem to be anticorrelated with enhanced cortical power (Di Pietro et al., 2018), suggesting chronic pain-induced thalamic disinhibition (Krause et al., 2019). The somatosensory cortex is subjected to increased spine motility and changes in synaptic button density which may reflect a synaptic reorganization of the cortical area (Kim et al., 2017). Additionally, astrocytes increase in mGluR5 elicit spontaneous Ca^{2+} transient (Kim et al., 2017), which together with increases in N-type voltage-dependent Ca^{2+} channel subunits (Okada et al., 2021) may contribute to increased synapse formation.

Molecular changes are undoubtedly behind the chronification of pain. Immunohistochemistry and *ex vivo* electrophysiology are very informative techniques, yet these imply the termination of the experiment. Those molecular changes, however, impact electrophysiological oscillations, which can be easily and reliably measured in a range of species, including mice and humans. Therefore, *in vivo* electrophysiology shows great potential to track the changes of the chronification of pain. In addition, changes that acute pain inflict in the network are very difficult to impossible to track in *ex vivo* experiments, reinforcing the utility of neuroelectrophysiological recordings.

4. Sleep

*"**Narrator:** In the living room, an evening after a rainy autumn day.*

***Marc:** Are you sure you want to start watching a movie now?*
***Laura:** Yes, of course! I've been wanting to watch this movie for months!*
***Marc:** But you always fall asleep when it's this late.*
***Laura:** (giggling) You're exaggerating Marc!*
***Marc:** Alright… alright…*

***Narrator:** She fell asleep 15 minutes after the movie started."*

All animals oscillate between two main states: wake and sleep. High muscle tone and active cognitive processing characterize the former. The latter features very low muscle tone (or muscle atonia), decreased responsiveness to external stimuli, decreased temperature, and lower levels of consciousness. In addition, the cerebral metabolic rate of glucose decreases in sleep by 44%, and that of oxygen, by 25%, setting the brain into an anabolic state.

Although sleep is usually aligned to the circadian clock of the individual, factors such as age and disease vary sleep architecture, characteristics, times, and needs. For instance, in developing animals, sleep, particularly REM, is the dominant behavioral state and the cycling between states is faster than in adults.

4.1. The two-process model of sleep regulation: Sleep homeostasis and circadian clock

In 1982, Bobérly proposed that transitions between sleep and wake depended in two processes: C and S. The *process C* is defined by the circadian cycle driven by the suprachiasmatic nucleus (SCN) and does not depend on the previous sleep history. The *process S* rides on top of process C and is known as sleep homeostasis. Process S strengthens as sleep pressure – measured as increases in the power of slow wave activity (SWA, 0.5-4 Hz) – intensifies as a function of time. In other words, time awake increases Process S, and sleep, decreases it. Naps can alleviate sleep pressure, resetting process S and decreasing the need for sleep. Inversely, prolonged wake keeps the rise of process S. However, if process C is low even when process S is high, falling asleep may be more difficult. This is an effect commonly seen in night workers (**Fig. 7**).

Note that additional factors also modulate sleep-wake transitions. These include environmental cues (i.e. auditory stimuli), internal needs (i.e. emotional status), breathing (i.e. sleep apnea) and immune system (i.e. fever) (Eban-Rothschild et al., 2017).

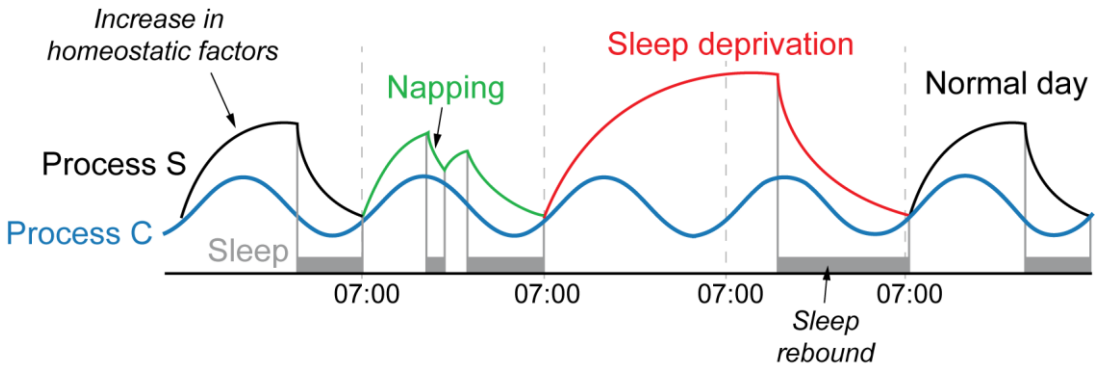


Figure 7. Two-process diagram. *Process C (blue) is usually invariable. Process S changes over time as a factor of events during the day such as napping (green) and sleep deprivation (red). Increases in process S indicate sleep pressure and these are driven by accumulation of homeostatic factors in the cortex and basal forebrain. Homeostatic factors clear out during sleep.*

4.2. Sleep and wake: Oscillations, circuits, and neuromodulators

Sleep and wake are two very distinct physiological states that in the brain manifest as differences in:

1) the network functional connectivity
2) the composition of the extracellular milieu
3) the proportion of neuromodulators and neurotransmitters
4) blood and glymphatic flow

These physiological differences in wake and sleep set the neurons to fire in different patterns, generating characteristic electrical activity that electroencephalography (EEG) can measure. These oscillatory patterns (EEG) together with muscle activity (electromyogram, EMG) define three main arousal states (**Fig. 8**):

1) Wakefulness
2) NREM sleep
3) REM sleep

Detailed subclassification of behavioral states is commonly performed in human studies. For instance, NREM is divided in four subtypes (N1 or light sleep, N2 and N3-N4 or deep sleep) and cognitive processes like attention, drowsiness, eyes-closed, and mind wandering are often distinguished in wake. Animal research lacks this detailed subclassification (exemplified in the absence of NREM substaging in rodents despite obvious fluctuations). Yet, new equipment and analysis tools provide the power to classify behavioral states more accurately.

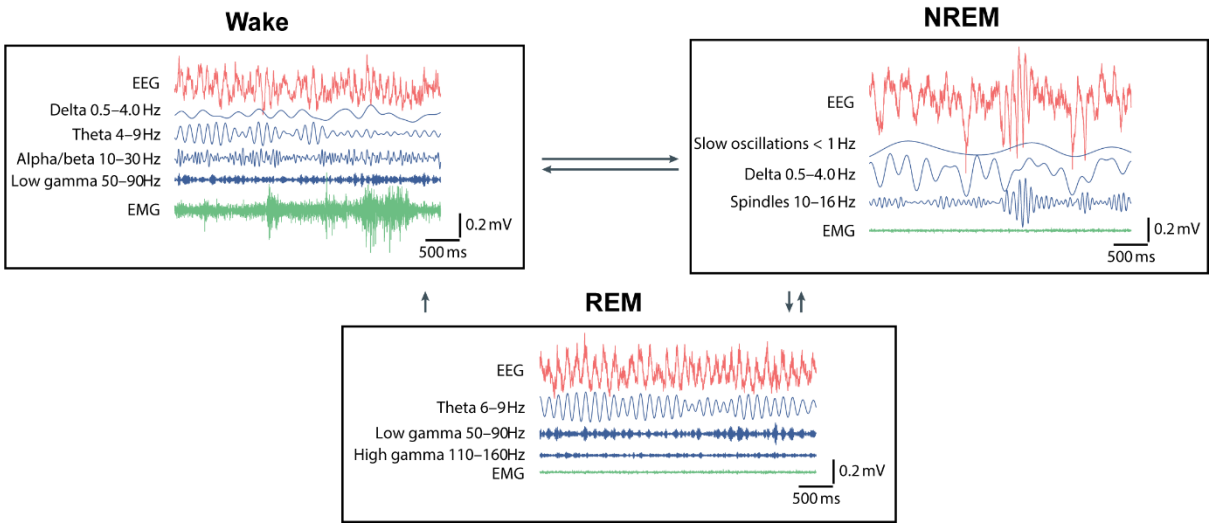


Figure 8. Oscillations in wake and sleep. *Electrophysiological signatures featuring the most prominent oscillations of each arousal state. Modified from Adamantidis et al., 2019.*

4.2.1. Wake

Wakefulness is defined by low voltage (low amplitude) and high frequency EEG activity. During wake, lower frequency rhythms such as theta (4-8 Hz) arise over widespread areas and synchronize faster, locally generated rhythms (beta (16-30 Hz) and gamma (>30 Hz)) of functionally interrelated areas. These rhythms arise from interactions between GABAergic neurons in the thalamoreticular nucleus (TRN), thalamocortical (TC) neurons and corticothalamic (CT) pyramidal neurons (Adamantidis et al., 2019; Crunelli et al., 2018).

The intermediate state from sleep to wake receives the name of *sleep inertia* and contains elements of sleep such as slow oscillations and theta waves. This is characterized by low levels of vigilance and awareness, together with confusion and cognitive impairment (Adamantidis et al., 2019; Brown et al., 2012).

4.2.1.1. *Gamma*

The transition from sleep to wake is characterized by the release of acetylcholine (ACh), norepinephrine (NE) and histamine (His) onto TC relay neurons. The resulting depolarization causes a switch in the firing pattern from bursting to tonic mode, favoring gamma activity. Additionally, medium spiny neurons in the striatum projecting to TC neurons also promote tonic firing.

Another mechanism of gamma activity are the inhibitory projections from fast-spiking amino acid γ-aminobutyric acid (GABA) neurons in the basal forebrain (BF) to cortical and hippocampal PV interneurons, which generate cortical and hippocampal gamma.

4.2.1.2. *Wake-promoting circuits and neurotransmitters*

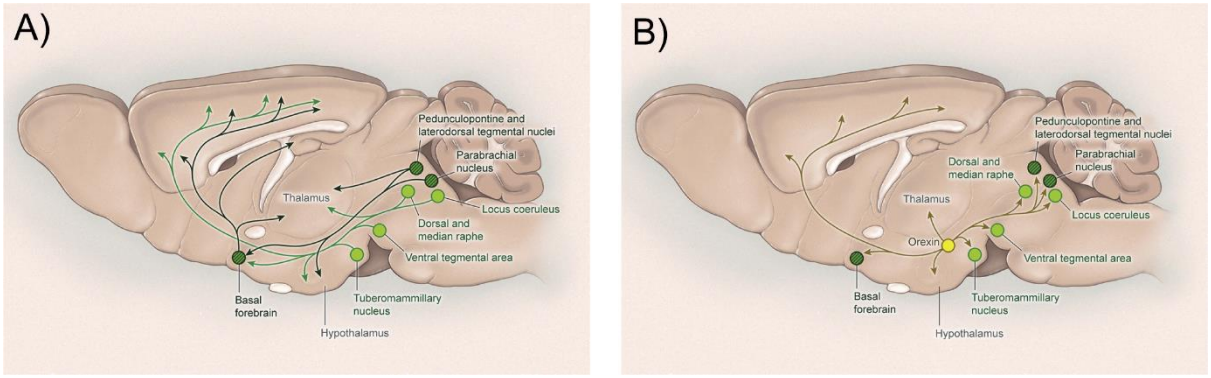


Figure 9. Wake-promoting circuits. *A) Monoaminergic pathways (light green) and cholinergic pathways (dark green) promoting awakening. B) Orexigenic projections from the lateral hypothalamus that maintain wakefulness. Modified from Scammell et al., 2017.*

Redundant diffuse circuits of multiple neurotransmitter systems promote wakefulness (**Fig. 9**). These are interconnected in a mutually mostly excitatory (glutamatergic) network and none of them is essential for wake. Wake-promoting brain areas are located throughout the brainstem, hypothalamus, and basal forebrain (Brown et al., 2012). These brain areas are primarily innervated by a range of neurotransmitters (**Table 1**), indicating that the transition between sleep and wake is complex; there is no single "switch" between these states.

Brain area	*Neurotransmitter*
Parabrachial nucleus (PBN)	Glutamate
Locus coeruleus (LC)	Norepinephrine (NE)
Laterodorsal tegmental nuclei (LDT)	Acetylcholine (Ach)
Pedunculopontine tegmental nuclei (PPT)	Acetylcholine
Tuberomammillary nucleus (TMN)	Histamine (His)
Dorsal raphe nucleus (DRN)	Serotonin (5HT)
Ventral tegmental area (VTA) and periaqueductal gray (vPAG)	Dopamine (DA)
Lateral hypothalamus (LH)	Orexins Melanin-concentrating hormone

Table 1. *Wake promoting areas and their neurotransmitters.*

These neurotransmitter systems convergently project to the thalamus, basal forebrain (BF) and cortex and seem to be required for different aspects of wakefulness (Brown et al., 2012) (**Table 2**).

Function	*Neurotransmitter*
Inhibition of sleep active neurons	NE, 5HT, ACh
Facilitation of arousal	ACh, His
Reward-induced arousal	DA, ACh
Aversion-induced arousal	NE, 5HT, His
Wake consolidation	Orexins
Maintenance of high muscle tone	NE
Enhancement of synaptic plasticity for memory consolidation	ACh, NE, 5HT, His, DA, orexins

Table 2. *Functions of neurotransmitters during wake.*

4.2.2. NREM sleep

NREM stands for Non-Rapid Eye Movement as it only presents slow rolling eye movements compared to the fast eye movements found in Rapid Eye Movement (REM) sleep. NREM ensues from an increase in homeostatic factors (i.e. adenosine, nitric oxide (NO) and GABA) that inhibit wake-promoting systems. NREM characteristic large amplitude and low frequency oscillations (< 4 Hz), as well as sleep spindles, originate in reverberating thalamo-cortical loops (Adamantidis et al., 2019; Brown et al., 2012; Crunelli et al., 2018) where T-type Ca^{2+} channels in the thalamus greatly influence the oscillation type (**Fig 10**).

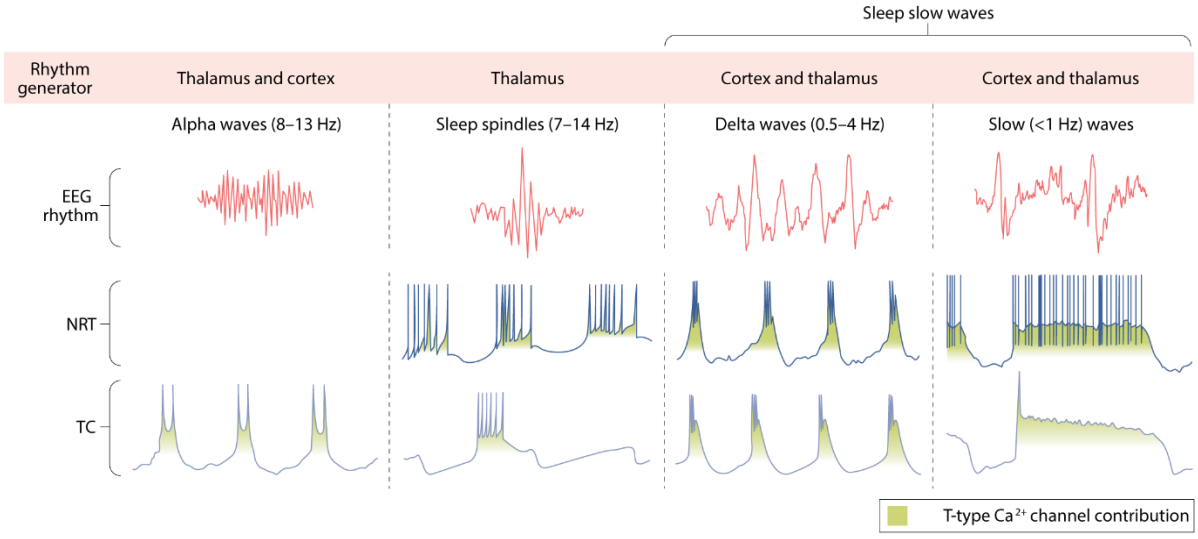


Figure 10. Thalamocortical generation of NREM rhythms. *Contribution of the different thalamocortical components that generate the oscillations of states of low vigilance together with the contribution of T-type Ca^{2+} channels activity. NRT, nucleus reticularis thalami (a.k.a. TRN); TC, thalamocortical neurons. (Crunelli et al., 2018)*

The four NREM categories in humans are based on changes in oscillatory patterns and levels of arousability (Adamantidis et al., 2019). Stage N1 is initiated by cortical alpha waves (8-11 Hz) followed by theta (4-8 Hz) oscillations, resembles drowsy wake, and awakening is highly likely. The presence of sleep spindles (transitory 11-14 Hz oscillations) and K-complexes marks the transition into stage N2. Stages N3 and N4 are known as deep sleep and are characterized by prominent delta waves (1-4 Hz), slow oscillations (< 1Hz) and low probability of awakening.

4.2.2.1. Slow waves

Slow waves (0.5-4 Hz) tend to originate in frontal regions and propagate along a frontoparietal axis, but they attenuate over time and only a small portion reach posterior cortices. These include slow oscillations (SO, < 1Hz) and delta waves (1-4.5 Hz) (Adamantidis et al., 2019; Brown et al., 2012).

SO arise from the synchronization of large groups of neurons that alternate between a depolarized (a.k.a UP or ON state) and a hyperpolarized state (a.k.a DOWN or OFF state). During the UP states, increased cell depolarization promotes excitatory and inhibitory thalamocortical and corticothalamic cells to fire bursts of action potentials. Inversely, during the DOWN states, cell hyperpolarization induces a state of relative quiescence. The duration of the UP and DOWN states – what defines SO frequency – can differ between animal species, previous sleep history and site of the recordings. Mechanistic studies suggest that UP states arise from L5 cortical neurons and are extinguished by astrocytes and inhibitory cortical interneurons. It is still unclear whether the ontogeny of SO stems from the thalamus, the neocortex or a combination of the two.

There are two proposed mechanisms for the generation of delta waves:

1) Cortical origin dependent on acetylcholine-sensitive potassium conductance.
2) Thalamic origin. Membrane hyperpolarization activates the hyperpolarization-activated cation current (I_h) progressively increasing membrane depolarization and subsequently opening T-type Ca^{2+} channels. This, in turn, activates low threshold Ca^{2+} currents (I_T), resulting in low-threshold Ca^{2+} spikes.

4.2.2.2. *K-complexes and Sleep spindles*

K-complexes – brief high voltage negative potential followed by a positive peak – are common in humans but remain underinvestigated in rodents. These may be spontaneous or evoked by external stimuli, resembling evoked-potentials (EP). However, stimulus-induced K-complexes seem to be independent of thalamo-cortical relay and dependent on the timing of the stimulation relative to the cortical UP and DOWN states.

Sleep spindles are transient 6-15 cycles of 11-15 Hz oscillations that last between 0.2 and 2 seconds, with variable amplitude that peaks at 100 μV. Hence, their description as "*transient, waxing and waning"* (Adamantidis et al., 2019). Sleep spindles frequently appear during the UP states of SO and are distributed across frontal (fast sleep spindles) and occipital (slow sleep spindles) areas. The decrease of aminergic input to the thalamus initiates sleep spindles through activation of low-threshold T-type Ca^{2+} channels in the TRN. The TRN, then, generates bursts of activity in the spindle range, consequently hyperpolarizing TC neurons. Through I_h currents and I_T currents, TC will generate a low-threshold calcium spike followed by a burst of sodium-dependent action potentials that will excite L4 pyramidal neurons, giving rise to a sleep spindle. The cortex as well as an increase in the aminergic tone in the thalamus drive termination of sleep spindle activity. Spindles are implicated in memory consolidation and

cognition and appear shortly before the transition to REM sleep (Adamantidis et al., 2019; Brown et al., 2012; Crunelli et al., 2018; Eban-Rothschild et al., 2017).

4.2.2.3. *NREM-promoting circuits and neurotransmitters*

Most NREM generating circuits consists of GABA- and galanin-releasing neurons that inhibit wake-promoting circuits and cortical glutamatergic neurons (**Fig. 11**). These neurons reside in the ventrolateral preoptic area (VLPO), the anterior hypothalamus and the BF. GABAergic neurons in the parafacial zone (PZ) in the brain stem also promote NREM by inhibiting glutamatergic PB neurons, which, in turn project to wake-promoting magnocellular BF neurons. Adenosine receptor-expressing cells in the NAcc and zona incerta (ZI) also induce NREM. Interestingly, VLPO neurons receive direct inputs from the retina and indirect inputs via the dorsomedial hypothalamus from the suprachiasmatic nucleus, the driver of Process C. Additionally, many of the neurons in VLPO are sensitive to temperature, explaining the coupling of sleep and body temperature.

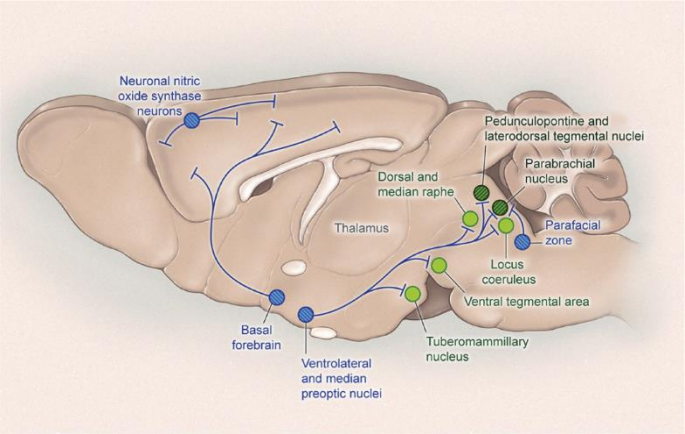


Figure 11. NREM-promoting circuits. *GABAergic neurons in the preoptic area and the basal forebrain inhibit wake-promoting areas. Modified from Scammell et al., 2017.*

Wake generates molecules that accumulate in the BF and cortex inducing sleep. These are termed *homeostatic factors* and comprise neuromodulators (nitric oxide (NO) and adenosine), cytokines (TNF-α, IL-1), prostaglandin D2 (PDG2), peptides, growth factors (BDNF, GHRH), delta sleep inducing peptide, uridine, oxidized glutathione and muramyl peptide.

1. **NO**. Synthesized by NO synthase in neurons (nNOS) and endothelial cells (eNOS). Production is highest in wake, and cortical neurons containing nNOS seem to underlie homeostatic sleep regulation. NO additionally stimulates adenosine release, which further contributes to sleep pressure.

2. **Adenosine**. Byproduct of ATP utilization linking neuronal energy metabolism with sleep. Adenosine binds to adenosine 1 receptors (A1R) in wake-promoting neurons, causing inhibition and inducing sleepiness and decreased vigilance. It also binds to A2R in sleep-active neurons, which mediates its somnogenic effects. Furthermore,

adenosine activates P2 purinergic receptors in astrocytes, consequently producing **IL-1** and **TNF- α**.

3. **PDG2.** Lipid signaling molecule generated from arachidonic acid through the cyclooxygenase pathway. Its levels in the brain progressively increase during wake and its somnogenic effects are mediated via A2A receptors in the VLPO as well as in the leptomeninges and the arachnoid space. Interestingly, PGD2 contributes to neuropathic pain in the spinal cord (Kanda et al., 2013).

4.2.3. REM sleep

REM, also known as "paradoxical sleep", is characterized by a complete loss of muscle tone (muscle atonia) coupled with heightened cortical activation represented as dominant low amplitude theta (4-8 Hz) and gamma (>30 Hz) oscillations. This is coupled with a decrease of slow waves in frontal cortices.

4.2.3.1. *Theta rhythm*

The theta rhythm occurs both in wake and in REM sleep and has been classified as type I and type II. Type I is insensitive to atropine and predominantly arises during locomotion and cognitive tasks as well as in REM sleep. In the latter case, it has been linked to contextual memory consolidation. Type II is affected by atropine and occurs during fear-induced freezing and urethane-induced anesthesia. Type II will not be further discussed here.

The hippocampus seems to drive the theta rhythm via hyperpolarization-activated and cyclic nucleotide-gated non-selective cation channels expressed in the medial septum GABAergic neurons, which inhibit hippocampal pyramidal cells. Cholinergic neurons in the medial septum fine-tune the hippocampal theta rhythm by either increasing the amplitude or inhibiting circumventing oscillations (i.e. slow waves and beta range frequencies). Other modulatory mechanisms of hippocampal theta rhythm include the entorhinal cortex, posterior hypothalamus (supra-mammillary glutamate neurons, which are also involved in wakefulness), and brainstem circuits (pontine cholinergic cells).

4.2.3.2. *Gamma oscillations*

Gamma oscillations are ubiquitously recorded during wakefulness and REM. Gamma arises from the synchronization of local neuronal assemblies of inhibitory interneurons. Inhibition is mediated by postsynaptic $GABA_A$ receptors in either other local interneurons (I-I model) or in pyramidal cells (E-I model) or by both. High gamma activity during sleep prevents awakening and seems to be related to consolidation of awake experiences as memories.

4.2.3.3. *REM-promoting circuits and neurotransmitters*

REM promoting circuits reside in the brainstem and are modulated by hypothalamic and basal forebrain circuits (**Fig. 12**). For instance, melanin-concentrating hormone neurons in the lateral hypothalamus modulate frequency and duration of REM bouts, and PPT and LDT cholinergic cells time the REM bouts.

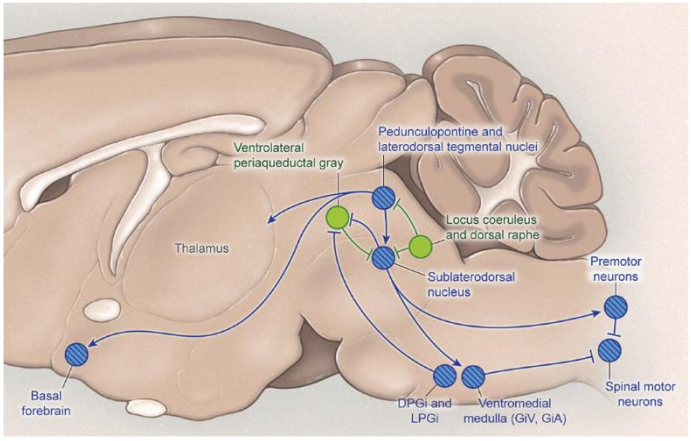


Figure 12. REM-promoting pathways. *Neurons in the sublaterodorsal nucleus (a.k.a. subcoeruleus nucleus) activate inhibitory premotor neurons and neurons in the ventromedial medulla (VMM) to inhibit spinal motor neurons. Neurons in the pedunculopontine and laterodorsal tegmental nuclei promote theta oscillations. Modified from Scammell et al., 2017.*

The brainstem houses the circuits that promote REM and execute sleep paralysis. Glutamatergic cells in the subcoeruleus nucleus (SubC) are responsible for the complete muscle atonia of REM at the same time that control the timing of REM.

SubC glutamate neurons excite GABA and glycinergic cells in the ventromedial medulla (VMM) that inhibit somatic and motor neurons that drive muscle movements. Simultaneously, SubC glutamatergic cells also project to the basal forebrain, causing the cortical activation observed in REM.

The characteristic muscle twitches in REM result from glutamatergic bursts that activate AMPA/kainite receptor.

REM is terminated when GABAergic neurons in the ventrolateral periaqueductal gray (vlPAG) inhibit glutamatergic SubC neurons.

5. Sensory perception during sleep

"That was the only thing Oliver had in mind. He just couldn't make sense of it.
He would fall asleep at night and suddenly it would be morning again!
What had happened all that time he was asleep?

Oliver had no recollection. Or did he?"

Sleep is no longer understood as a state of total disconnection from the environment (Hennevin et al., 2007). Furthermore, Osorio-Forero et al., 2021 recently revealed in mice the presence of periods of higher arousability during NREM driven by activation of the Locus Coeruleus with the putative role of sampling the environment. While low reactivity to external stimuli is indeed one of the hallmarks of sleep (Steriade, 2003), cumulating evidence is showing that despite stimuli not always evoking an overt behavioral reaction, peripheral sensory information can reach the cortex and be processed during sleep.

In particular, auditory stimuli are well known to activate cortical neurons in the primary auditory cortex and other upstream areas in NREM and REM sleep in rodents (Nir et al., 2015; Sela et al., 2020) and primates (Issa & Wang, 2008). Further studies in humans have confirmed these findings and have additionally shown the preserved ability to classify words based on their semantic meaning during light NREM (Andrillon et al., 2016), as well as to process simple narratives during REM (Fogel et al., 2022). Interestingly, calling the participant's name as well as the sound of unfamiliar voices during sleep evoke awakenings more often than any other sound, suggesting that the social, and probably biological, relevance of the stimulation can also be processed during sleep (Ameen et al., 2022; Perrin et al., 1999).

Although sensory perception during sleep has mostly been studied using auditory paradigms, some studies have also used visual cues (Sharon & Nir, 2018), extending the knowledge about sensory modalities that can be processed during sleep. In addition, targeted memory reactivation using olfactory cues confirms that odor is another sensory modality that is perceived and processed during sleep.

Research on sensory perception during sleep has bloomed in the past decade. However, nociceptive processing during sleep has remained under-investigated, even though the relationship between sleep and pain is well established (Bentley et al., 2003; Byers et al., 2016; Cardis et al., 2021; Chouchou et al., 2011; Dalanon et al., 2021; Finan et al., 2013; Krause et al., 2019; Rosseland et al., 2018; Smith & Haythornthwaite, 2004; Staffe et al., 2019; Wilson et al., 2002).

Overarching aims of this thesis

"No plan survives contact with the enemy"

Adapted from Helmuth von Moltke

This thesis aims at providing *in vivo* electrophysiological measurements to advance the understanding of neural correlates reflecting the bidirectional interaction of pain and sleep.

5.1. Manuscript #1: Somatosensory processing in sleep and wake

In the first part of the thesis, I took a naturalistic approach to assess how sleep modulates the processing of nociception at the cortical end of the medial (ACC) and the lateral (S1) pain pathway. In addition, I used the EEG to evaluate whether intra-cortical oscillatory features spread globally in the network. Furthermore, correlating autonomic responses with neural frequency bands associated with pain processing (gamma) and attention (alpha) I showed a new method to distinguish a noxious stimulation from a non-noxious one. The results of the first part of the thesis are collected in Manuscript #1 "*Interactive Effects of Pain and Arousal State on Heart Rate and Cortical Activity in the Mouse Anterior Cingulate and Somatosensory Cortices"*, in preparation to be submitted to Journal of Neuroscience.

5.2. *Manuscript #2: Effect of chronic pain in the S1 and ACC in sleep and wake*

In the second part of the thesis, I evaluated how permanent pain affects sleep and brain dynamics. To this aim I used a mouse model for chronic pain to investigate the effects of pain on sleep. Chronic pain disrupted the sleep phenotype only for a limited time. For this study, I also evaluated the receiving ends of the medial (ACC) and lateral (S1) pain pathway. Interestingly, the EEG – representing global neural activity – did not recapitulate the changes in power spectral density profile and signal autocorrelation recorded in the ACC and S1. Moreover, here I show that features of the thalamo-cortical dysrhythmia described in humans are predominantly found in the ACC. Manuscript #2 *"Effects of Chronic Pain in the Sleep Phenotype and in the ACC and S1 Activity during Sleep and Wake in Mice"* collects the results of the second part of the thesis and presents preliminary data.

Results

"The most exciting phrase to hear in science, the one that heralds new discoveries, is not 'Eureka!' but 'That's funny...'"

Attributed to Isaac Asimov

1. Manuscript #1

Interactive Effects of Pain and Arousal State on Heart Rate and Cortical Activity in the Mouse Anterior Cingulate and Somatosensory Cortices

My contribution

I conceptualized and designed the experiments, as well as designed and built the recording rig and analysis pipeline.

I carried out all aspects of the experiments, from implant assembly and implantation surgeries to data collection, management, curation, and analysis, in addition to the termination of experiments, tissue collection and histological verification.

I self-taught Python and wrote the entire analysis pipeline and the statistics in this programing language. Dr. Mike X Cohen provided the knowledge on data curation, analysis methods and statistics. Mike and I together developed the algorithms measuring the heart rate and its variability.

I trained Margot Renard to assist in all the steps of the experiments and I taught her sleep scoring. For a batch of 8 animals, Margot assisted in the implantation surgeries and participated in the termination of the experiments, tissue retrieval and histological verification for these animals. She also collected the data for this batch and performed the sleep scoring, which I later revised.

I wrote the entirety of the manuscript, performed the analysis and statistics, and designed and generated the figures using Python.

Interactive Effects of Pain and Arousal State on Heart Rate and Cortical Activity in the Mouse Anterior Cingulate and Somatosensory Cortices

Sandoval Ortega RA[1], Renard M[1], Cohen MX[2], Nevian T[1]

[1] Neuronal Plasticity Lab, Department of Physiology, University of Bern, Buehlplatz 5, 3012 Bern, Switzerland

[2] Synchronization in Neural Systems Lab, Donders Centre for Medical Neuroscience, Radboud University Medical Center, Houtlaan 4, 6525 XZ Nijmegen, the Netherlands

Abstract

Sensory disconnection is a hallmark of sleep, yet the cortex retains the ability to process sensory information such as auditory inputs. Acute painful stimulation during sleep increases the likelihood of awakening, indicating that certain mechanisms for pain processing remain active. However, processing of somatosensensation, including pain, during sleep remains underexplored.

To identify cortical spectral fingerprints of somatosensation during sleep, we simultaneously recorded local field potentials in the anterior cingulate (ACC) and the somatosensory (S1) cortices in mice while animals received noxious and non-noxious (control) mechanical stimuli to the hind paws throughout their sleep cycle.

Noxious stimuli evoked stronger heart rate increases compared to non-noxious in both wake and NREM sleep. Somatosensation reached the S1 and ACC in sleep and elicited complex transient and sustained spectral responses in the delta, alpha, and gamma. These dynamics were modulated by the sleep state, behavioral reactivity to the stimulation, and the stimulation intensity (non-noxious vs. noxious). Furthermore, correlations of the heart rate with the alpha band suggested sustained attention in wake and increased awareness during sleep, while correlations with the gamma band allowed to disentangle saliency from pain.

This study is the first to use freely behaving animals to evaluate somatosensory processing in natural sleep. Furthermore, it highlights the potential of combining physiology measures and neural data to gain a more comprehensive understanding of brain processes sharing neural signatures (i.e. saliency and pain). This approach can also be useful in detecting pain in individuals or animals that are unable to communicate.

Significance statement

Here we investigated how sensory and affective components of somatosensory information are processed in naturally sleeping mice, by recording heart rate and local field potential activity from the S1 (sensory component) and ACC (affective/cognitive component) while providing noxious or non-noxious stimulation of the hind paw. Increases in heart rate

confirmed the perception of the stimuli during sleep, whereas increases in heart rate variability may contribute to prevent awakening. Stimuli elicited a complex spectral response that included a sustained alpha suppression and transient delta/gamma enhancement. These cortical responses differentially correlated with heart rate dynamics, suggesting that brain-physiology correlations may provide dissociable signatures of saliency and attention during somatosensory processing.

Introduction

Sleep is no longer described as a state of total disconnection from the environment (Hennevin et al., 2007). Auditory and visual stimuli have been shown to activate primary and supplementary cortical areas during sleep (Andrillon et al., 2016; Nir et al., 2015; Sela et al., 2020; Sharon & Nir, 2018). Yet, evidence on somatosensory processing while asleep is lacking.

Pain-induced sleep disruptions (Finan et al., 2013) hint at pain processing during sleep. Most research, however, has focused on pain processing during wake (Su et al., 2020) and only few studies compared noxious evoked potentials in sleep and wake. In humans, laser-evoked potentials in the cingulate, insula, and operculum were smaller in NREM compared to wake (Bastuji et al., 2012). Electrical stimuli displayed the same trend in the secondary somatosensory cortex (S2) (Kakigi et al., 2003) as well as in the primary somatosensory cortex (S1), motor cortex, cingulate, insula and medial temporal area (Wang et al., 2004). However, the opposite effect was observed in S1 and S2 after median nerve stimulation (Kitamura et al., 1996) and in EEG recordings from rats (Shaw et al., 2006). Despite the discrepancies, these studies confirm that pain during sleep engages cortical areas belonging to the "pain matrix" (de Ridder et al., 2022; Legrain et al., 2011).

Areas recruited by painful stimulation are also recruited upon salient stimuli from other sensory modalities (Liberati et al., 2016), questioning whether activation of the "pain matrix" follows saliency and directed attention, in addition to pain (de Ridder et al., 2022; Legrain et al., 2011).

A physiological measure of pain used for the objective assessment of pain perception in the clinic is the heart rate (HR) (Lechner et al., 1998). Increases in HR follow acute painful stimuli (Hilgard et al., 1974; Möltner et al., 1990; Tousignant-Laflamme et al., 2005) as a result of autonomic nervous system (ANS) activation, particularly, of the sympathetic system (Kramer et al., 2012; Loggia et al., 2011; Terkelsen et al., 2005). Heart rate variability (HRV), which indicates the variance in the interval between heartbeats, is also used as an indicator of ANS activation (Forte et al., 2022). Furthermore, correlations between HR and neural activity suggest that brain-physiology correlations have the potential to better contextualize neural activity (Al et al., 2020).

The S1 and ACC are consistently activated upon painful stimuli (Apkarian et al., 2005; da Silva & Seminowicz, 2019; Garcia-Larrea & Peyron, 2013; Peyron et al., 1999). The S1 receives the terminal projections of the lateral pain pathway and processes the sensory component of pain. The ACC, on the other hand, receives the terminal projections of the medial pain pathway and processes the affective-motivational component of pain. This anatomical and

functional separation allows for the dissociation of the sensory and emotional experience of pain. This dichotomy is demonstrated by anterior cingulotomy, which preserves the sensation of pain while eliminating discomfort (Agarwal et al., 2016).

Given the differential role of S1 and ACC in pain processing, we implanted mice in these two cortices and simultaneously recorded the heart rate. We hypothesized that correlations between the heart rate and known markers of sensory processing and pain intensity, such as increases in gamma activity in the S1 (Heid et al., 2020; Zhang et al., 2012b) can differentiate painful from not painful stimuli. In addition, suppression of alpha in sensory cortices contralateral to the stimulation site is interpreted as increased attention to the origin of the stimulation (Bacigalupo & Luck, 2019; Ikkai et al., 2016). Hence, alpha-HR correlations can potentially indicate changes in attentional processes in wake and changes in brain state in sleep, even in the absence of a behavioral response.

Our results confirm the processing of nociception in the S1 and ACC during sleep. Furthermore, we show the potential of simultaneously recording neural activity and the heart rate to disentangle noxious from non-noxious processing in addition to remove the effect of saliency.

Materials and Methods

Animals

Male C57BL/6J mice aged 6 weeks were grouped together, caged with food and water *ad libitum*, in a 12:12 light-dark cycle with lights on at 06:30 (corresponding at ZT0) and lights off at 18:30. At 12-14 weeks of age, 17 animals were implanted in the ACC and the S1HL. Misplaced tetrodes were excluded post-hoc. Following implantation, animals were kept housed together. Recordings started 14-16 days after the implantation surgery. All experiments were conducted after the approval of the cantonal veterinary office of Bern, Switzerland.

Surgery for chronic neural recordings

Anesthesia was initially induced with 5% isoflurane at a flow of 2l/min of O2 and analgesia was achieved with an initial injection of 2.5 mg/kg, 0,5 mg/ml of carprofen. During surgery animals were maintained at 1.5 – 2% isoflurane at a flow of 1.5l/min of O2. Animals were fixed on a stereotaxic frame and 0.3 mm craniotomies were performed to implant tetrodes bilaterally in the ACC (AP: + 0.6, ML: +/- 0.3, DV: - 1.4 from brain surface) and the S1HL (AP: -0.1, ML: +/- 1.9; DV: -0.4). Craniotomies of 0.9 mm were used to insert stainless steel screws to measure the frontal electroencephalogram (EEG) (AP: +2.5, ML: - 1.4), the parietal EEG (AP: -2.6, ML: - 2.5) and the ground (GND; AP: -5.2, ML: +/- 1.5). Finally, two bare-ended electromyogram (EMG) wires were sutured to the epaxial neck muscles to record muscle tone and two additional bare-ended EMG wires were positioned at the lower back to record the electrocardiogram (ECG). The GND screws on the cerebellum were used as reference.

Data acquisition

Neural signals were acquired and amplified using RHD2000 amplifier boards and digitized at a rate of 20'000 Hz by the Intan RHD USB Interface board (Intan Technologies, Los Angeles, CA). Animals were habituated to the recording setup 1 – 3 days previous to the first recording session. Recording sessions were performed on the second half of the light cycle and the first stimulation was performed between ZT6 and ZT7. One recording session consisted of 100 stimuli of one type (either Nox or NN). The inter-trial interval as well as the stimulated hind paw were random. Each animal received a total of 200-300 stimuli per stimulation type. The experimenter marked stimuli that induced paw withdrawal with a TTL pulse. To synchronize the stimuli with the electrophysiological data, either a 20 G needle (for the painful stimulation) or a yellow pipette tip (for non-painful stimulation) were attached to a load cell, which in turn was connected to the Intan RHD USB Interface board. This system allowed defining the precise start and end of each stimulation. Stimuli were applied to the planar surface of the hind paws.

Histological verification of intracranial recording sites

After the recording experiments, animals were deeply anesthetized with 5% isoflurane at a flow of 2l/min of O2 followed by an intraperitoneal injection of 80/10 mg/kg Ketamine/Xylosine mixture. To confirm the tetrodes location, electrolytic lesions were performed by applying 2 seconds long 30 µA current 5 times per electrode. After transcardial perfusion of 4% paraformaldehyde (PFA), heads were kept in PFA at 4ºC for 4 days. Brains were retrieved on day 5 and further post-fixed in PFA for 12h at 4ºC. Brains were washed in PBS and sliced at 70 µm. Brain slices were stored in PBS. For imaging, brain slices were mounted with Mowiol ® 4-88 mounting medium and imaged to confirm the location of the tetrodes.

Analysis

Stimulation detection

The voltage deflection signals of the stimuli were down-sampled at 1000 Hz. Using a custom-made software, the signal was displayed and the researcher marked the onset and offset of each stimulation.

Sleep scoring

The signals used for sleep scoring were the two EMG of the neck, the frontal EEG and the parietal EEG. The scoring of the different arousal states was done manually using a custom-made software written in Python. This software was designed to score without a scoring window, allowing for high temporal precision and precise marking of transition periods, microarousals and quick state transitions. Artifacts and movement-related noise were marked during the scoring and discarded a posteriori.

Data preparation

EEG and local field potentials (LFP) were low-pass filtered at 300 Hz. EMG was bandpass filtered between 100 Hz and 500 Hz. LFP, EEG, EMG and ECG were all down-sampled to 1000

Hz. Filters for EEG, LFP and EMG were applied before down-sampling. ECG filters were applied after down-sampling. To process the ECG, first, slow oscillations were removed, then high frequency muscle activity smoothed with the *scipy.signal.wiener* filter.

Data epochs were cut from the continuous signals from 10 seconds pre- to 20 seconds post-stimulation onset. Automated detection of noisy trials was done per animal using z-scoring on the time series. Any trial which z-score surpassed ± 3 std was eliminated.

Quantification of Somatosensory Evoked Potentials and peaks detection

Somatosensory evoked potentials (SEPs) were lowpass filtered below 20 Hz using *scipy.signal.filtfilt*. The resulting time-series were cut between -0.2 and 1 sec. Baseline normalization was done by subtracting the mean of the pre-stimulus window (-0.3 to -0.1 sec).

We were interested in extracting peak amplitude and timing from the first and last voltage deflections of the SEPs. However, variance in the signals was high. Therefore, a Gaussian fit using *scipy.optimize.curve_fit* was applied to the signal to obtain the peak time. We centered a window around the peak time to collect the peak amplitude from the empirical data. The ampltitude was the mean of the values that fell within that window. The length of the window was 10% of the duration of the peak set by the time boundaries manually chosen for the modeling. However, if the width of the fitted Gaussian exceeded 40% of the duration of the peak, the window would be expanded to 30% of the peak duration.

Boundaries for the modeling of each peak of SEPs were chosen manually for each recording site using the global SEPs time series. We fitted each peak to a Gaussian curve using *scipy.optimize.curve_fit*.

Spectro-temporal data collection

Extraction of spectral features was performed using a custom-written Complex Wavelet Transform (CWT) function (Cohen, 2014). Power of 64 logarithmically spaced frequencies between 0.5 and 160 Hz were extracted for each trial.

In each spectro-temporal analysis, trials were baseline normalized (in dB), if necessary, and later cut at the time points of interest.

For the analysis of sustained responses, the spectrograms of stimuli without a behavioral response were subtracted from the spectrograms with a behavioral response per animal and condition. If an animal only presented one behavioral response per condition, the data from that animal was excluded from the final analyses of the condition.

Heart rate analysis

R peaks of the ECG were detected using *scipy.signal.find_peaks* and the peak times were used to create a binary signal to compute the heart rate (HR). A second signal with the R-R distance for each beat time was created to compute the heart rate variability (HRV). Convolution with a unit kernel of 4 seconds was used to retrieve the HR and the HRV from the previous signals.

Baseline normalization of HR was performed using % change (hr - bsl) / bsl * 100. Baseline normalization of HRV was performed by z-scoring (hrv - bsl) / bsl.
A window between 2 and 10 seconds was used to calculate the mean HR and HRV.

Heart rate-frequency band correlations

Trial-by-trial correlations of HR with either alpha (8 – 15 Hz) or broad gamma (30 – 160 Hz) frequency bands were done by feeding the mean value of a window (alpha: 0-3 seconds and 6-16 seconds; gamma: 0-2 seconds and 6-16 seconds) to the function *scipy.stats.stats.pearsonr*. Correlation coefficients exceeding ±0.98 were defined as outliers and excluded from the analysis. Data from animals with fewer than 5 stimuli in a condition, were excluded from the final analyses of that condition.

Statistics

All data are presented as mean ± SEM unless otherwise specified. ANOVA models were created with *statsmodels.formula.api.ols* and were later fed to *statsmodels.api.sm.stats.anova_lm*. All post-hoc analysis as well as comparisons between two groups used *scipy.stats.ttest_ind*.

Time series and time-frequency statistics

Statistics on the time domain were performed using *scipy.stats.ttest_1samp* and corrected for multiple comparison using false discovery rate (FDR) correction with the function *statsmodels.stats.multitest.fdrcorrection*.

Spectrograms were smoothed with *scipy.signal.convolve2d* with a normalized 2-D Gaussian. Time series statistics were applied for each single frequency vector.

SEP peaks

A 4-factor ANOVA type 1 was used to identify which factors contributed to differences in the peak time and amplitude. We assumed most variance was explained by the stimulation type, followed by the recording site, arousal state and behavioral response. Behavioral response showed no effect on the dependent variables. Therefore, subsequent analysis of peaks was done with a 2-way ANOVA type 2 within brain area given that no dependency was expected between stimulation type and arousal state.

Heart rate statistics

A two-way type-2 ANOVA was used to evaluate the stimulation type and the arousal state for HR and HRV as no interaction was expected between the two factors. Later, to evaluate the effect of arousal state and behavioral response, a two-way type-1 ANOVA was used, assuming the arousal state would explain more variance than the behavioral response.

Within-group statistics for correlations of HR with frequency bands were performed using *scipy.stats.ttest_1samp* and corrected for multiple comparison with *statsmodels.stats.multitest.fdrcorrection*.

Results

Behavioral responses to mechanical stimuli in sleep and wake

In order to assess the processing of somatosensory information and, particularly, nociception, during sleep and wake, we simultaneously recorded local field potentials (LFP) in the hindlimb area of the primary somatosensory cortex (S1HL) and the anterior cingulate cortex (ACC) together with EEG and ECG in freely moving male mice placed on a grid (**Fig 1A,B**). Animals on the grid received mechanical stimulation of the plantar sole. Stimuli were performed with a needle or a yellow pipette tip mounted on a dynamic von Frey device for the noxious (Nox) and the non-noxious (NN) stimulation, respectively. Sessions where non-noxious stimuli were delivered lasted 4h 24min ± 15min, and sessions of noxious stimulation, 4h 58min ± 11min. Stimuli were delivered at random intervals between 17sec and 51min. The inter-trial intervals were equally distributed for both stimulus types (**Fig 1C**, NN: 35±5 min; Nox: 35±3 min; T(32)=0.077, p=0.939). Repeated manual mechanical stimulation varied in duration and was significantly shorter for noxious than for non-noxious stimuli due to shorter response latencies to noxious stimuli (**Fig 1D**, NN: 460±21 msec; Nox: 350±25 msec; T(32)=3.227, p=0.002). Animals underwent the natural stages of wake and sleep (**Fig 1E**). With our stimulation paradigm, the time spent in each arousal state was comparable to undisturbed recordings (Soltani et al., 2019). Thus, the distribution of stimuli in the different arousal states followed the prevalence of the corresponding arousal state in the light phase (**Fig 1F**). Given the low number of stimuli performed during REM, we excluded this sleep state from further analysis.

Stimuli that did not evoke any type of behavioral response were classified as “no response”. Paw withdrawal, flinching or licking of the stimulated paw were categorized as “motor response”. For stimuli delivered during NREM sleep, the subsequent presence of wake EEG and EMG characteristics within 5 seconds after stimulation onset were classified as awakening, which could coexist with a motor response (**Fig 1G**). As expected, noxious stimuli in wake evoked motor responses more often than non-noxious stimuli (Nox: 68.4±4.9%, NN: 29.9±4.7%, T(32)= 5.497, p=4.6x10^{-6}). In NREM, the occurrence of motor responses were not different between stimuli (Nox: 13.7±4.1%, NN: 4.3±1.2%, T(27)=1.788, p=0.08), but noxious stimulation caused simultaneous motor response and awakening more often as compared to non-noxious stimuli (Nox: 39.1±3.3%, NN: 10.2±1.7%, T(32)=7.450, p=1.7x10^{-8}). Non-noxious stimuli administered in NREM evoked behavioral reactions less often than noxious stimuli as the number of no responses was significantly larger in this case (Nox: 21.9±2.9%, NN: 52.9±3.1%, T(32)=-7.116, p=4.4 x10^{-8}). However, the rate of awakenings remained equal between both stimuli (Nox: 29.5±2.8%, NN.: 35.1±3.7%, T(32)= -1.168, p= 0.251). Yet, animals awoke faster for noxious compared to non-noxious stimulation (**Fig 1H**) but the duration of the stimulus-evoked awake bouts did not differ between stimulus types (**Fig 1I**). We conclude that noxious stimuli had a stronger effect on animal behavior and lead in total to more frequent awakening.

Given the unbalanced distribution of the different behavioral responses in NREM, in subsequent analysis we grouped the behavioral responses as “Response”, which included motor responses, awakening and the combination of the two, and “No Response”.

Noxious and non-noxious somatosensory stimuli reach the ACC and S1 during NREM sleep

Rodent studies permit direct interrogation of neuronal populations and use of intracranial electrodes that allow recording of neural activity at high temporal resolution. While Local Field Potentials (LFP) are the linear sum of the neural inputs (local as well as long-range axonal terminals) nearby the location of the recording intracranial electrode, the EEG is a linear weighted combination of the activity of multiple brain areas (Buzsáki et al., 2012; Herreras, 2016). Therefore, we used the LFP to infer the strength of cortical recruitment, and the EEG to investigate the confluent activity of fronto-parietal regions.

Somatosensory evoked potentials (SEPs) could be observed in S1 and ACC, both in wake and in NREM, confirming that peripheral somatic stimuli reached both cortical areas in both arousal states, independently on whether the animals responded to the stimulation (**Fig 2A**). Of note, the EEG only detected SEPs in NREM (**Table 2**), suggesting that response to external stimuli evoked synchronized temporal dynamics in multiple fronto-parietal regions in NREM, but not in wake.

The global averaged SEP (**Fig 2B**) allowed us to define the boundaries of the first and last peaks for each recording site. All SEPs were characterized by a rapid first voltage deflection (positive in ACC and negative in S1HL), and a final negative deflection, previously attributed to the thalamo-cortical and the local feed-back circuits, respectively (Woodman, 2010).

A 4-way ANOVA revealed that the factor that most influenced peak amplitude and latency was the brain region, followed by the type of stimulation and then the arousal state (**Table 1**). Therefore, these factors were used to group the data for the analysis of peak amplitude and latency.

The latency to the first peak in wake revealed that peripheral information is first relayed to the S1HL and then to the ACC, for both noxious (S1HL: 41.2±3.3 msec, ACC: 74.4±10.3 msec, T(32)=2.9, p=0.005) and non-noxious stimuli (S1HL: 32.7±3.1 msec, ACC: 51.9±6.2 msec, T(32)=2.6, p=0.01) (**Figure 2C**). These temporal dynamics were preserved in NREM in noxious (S1HL: 30.1±2.1 msec, ACC: 94.6±9.7 msec, T(32)=6.2, $p=0.5x10^{-7}$) and non-noxious stimuli (S1HL: 28.2±2.4 msec, ACC: 89.4±8.2 msec, T(32)=6.9, $p=0.7x10^{-8}$). The feedback circuits, measured as the latency to the last peak, were first recruited in S1HL and then in ACC in wake ($S1HL_{Nox}$: 217.4±12.7 msec, ACC_{Nox}: 327.4±26.6 msec, T(32)=3.6, p=0.001; $S1HL_{NN}$: 263.4±16.9 msec, ACC_{NN}: 377.6±32.4 msec, T(32)=3.02, p=0.004) as well as in NREM sleep ($S1HL_{Nox}$: 296.3±10.9 msec, ACC_{Nox}: 373.9±19.1 msec, T(32)=3.4, p=0.001; $S1HL_{NN}$: 343.5±10.8 msec, ACC_{NN}: 448.1±11.2 msec, T(32)=6.4, $p=0.2x10^{-7}$). These results demonstrated that the temporal recruitment of different circuits within the S1HL and ACC was preserved across arousal states.

SEP temporal dynamics within each brain area showed an effect of the arousal state on the latency of both peaks (**Table 2**) (**Fig 2C**). Post-hoc analysis revealed that NREM sleep delayed the first peak in ACC for non-noxious (wake: 51.9±6.2 msec, NREM: 84.4±8.2 msec, T(32)=3.5, p=0.001) but not noxious stimulation (wake: 74.4±10.3 msec, NREM: 94.6±9.7 msec, T(32)=1.3, p=0.1). In S1HL, NREM surprisingly had the opposite effect and sped up the first peak when the stimulation was noxious (wake: 41.2±3.3 msec, NREM: 30.1±2.1 msec, T(32)=2.7, p=0.01) while it had no effect on non-noxious stimulation (wake: 32.7±3.1 msec,

NREM: 28.2±2.4 msec, T(32)=1.09, p=0.2). Post-hoc analysis on the second peak showed that NREM delayed the peak time in S1HL independently of the stimulation type ($wake_{Nox}$: 217.4±12.7 msec, $NREM_{Nox}$: 296.3±10.9 msec, $T_{Nox}(32)$=-4.5, p_{Nox}=0.00007; $wake_{NN}$: 263.4±16.9 msec, $NREM_{NN}$: 343.5±10.8 msec, $T_{NN}(32)$=-3.8, p_{NN}=0.0005). The numerical delays in the ACC, however, did not reach statistical significance for neither noxious (wake: 327.4±26.6 msec, NREM: 373.9±19.1 msec, T(32)=1.3, p=0.1) nor non-noxious-stimuli (wake: 377.6±32.4 msec, NREM: 448.1±11.2 msec, T (32)=1.9, p=0.05). Summarizing, the effect of the arousal state on the peak latencies was specific to the brain area. The fact that the first peak was delayed in ACC for non-noxious stimulation and sped up in S1HL for noxious stimulation suggests that the characteristics of the stimulation, such as salience and valence, can be distinguished in NREM sleep.

The type of stimulation additionally influenced the latency to the last peak (**Table 2**). In wake, noxious stimulation evoked faster second peaks in S1HL (Nox: 217.4±12.7 msec, NN: 263.4±16.9 msec, T(32)=-2.1, p=0.04) but not ACC (Nox: 327.4±26.6 msec, NN: 377.6±32.4 msec, T(32)=-1.1, p=0.2) (**Fig 2C**), suggesting that the stimulation type determines the recruitment speed of local circuits only in S1HL. In NREM, this effect is preserved in S1HL (Nox: 296.3±10.9 msec, NN: 343.5±10.8 msec, T(32)=-2.9, p=0.005) and extended to ACC (Nox: 373.9±19.1 msec, NN: 448.1±11.2 msec, T(32)=-3.236, p=0.002). Given that noxious stimulation evoked faster last peaks in both S1HL and ACC in NREM, and only in S1HL in wake, the recruitment speed of local circuits in NREM may indicate the urgency to process salient stimulation and generate an adequate response.

We additionally evaluated the amplitude of the peaks to infer the recruitment strength of S1HL and ACC for each condition (**Fig 2D**). We found that the impact of the arousal state and stimulation type on peak amplitude was less pronounced than on peak timing (**Table 2**). The arousal state exclusively affected the amplitude of the first peak in ACC, by generating larger amplitudes in NREM than in wake for noxious (wake: 27.3±4.3 μV, NREM: 54.4±5.7 μV, T(32)=3.64, p=0.0009) and non-noxious stimuli (wake: 25.7±6.2 μV, NREM: 48.4±5.7 μV, T(32)=2.6, p=0.01). The stimulation type, on the other hand, only impacted the second peak in S1HL as noxious stimuli evoked larger negative amplitudes than non-noxious stimuli in NREM (NN: -56.6±5.6 μV, Nox: -99.8±10.2 μV, T(32)=-3.57, p=0.001), but not in wake (NN: -77.3±14.9 μV, Nox: -80.5±8.4 μV, T(32)=-0.1, p=0.8). The fact that the amplitude of the first peak in S1HL was unaffected by either arousal state or stimulation type suggests that the thalamo-cortical connections remained active in NREM sleep and did not distinguish between types of stimulations.

Overall, these results suggest that thalamo-cortical connections transmit noxious and non-noxious information equally in wake and NREM and that the information flows from S1HL to ACC. Furthermore, these findings indicate that the type of stimulation can be distinguished in NREM sleep.

Stimulus-evoked spectro-temporal dynamics depend on the arousal state and behavioral response

After having established that nociceptive stimuli reached cortical areas during sleep, we studied their influence on neuronal network oscillations. Analyzing the EEG and the LFP of the ACC and S1HL in the spectral domain allowed to determine the temporal evolution in each frequency band after sensory stimulation. Frequency bands have been associated with multiple cognitive processes (Başar et al., 1999, 2001). Therefore, stimulus-induced spectro-temporal changes provide insight into local circuit dynamics and may lead to biomarkers of pain processes.

Stimulus-averaged spectrograms showed strong differences between wake and NREM while non-noxious and noxious stimuli evoked similar spectro-temporal profiles (**Fig 3A**). In all conditions, stimuli increased gamma (> 30 Hz) power right after the stimulation onset, according to the role of gamma in sensory processing (Başar-Eroglu et al., 1996; Karakaş et al., 2001).

In wake, stimuli evoked a transient decrease in the 2-16 Hz range power (**Fig 3A, top row**). This effect may capture increased attentional processes given that, in humans, stimulus induced decrease in alpha (8-16 Hz) power has been correlated to increased attention to the location of the stimulation (REF). Following the desynchronization in the 2-16 Hz range, particularly for noxious stimuli, a rebound activation of low frequencies (< 16 Hz) extended beyond 16 seconds.

In contrast, stimuli in NREM showed long-lasting, sustained responses (**Fig 3A, bottom row**). Gamma responses were biphasic in time, showing a first transient increase that evolved into a depression of 3 to 4 seconds and a later second increase starting between 5 and 6 seconds. Furthermore, a desynchronization of low frequencies (<16 Hz) was observed. This was bimodal in frequency given that the decrease in power was transient for frequencies below 6 Hz and sustained between 6 and 16 Hz. Because behavioral responses were pooled in these spectrograms, the sustained activity pattern in NREM could be a result of motor responses or changes in arousal state. Therefore, we isolated stimuli performed in NREM that did not evoke any kind of behavioral response (**Fig 3B**). For these stimuli, the increases in gamma as well as the desynchronization between 6 and 16 Hz were maintained.

To determine whether the initial phasic sensory response differed from a later, perhaps more affective, response, we separately averaged the spectra from the first 400 ms post-stimulation vs. 0.5 to 16 seconds, respectively. Furthermore, given that the spectrograms of stimuli without a behavioral response (**Fig 3B**) have different spectral characteristics than the global spectrograms (**Fig 3A**), we separated stimuli with and without response.

Phasic responses showed differences between response vs. no response (**Fig 3C**) and non-noxious vs. noxious (**Fig 4A**) for stimuli performed during NREM sleep. The presence of a behavioral response was associated with a higher increase in frequencies above 40Hz for both non-noxious and noxious stimuli, and stronger depression of low frequencies (2 – 13 Hz) for non-noxious stimuli (**Fig 3C, right**). Comparing non-noxious and noxious stimuli (**Fig 4A**), we found that noxious stimuli with an overt behavioral response evoked larger power increases in frequencies above 16 Hz in the S1HL and in the beta (16-30 Hz) range in the ACC. In addition,

larger increases of frequencies above 50 Hz were recorded in the EEG for noxious stimuli, indicating that most fronto-parietal brain regions had a stronger gamma activation following a noxious stimulus than a non-noxious one.

To isolate a longer-lasting sustained response, we subtracted spectrograms of stimuli without a behavioral response from those with a behavioral response (**Fig 3D**) and non-noxious stimuli spectrograms were from noxious stimuli ones (**Fig 4B**), allowing the study of behavior-associated changes and stimulus type-specific changes, respectively. In wake, behavioral responses evoked larger power increases in slow frequencies (< 2Hz) for noxious stimuli (**Fig 3D, top row**). Comparing both types of stimuli, noxious stimuli evoked larger increases on slow frequencies than non-noxious stimuli when a behavioral response was present, yet significance was not reached (**Fig 4B, top row**). In NREM sleep, behavioral responses significantly increased gamma (> 30 Hz) power more than stimuli without behavioral responses (**Fig 3D, bottom row**). Differences in power increases were specific to the recording site given that gamma differences were broadband in the S1HL and between low and medium gamma (30-80 Hz) in the ACC. The EEG captured broadband gamma activation of shorter duration than S1HL indicating that while fronto-parietal areas increased gamma activity following a stimulation with a behavioral response, in most areas, this response was shorter than in S1HL and ACC. Desynchronization of frequencies below 16 Hz was ubiquitous. However, differences in this desynchronization lasted longer when stimuli were non-noxious.

In NREM, no differences between noxious and non-noxious stimuli were observed in the sustained response (**Fig 4B, bottom row**).

These results suggest that even in the absence of an overt behavioral response, stimuli in NREM evoked changes in frequency bands associated with stimulus processing (gamma increase (REF)) and attention (alpha suppression (REF)). Furthermore, differences between stimuli and behavioral responses are more evident in NREM than in wake.

Differential engagement of the autonomic response in wake and NREM

Heart rate (HR) and heart rate variability (HRV) are physiological parameters that vary in response to different arousal states, as a result of the differential activation of the autonomic nervous system (Azarbarzin et al., 2014). Nociceptive stimuli have been shown to trigger an autonomous response that is reflected by changes in HR (Forte et al., 2022; Hilgard et al., 1974; Loggia et al., 2011; Möltner et al., 1990; Terkelsen et al., 2005; Tousignant-Laflamme et al., 2005). As such, heart rate measures are used in the clinic as an objective measure of painful sensation (Hilgard et al., 1974; Loggia et al., 2011; Möltner et al., 1990), and can indicate painful perception in instances where communication is not possible. We used these physiological parameters to confirm painful perception in mice in the absence of an overt behavioral response. We found that the baseline HR of animals was xxx±xxx in wake and xxx±xxx in NREM. In contrast, we observed an inverse pattern for HRV, with values of xxx±xxx during wakefulness and xxx±xxx during NREM sleep.

Somatic stimulation increased the HR independently of the arousal state (**Fig 5A**). A 2-way ANOVA (**Table 3**) revealed that the stimulation type modulated the increases in HR by noxious

stimulation evoking larger increases than non-noxious in NREM (Nox: 10.7±1.4%, NN: 6.1±0.7%, T(26)=-2.6, p=0.01) but not in wake (Nox: 2.9±0.5%, NN: 2.3±0.4%, T(26)=-0.8, p=0.4). The arousal state also modulated stimulation-evoked HR changes by NREM showing larger increases than wake for both noxious (wake: 2.9±0.5%, NREM: 10.7±1.4%, T(26)=4.8, $p=0.5x10^{-5}$) and non-noxious stimulation (wake: 2.3±0.4%, NREM: 6.1±0.7%, T(26)=4.01, p=0.0004). Furthermore, the effects of the arousal state at the time of the stimulation interacted with the effects of the stimulation type (**Table 3**) by noxious stimulation increasing HR more in NREM than in wake (**Fig 5A**). The HRV, however, was exclusively modulated by the arousal state (**Table 3**) as increases in HRV were more prominent in NREM compared to wake in both noxious (wake: 2.3±0.4%, NREM: 6.1±0.7%, T(26)=2.5, p=0.01) and non-noxious stimulation (wake: 1.2±0.3%, NREM: 4.5±1.2%, T(26)=-3.8, p=0.0006) (**Fig 5B**). These results indicate that HR can be used as a marker for nociceptive perception during NREM.

During NREM, awakening following a stimulation was observed in 29.5%±2.8% of noxious stimuli and 35.1%±3.7% of non-noxious stimuli (**Fig 1G**). Given the different HR in wake and NREM, we evaluated the effect of overt behavioral responses in each stimulation type for HR (**Fig 5C**) and HRV measurements (**Fig 5D**). HR and HRV responses were similar between noxious and non-noxious stimulation under all conditions, although we observed a striking difference between wakefulness and NREM in the time course of HR responses to stimuli. While HR responses to stimuli during wake were transient, those elicited during NREM resulted in sustained responses lasting longer than 16 seconds, even in instances where the animals did not show observable behavioral responses. This suggests that the stimuli elicited changes in the animal's physiological state that were not detectable through behavioral observation alone, highlighting the importance of using multiple measures to infer pain perception.

Increases in HRV were only present during NREM. These increases were numerically larger when animals did not exhibit behavioral responses to both noxious (React: 3.6±1.1, NoReact: 6.7±1.6) and non-noxious stimulation (React: 7.6±1.4, NoReact: 3.6±1, T(26)=2.2, p=0.03). However, the effect of the behavioral response was only significant for non-noxious stimulation (**Table 4**). Interestingly, the increase in HRV of those stimuli applied during NREM that did not evoke a behavioral response (**Fig 5D**), coincided with a decrease in the HR (**Fig 5C**). This suggests that, in these cases, the parasympathetic activity (Goldberger et al., 2001), as measured by increases in HRV, may have been stronger than the sympathetic activity.

A 2-way ANOVA revealed that modulations of HR by arousal and behavioral reaction were present in both types of stimulation (**Table 4**). A significant interaction effect was driven by larger HR increases following a behavioral response during NREM compared to wake in both noxious ($NREM_{Resp}$: 12.3±1.4, $wake_{Resp}$: 3.3±0.5, T_{Resp} (26)=5.6, $p_{Resp}=0.6x10^{-6}$; $NREM_{NoResp}$: 4.1±1, $wake_{NoResp}$: 1.4±0.7, T_{NoResp} (26)=1.9, p_{NoResp}=0.05) and non-noxious stimuli ($NREM_{Resp}$: 9.3±1, $wake_{Resp}$: 4.4±1, T_{Resp} (26)= 3.1, p_{Resp}=0.003; $NREM_{NoResp}$: 2.8±0.5, $wake_{NoResp}$: 1.8±0.4, T_{NoResp} (26)= 1.2, p_{NoResp}=0.2). A second significant interaction effect was observed in the HR response to noxious stimulation applied during NREM. Specifically, stimuli that evoked a behavioral response were associated with larger HR increases compared to stimuli that did not elicit a behavioral response (**Fig 5C**).

Heart rate correlations with alpha and gamma bands

After having established that HR can be used to distinguish noxious from non-noxious stimulation during NREM, and that overt behavioral responses caused greater increases in HR, we correlated the HR with local oscillatory activity for ACC and S1HL contralateral to the stimulated paw. Given the different levels of activations of these neuro- and cardio-physiological responses, these correlations should aid to better disentangle noxious from non-noxious processing.

We focused on the correlations between HR and gamma band because of the increase of gamma with pain intensity (Zhang et al., 2012b) and the larger HR increases evoked by noxious stimulation (**Fig 4**). We also correlated the HR with the alpha band. Decreases of alpha in wake have been linked to increased attention to the contralateral side (Bacigalupo & Luck, 2019; Ikkai et al., 2016) and HR modulations have been found in heightened attention (Billings & Shepard, 1910; Laumann et al., 2003). Furthermore, sleep spindles fall within the alpha band and their termination upon stimulation would generate a decrease of alpha power. Interestingly, decreases of alpha in different states of consciousness has been additionally linked to information processing (Darracq et al., 2018; Griffiths et al., 2019; Klimesch, 2012).

Given that both spectral (**Fig 3**) and HR (**Fig 5**) responses show a transient and a sustained response, we performed these correlations in an early window to capture phasic dynamics and a later time window to investigate the long-lasting responses.

Single-trial correlations of heart rate with the alpha power showed significantly negative correlations in the late window independently of the behavior of the animal in both recording sites across arousal states (**Fig 6A**). In the early window, however, noxious stimulation delivered during NREM that evoked an overt behavioral response showed significantly negative HR-alpha correlations in S1HL (-0.12±0.03, T(13)=-3.7, p=0.002) and ACC (-0.14±0.04, T(13)=-2.8, p=0.01). Significant negative correlations for non-noxious stimulation were exclusive to the S1HL. These followed stimuli in NREM without a behavioral response (-0.12±0.05, T(13)=-2.2, p=0.04), as well as in wake in the presence of a behavioral response (-0.28±0.1, T(9)=-2.3, p=0.04).

Correlating heart rate measurements with gamma in the early window, we found significantly positive correlations in S1HL that were exclusive for noxious stimulation without a behavioral response in both wake (0.2±0.07, T(11)=2.5, p=0.02) and NREM (0.27±0.08, T(12)=3.08, p=0.009) (**Fig 6B**). These results suggest that correlating S1 gamma with heart rate immediately after a stimulation can remove the saliency component of the stimulation and aid to disentangle noxious from non-noxious somatosensation. Furthermore, the absence of correlation in the presence of a behavioral response suggests that the neural processes engaged in the response disrupts this correlation. In the late window, only non-noxious stimulation delivered in NREM evoked significantly negative correlations in the presence (-0.14±0.05, T(13)=-2.8, p=0.01) and absence (-0.18±0.06, T(13)=-2.8, p=0.01) of a behavioral response.

In the ACC, significantly positive correlations by non-noxious stimulation were found in wake if no behavioral response was evoked. These started in the early window (0.12±0.04, T(13)=2.6, p=0.01) and were kept in the late window (0.23±0.05, T(13)=4.08, p=0.001).

Nevertheless, noxious stimulation tended to evoke positive correlations in wake, although significance was achieved only in the late response by those stimuli that evoked a behavioral response (0.23±0.06, T(13)=3.2, p=0.006).

Discussion

In this study, we demonstrated that somatic stimulation reaches S1HL and ACC during NREM. The latency to SEP peaks indicated that the information flow from S1HL to ACC is preserved during NREM. Furthermore, consistent with human studies, the stimulation-dependent variation in latency to the last SEP, suggests that the characteristics of the stimulation can be distinguished during NREM (Imbir et al., 2021). We also observed that the latency to the first SEP peak, thought to reflect the activity of the thalamo-cortical relay (Woodman, 2010), did not distinguish between stimuli, as also reported in humans (Thorpe et al., 2021). Additionally, increases in gamma activity, a marker for sensory processing (Başar-Eroglu et al., 1996; Karakaş et al., 2001), were kept in stimuli performed during NREM without an overt behavioral response. Overall, these findings indicate that neural mechanisms identified in rodents may be conserved in humans. Furthermore, this study provides further evidence that sensory information can be processed during sleep and expands the sensory modalities previously demonstrated to be processed during NREM (Andrillon et al., 2016; Nir et al., 2015; Sela et al., 2020; Sharon et al., 2021).

Despite the recruitment of S1HL and ACC indicated that the flow of information is kept across arousal states, we found that the activation of local circuits, measured as the latency to the second peak, was generally delayed in NREM compared to wake (**Figure 2C**, **Table 2**). This effect could be attributed to a lower neuromodulatory tone in NREM compared to wake resulting from lower levels of serotonin (5HT), dopamine (DA), Acetylcholine (Ach) and Noradrenaline (NE) (Eban-Rothschild et al., 2017; Lee & Dan, 2012). Neuromodulators change the firing properties of neurons, consequently modifying the functional network (Marder, 2012). For instance, DA and ACh deficiency delay visual evoked potentials (Bodis-Wollner et al., 1982; Daniels et al., 1994; Onofrj et al., 1986), and NE has been proposed to modulate sensory processing (Adler et al., 1988). In the first peak, however, the putative effect of neuromodulators was observed only in ACC following non-noxious stimulation. Furthermore, while in S1HL the arousal state did not influence peak latencies for non-noxious stimulation, noxious stimuli in NREM presented earlier peaks. Hence, the effects of the arousal on the first peak suggest that mechanisms during NREM are able to distinguish noxious from non-noxious stimuli. Additional effects of the arousal state on SEPs were found in the ACC, as stimuli during NREM evoked larger amplitudes on the first peak. These results point to either a lower input diversity or a stronger recruitment strength in NREM for ACC, but not for S1HL. Hence, while recruitment of primary cortices is maintained across arousal states, the recruitment of higher cortical areas is under a wider range of influences.

It is important to note that the recruitment of a brain region, observed as SEPs in the LFP does not necessarily imply that the brain region is actively processing information. To infer the nature of the neural processes generated by an event, it is useful to analyze the spectral decomposition of the LFP. This method allows for the identification of neural activity patterns reflecting different types of neural processing (i.e. sensory processing and attention).

The spectrograms in Figure 3A and 3B show the complex responses of S1HL and ACC to somatic stimulation. The evoked spectral dynamics in wake and NREM are notably distinct, indicating significant differences in the neural activity of these arousal states. Nevertheless, similarities indicate that neural processing of somatic stimuli may be preserved in NREM.

In particular, gamma activation, associated with sensory processing (Başar et al., 2001; Başar-Eroglu et al., 1996; Heid et al., 2020; Karakaş et al., 2001), can be observed in both arousal states. Close visual inspection of gamma reveals differences in the sub-frequencies of activated gamma in wake and NREM. This suggests that gamma activity may raise from distinct cortical layers in each arousal state given that different cellular mechanisms in different cortical layers generate sub-bands of gamma (Han et al., 2021; Ray & Maunsell, 2011). Furthermore, the temporal dynamics of gamma during NREM exhibit a biphasic response. The initial activation, which is time-locked to the stimulation, primarily reflects sensory processes mediated by bottom-up mechanisms (Karakaş et al., 2001). After a transient desynchronization, the second, diffuse gamma activation likely reflects either perceptual or cognitive processes and is mediated by top-down mechanisms (Karakaş et al., 2001). The observation of these dynamics in both ACC and S1HL during NREM confirms their activation via bottom-up mechanisms and the engagement of top-down mechanisms. Therefore, S1HL, as part of the lateral pain pathway, likely processed the sensory component of the stimulation, while ACC, as part of the medial pain pathway, the emotional component. Nevertheless, given that both type of stimuli evoked similar dynamics in the ACC, the second gamma activation in ACC may not be exclusively related to emotional processing.

In NREM, a long lasting desynchronization in the 6-20 Hz range dominated the spectrograms. As in gamma, the spectral and temporal characteristics of this response differ between brain areas, suggesting that different mechanisms are involved. Within the 6-20 Hz range, there is alpha (8-16 Hz), which has been linked to attention in wake, particularly in sensory cortices (Bacigalupo & Luck, 2019; Clements et al., 2022; Mazaheri et al., 2014). Note that in wake, somatic stimulation evoked a transient alpha suppression (**Fig 3A**). Sigma (11-16 Hz) is another frequency band within the observed desynchronization. Sigma is a proxy for sleep spindles, which are transient oscillations involved in sleep preservation (Adamantidis et al., 2019). Sensory stimulation activates the Locus Coeruleus (LC), which releases NE in the thalamus, terminating sleep spindles (Osorio-Forero et al., 2021) and in turn, decreasing sigma power and promoting arousal (Osorio-Forero et al., 2022). Furthermore, decreases in alpha throughout the spectrum of consciousness has been linked to sensory processing (Darracq et al., 2018). Hence, the decrease in the 6-20 Hz range in NREM may be related to either attention, sensory processing or increased arousal.

Heart rate responses to somatic stimulation showed increases on both wake and NREM that were independent of the stimulation type (**Fig 5C**). As in human studies, the magnitude of the increase in heart rate was dependent on the intensity of the stimulation (Loggia et al., 2011) and the presence of a behavioral response (Chouchou et al., 2011), showing that our results confirm the perception of the stimulation during sleep and are transferable to humans.

Single-trials correlations of neural oscillatory activity with heart rate proved to have the potential to contextualize brain activity (**Fig 6**). Short unexpected stimuli, like the ones we delivered are intrinsically salient, particularly when these are noxious (Guo et al., 2019; Lee et al., 2020; Legrain et al., 2011). This property evokes neural dynamics time-locked to the

stimulation that mix with the neural activation driven by bottom-up processes. The correlation between heart rate and gamma band activity differentiated noxious from non-noxious stimuli in the S1HL but not in the ACC. Given that both types of stimuli are considered salient, these findings suggest that neural features of the gamma band specifically related to noxious processing can potentially be isolated using heart rate measurements.

Besides saliency, short, unexpected stimuli drive rapid directed attention to the stimulation site. Early negative correlations between heart rate and alpha were found in the S1HL for non-noxious stimulation in the absence of a behavioral response. This effect might indicate either localized increased attention or NE-driven arousal. However, the early response does not show a pattern of correlations, thus, these results are inconclusive. Nevertheless, significant negative correlations dominate the late response across conditions. In wake, this effect corresponds to high alpha power and low heart rate given that in the late window, alpha is increased in heart rate is back to baseline levels. Increases in alpha power reflect inhibition of the brain region (Klimesch, 2012). Therefore, our results likely indicate that S1HL and ACC are inhibited to allow activation of visual areas to localize the source of the stimulation, as previously shown in different experimental paradigms (Klimesch, 2012). Given that heart rate is low, which indicates sustained attention (Luque-Casado et al., 2016; Ribeiro & Castelo-Branco, 2019), the negative correlations in wake are likely due to increased sustained attention. Inversely, negative correlations in NREM rise form high HR and low alpha power. Heart rate increases together with increased arousal (Azarbarzin et al., 2014) and alpha power is lower in wake compared to NREM given that spindle activity is nested within this frequency band. Therefore, the effects observed in overt behavioral responses are clearly due to awakening. In the absence of behavioral responses, these negative correlations possibly indicate an increase in arousal within NREM, with the putative aim to increase attention or awareness of the surroundings to detect potential threads. A second stimulation within this window to evaluate the rate of awakenings could provide insights into this hypothesis.

To conclude, these results support previous claims showing that autonomic nervous system activity together with neural data can better contextualize neural processes (Lee et al., 2020).

Tables

Table 1. Results of 4-way ANOVA on ERP peak amplitude and peak timing. Statistics (F) and p-values for peak amplitude and time measures for each independent factor and their interactions. *: p < 0.05, **: p < 0.01.

	Amplitude			Time		
	F	**p-value**		**F**	**p-value**	
Independent factors						
Rec. site	81.921	4.75E-33	**	15.051	3.85E-07	**
Stim. Type	5.114	0.024	*	7.551	0.006	**
Arousal	2.842	0.092		10.420	0.001	**
Behav. Resp.	0.244	0.622		0.713	0.399	
Interactions						
Rec. site : Stim. Type	0.742	0.476		0.562	0.570	
Rec. site : Arousal	2.091	0.124		0.153	0.858	
Stim. Type : Arousal	2.847	0.092		0.110	0.741	
Rec. site : Behav. Resp.	1.566	0.210		0.160	0.852	
Stim. Type : Behav. Resp.	0.000	0.994		0.271	0.603	
Arousal : Behav. Resp.	0.734	0.392		0.183	0.669	
Rec. site : Stim. Type : Arousal	0.013	0.987		0.094	0.910	
Rec. site : Stim. Type : Behav. Resp.	0.118	0.889		0.046	0.955	
Rec. site : Arousal : Behav. Resp.	0.276	0.759		0.286	0.751	
Stim. Type : Arousal : Behav. Resp.	0.654	0.419		0.001	0.971	
Rec. site : Stim. Type : Arousal : Behav. Resp.	0.098	0.906		0.107	0.899	

Table 2. Results of 2-way ANOVA for the amplitude and latency of ERP peaks. Statistics and p-values for peak time and amplitude. *: p < 0.05, **: p < 0.01.

AMPLITUDE								
		Peak #1				**Last peak**		
		F	**p-value**			**F**	**p-value**	
ACC	Stim. Type	0.468	0.495			1.479	0.226	
	Arousal	19.744	1.90E-05	*		0.001	0.974	
	Stim. Type : Arousal	0.146	0.703			2.198	0.141	
S1HL	Stim. Type	1.329	0.251			5.036	0.026	*
	Arousal	1.489	0.225			0.007	0.936	
	Stim. Type : Arousal	0.182	0.671			3.812	0.053	
EEG	Stim. Type	0.154	0.695			8.401	0.004	**
	Arousal	17.327	5.70E-05	**		1.921	0.168	
	Stim. Type : Arousal	0.003	0.960			4.839	0.030	*
TIME								
		Peak #1				**Last peak**		
		F	**PR(>F)**			**F**	**PR(>F)**	
ACC	Stim. Type	2.644	0.106			9.182	0.003	**
	Arousal	11.438	0.001	**		8.125	0.005	**
	Stim. Type : Arousal	1.034	0.311			0.342	0.560	
S1HL	Stim. Type	2.713	0.102			11.361	0.001	**
	Arousal	6.186	0.014	*		32.983	6.10E-08	**
	Stim. Type : Arousal	1.113	0.293			0.002	0.968	
EEG	Stim. Type	3.260	0.073			21.681	8.00E-06	**
	Arousal	5.265	0.023	*		5.031	0.027	*
	Stim. Type : Arousal	0.236	0.628			0.139	0.710	

Table 3. HR and HRV by Stimulation type and Arousal. Statistics (F) and p-values for the mean of the HR and HRV of an 8 seconds window corresponding to Figure 5 A and B. *: p < 0.05, **: p < 0.01.

	HR				HRV		
	F	**PR(>F)**			**F**	**PR(>F)**	
Stim. Type	7.792	0.007	**		0.028	0.867	
Arousal	38.597	8.87E-08	**		20.611	3.40E-05	**
Stim. Type : Arousal	4.419	0.040	*		1.051	0.310	

Table 4. HR and HRV by Arousal and Behavioral Response. Statistics (F) and p-values for the mean of the HR and HRV of an 8 seconds window corresponding to Figure 5C and D. *: p < 0.05, **: p < 0.01.

		NN				Nox		
		F	**PR(>F)**			**F**	**PR(>F)**	
HR	Arousal	11.489	0.001	**		31.085	8.94E-07	**
	Behav. Resp	28.774	2.00E-06	**		23.157	1.32E-05	**
	Arousal : Behav. Resp	5.217	0.026	*		8.912	0.004	**
HRV	Arousal	30.607	1.00E-06	**		13.917	4.74E-04	**
	Behav. Resp	5.309	0.025	*		1.640	0.206	
	Arousal : Behav. Resp	3.336	0.074			2.684	0.107	

Figure legends

Figure 1. Setup and behavioral characterization. A) Recording sites (left) and examples of electrolytic lesions (right) in ACC (top) and S1HL (bottom). **B)** Illustration of the experimental setup with an animal on a grid receiving stimulation to the hind paw using a dynamic von Frey device. **C)** Distributions of stimulation durations. Vertical dashed lines indicate the most common stimulus duration. Grey horizontal bar, $p<0.05$, FDR corrected. **D)** Distributions of the inter-trial interval. **E)** Example recording of 30 minutes of a NN session. From top to bottom: Hypnogram, raw slow wave activity (SWA, cyan) and smoothed SWA (dark blue), EEG, EMG, spectrogram of the EEG. Vertical dotted lines indicate stimuli onset. **F)** Distributions of stimuli in the different arousal states. **G)** Behavioral responses. **H)** Distributions of time to awakening from stimulation onset for those stimuli in NREM that evoked awakening. Grey horizontal bar, $p<0.05$, FDR corrected. **I)** Distribution of time awake after stimuli onset for those stimuli in NREM that evoked awakening. Data is represented as mean ± SEM.

Figure 2. Somatosensory Event-Related Potentials. A) SEPs for each recording site, divided by type of stimulation (rows), absence (No Resp.) or presence (Resp.) of a behavioral response (columns) and arousal state (NREM, blue; wake, red). Yellow boxes represent $p<0.05$, FDR corrected. **B)** Global ERP for each recording site indicating first and last peaks. **C)** Latency to first peak (left) and last peak (right). **D)** Amplitude of first peaks (top) and last peaks (bottom). Data were represented as mean ± SEM. See Table 2 for statistics.

Figure 3. Spectral properties comparing presence and absence of a behavioral response. A) Grand average spectrograms independent of the behavioral response. **B)** Grand average spectrograms of only stimuli in NREM without a behavioral response. Recording sites are in the columns and stimulation types in the rows. **A, B)** Vertical dotted lines represent the stimulation onset. **C)** Spectral profiles of the phasic response (0 – 400 msec post-stimulation onset) in dB. Vertical dotted line at zero defines no change from baseline. Data were represented as mean ± SEM. Yellow shaded areas indicate statistically significant differences at $p<0.05$, FDR corrected **D)** Differential sustained response. Black contour lines delineate statistical significant differences between response and no response ($p<0.05$, FDR corrected). **A, C, D)** For each recording site, data are divided by arousal state (rows) and stimulation type (columns). Black contour lines delineate statistically significant differences between response and no response ($p<0.05$, FDR corrected).

Figure 4. Spectral properties comparing noxious and non-noxious stimulation. A) Spectral profiles of the phasic response (0 – 400 msec post-stimulation onset) in dB comparing NN and Nox. Vertical dotted line at zero defines no change from baseline. Data are represented as mean ± SEM. Yellow shaded areas indicate statistically significant differences at $p<0.05$, FDR corrected. **B)** Differential sustained response comparing NN and Nox. Black contour lines delineate statistical significant differences between Nox and NN ($p<0.05$, FDR corrected).

Figure 5. Heart rate and heart rate variability. A) Heart rate (HR) in percent change from baseline. **B)** Heart rate variability (HRV) in z-score from baseline. **C)** Time course of the percent change in HR on the left. Bar graphs of the mean HR of a 8 seconds window between 2 and 10 seconds after stimulation onset on the right. **D)** HRV time course as z-score. Bar graphs of the mean HRV of a 8 seconds window between 2 and 10 seconds after stimulation onset on the right. **C, D)** Horizontal bars near the bottom of the time series plots represent significant differences between response and no response in wake (pink horizontal bar) and

NREM (blue horizontal bar). Data were represented as mean ± SEM. See Table 3 and 4 for statistics.

Figure 6. Heart rate single-trial correlations with alpha and gamma frequency bands. A) Single-trial correlations between heart rate and power in alpha band. **B)** Single-trial correlations between heart rate and power in gamma band. Each data point represents one animal. Data were represented as mean ± SEM. *, $p < 0.05$, **, $p < 0.01$.

Figures

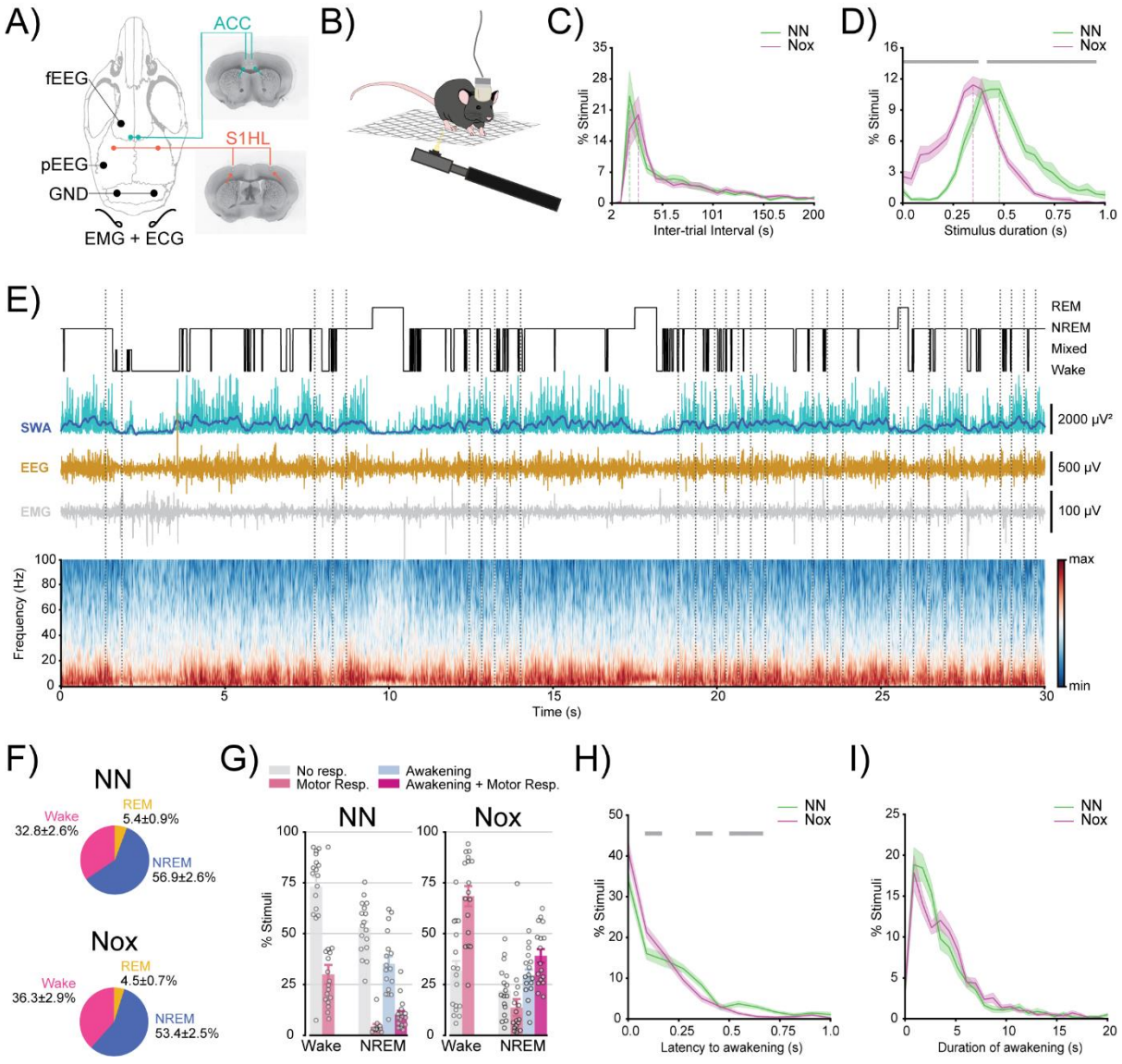


Figure 1. Setup and behavioral characterization.

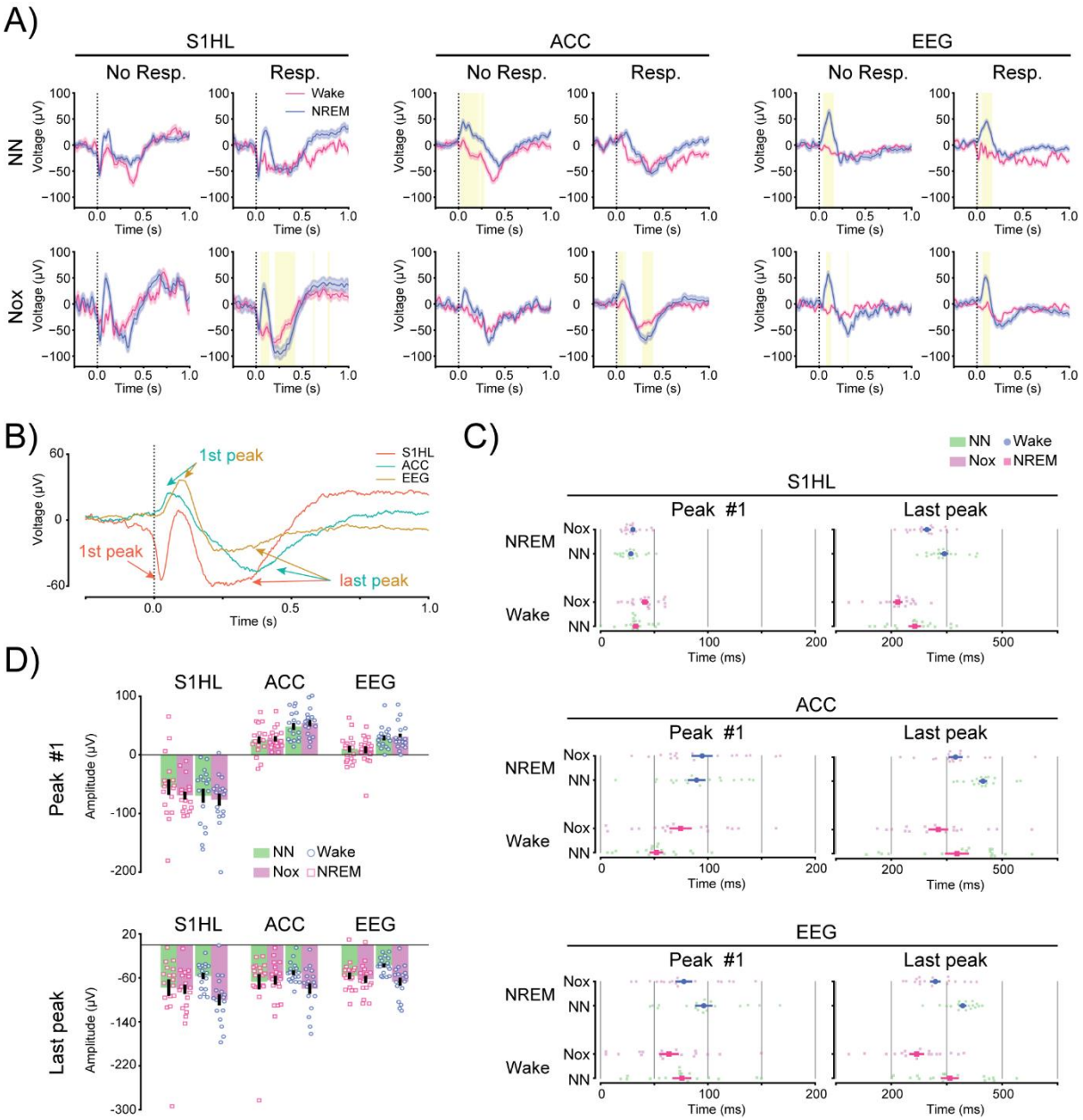


Figure 2. Somatosensory Event-Related Potentials.

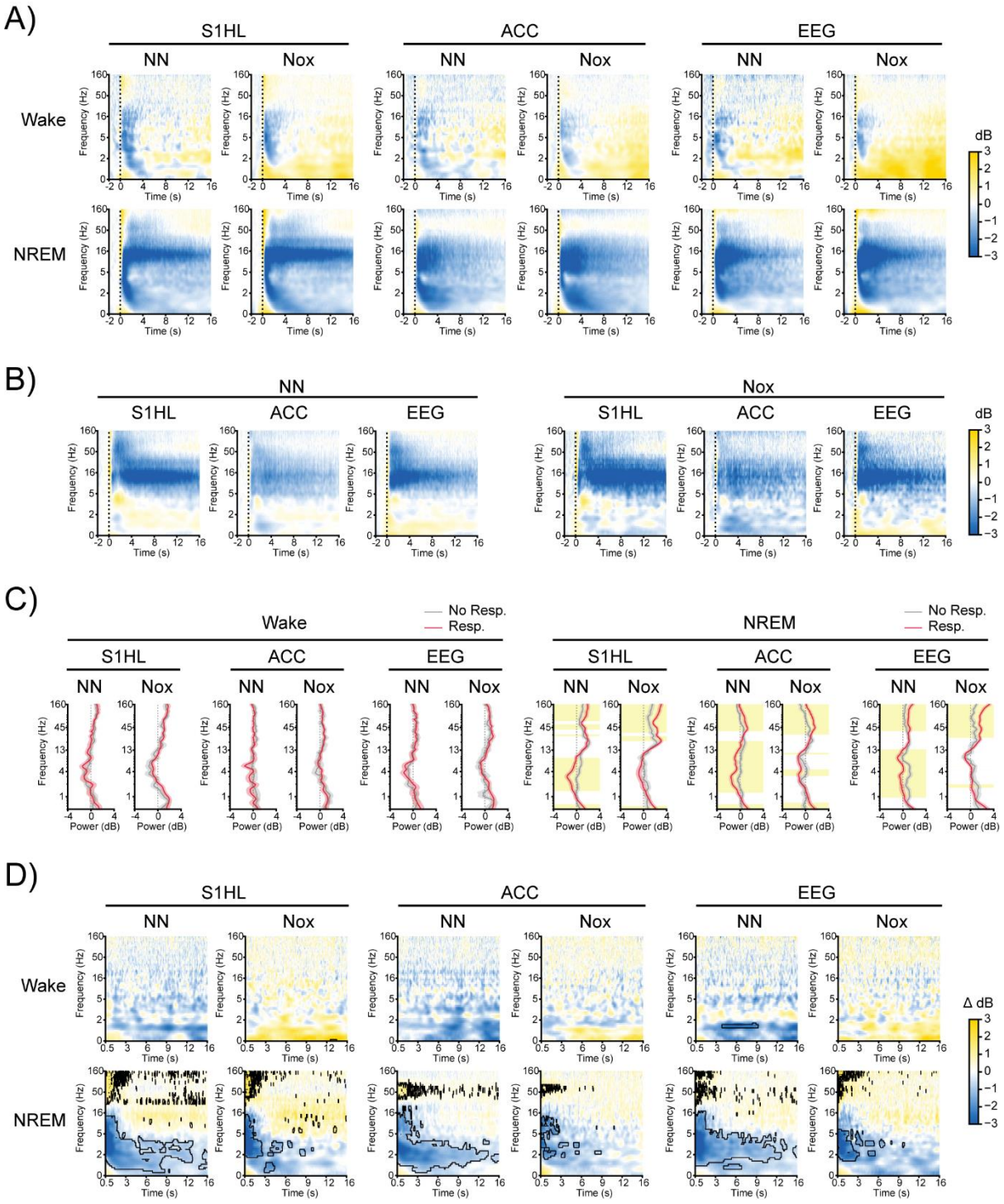


Figure 3. Spectral properties comparing presence and absence of a behavioral response.

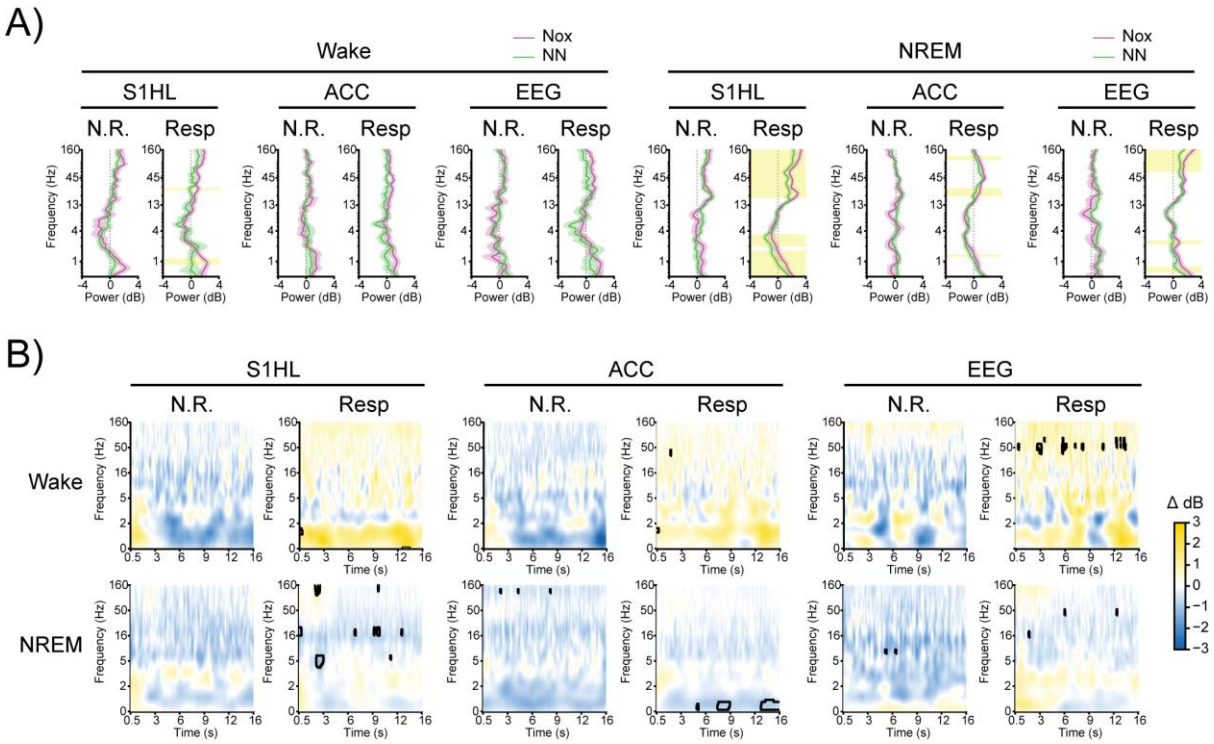


Figure 4. Spectral properties comparing noxious and non-noxious stimulation.

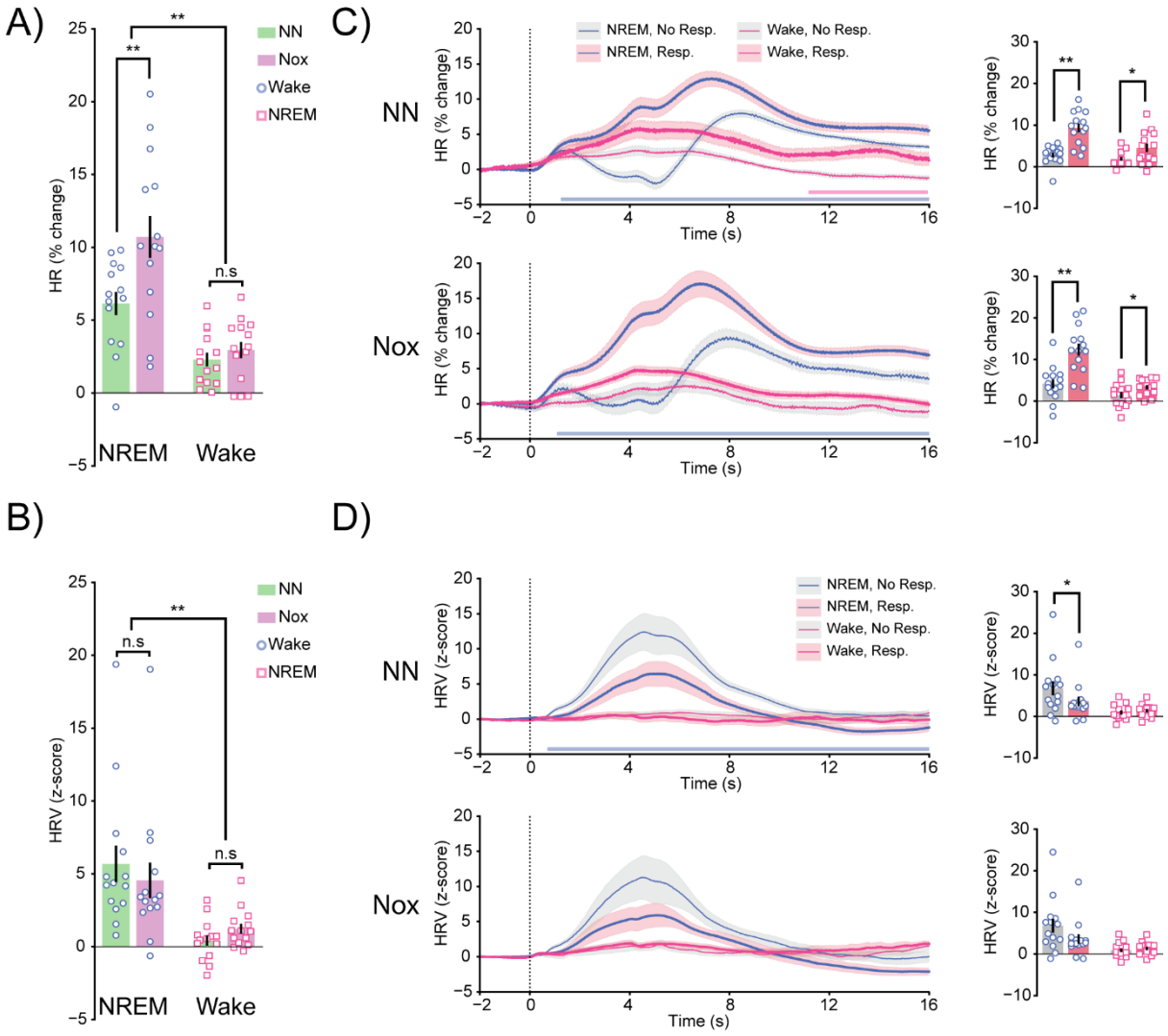


Figure 5. Heart rate and heart rate variability.

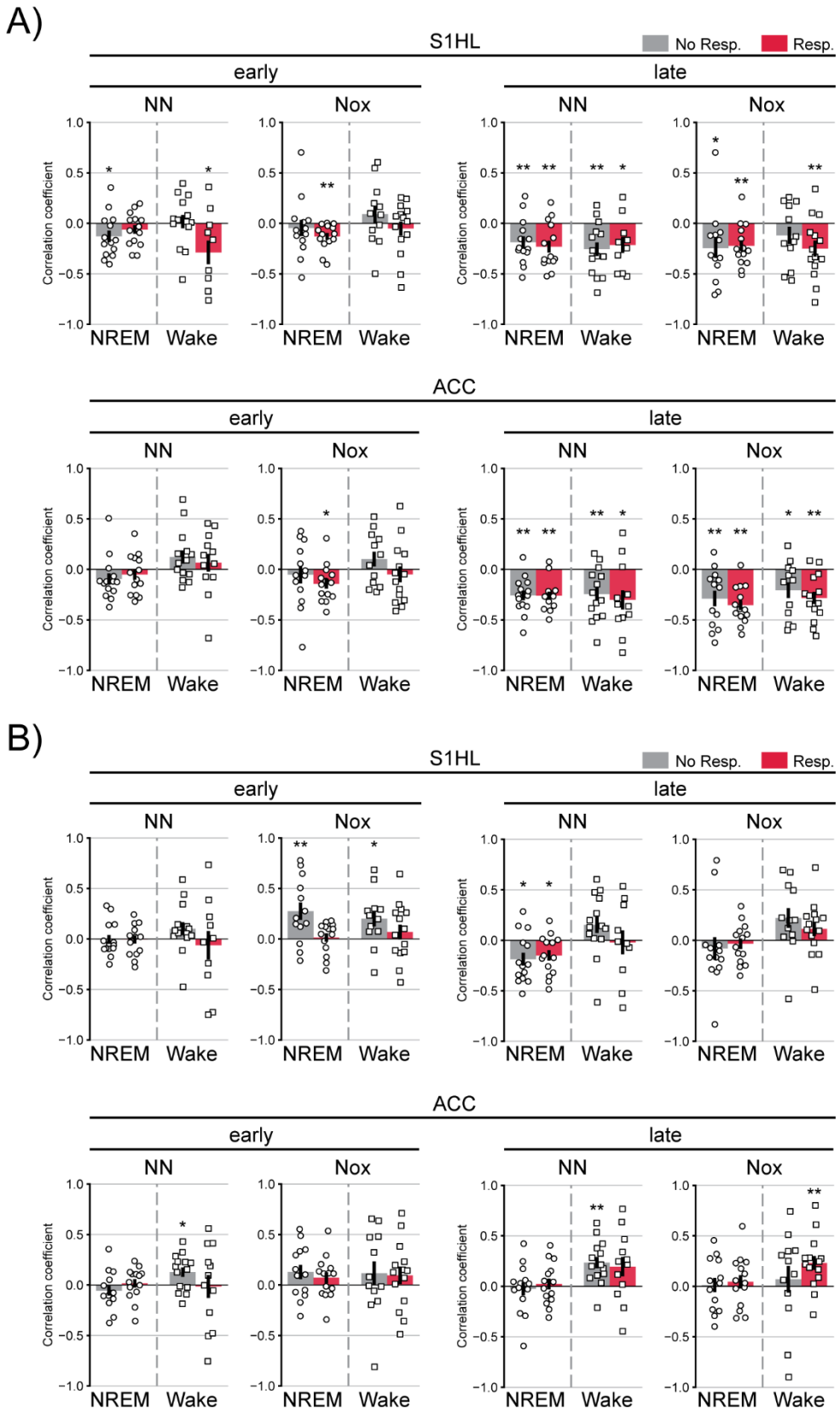


Figure 6. Heart rate single-trial correlations with alpha and gamma frequency bands.

2. Manuscript #2

Chronic Pain Induces Transient Sleep Disturbances and Long-Lasting Changes in Neural Dynamics in Mice

My contribution

I conceptualized and designed the experiment, as well as designed and built the recording rig and analysis pipeline.

I carried out all aspects of the experiments, from implant assembly and implantation surgeries to data collection, management, curation, and analysis, in addition to the termination of experiments, tissue collection and histological verification.

I wrote the entire analysis pipeline and the statistics in Python. Dr. Mike X Cohen provided the knowledge on data curation, analysis methods and statistics. Dr. Cohen provided the algorithm used for synchronization analysis in MATLAB. I ported the script to Python, tested it and implemented it.

I trained Margot Renard to assist in all the steps of the experiments and I taught her sleep scoring. For a batch of 8 animals, Margot assisted in the implantation surgeries and participated in the termination of the experiments, tissue retrieval and histological verification for these animals. She collected the full dataset for this batch and performed the sleep scoring, which I later revised.

I wrote the entirety of the manuscript, performed the analysis and statistics, and designed and generated the figures.

Chronic Pain Induces Transient Sleep Disturbances and Long-Lasting Changes in Neural Dynamics in Mice

Sandoval Ortega RA[1], Renard M[1], Cohen MX[2], Nevian T[1]

[1] Neuronal Plasticity Lab, Department of Physiology, University of Bern, Buehlplatz 5, 3012 Bern, Switzerland

[2] Synchronization in Neural Systems Lab, Donders Centre for Medical Neuroscience, Radboud University Medical Center, Houtlaan 4, 6525 XZ Nijmegen, the Netherlands

Abstract

Chronic pain perturbs cortical neural activity, as evidenced by changes in the power of theta, alpha, and gamma bands in awake electroencephalogram (EEG) recordings. Additionally, connectivity between the anterior cingulate cortex (ACC) and the somatosensory cortex (S1) – key brain regions involved in pain processing – is altered in chronic pain. Despite nearly 90% of chronic pain patients experience sleep disturbances, indicative of altered neural activity during sleep, the specific changes remain to be fully characterized.

To investigate the effects of chronic pain on neural dynamics during sleep, we conducted EEG and intracranial recordings from the S1 and ACC in mice, using the spared nerve injury (SNI) model to induce chronic pain. Recordings lasted up to seven weeks following induction of chronic pain.

Chronic pain evoked transient sleep disturbances along with long-lasting changes in neural activity and heart rate measures, indicating that compensatory mechanisms independent of S1 and ACC normalized the sleep phenotype. Changes in theta, alpha, and gamma were exclusive to the ACC and affected all arousal states. Additionally, shifts in peak frequency only appeared in wake in the S1 and EEG. However, modulations of peak frequency power were unique to the ACC across all arousal states. Furthermore, chronic pain decreased the strength of interhemispheric synchrony of slow oscillations in the S1 and ACC.

These results provide insight into the neural mechanisms underlying chronic pain-induced sleep disturbances, and may aid in the development of therapeutic strategies aimed at improving pain ratings through sleep restoration in individuals with chronic pain.

Significance statement

We employed simultaneous EEG and intracranial recordings in the S1 and ACC in a mouse model of chronic pain to provide new insights into the effects of chronic pain on neural dynamics during sleep. We found that chronic pain evokes transient sleep disturbances in conjunction with long-lasting changes in neural activity and heart rate measures. Specifically, changes across all arousal states in the power of theta, alpha, and gamma in the ACC as well

as decreased interhemispheric synchrony of slow oscillations in the S1 and ACC, and shifts in peak frequency in S1 and EEG specific that were for wake. Our findings suggests that targeting sleep restoration may be an effective approach for reducing pain in individuals with chronic pain.

Introduction

Chronic pain is a significant public health issue that affects 20% of the population worldwide (2-40% in US, 12-30% in Europe) and significantly reduces the quality of life (Breivik et al., 2005; Hopp et al., 2014; Yong et al., 2022). It is well established that chronic pain evokes sleep disturbances such as frequent awakenings and poor restorative sleep (Breivik et al., 2005; Finan et al., 2013; Finan & Smith, 2013; Nicholson & Verma, 2004; Pereira et al., 2017; Wilson et al., 2002). These sleep disruptions, in turn, further exacerbate pain and decrease overall physical and mental well-being (Krause et al., 2019; Rosseland et al., 2018; Sivertsen et al., 2015; Skarpsno et al., 2021; Staffe et al., 2019).

During wakefulness, chronic pain has been associated with an imbalance between brain areas processing pain input (i.e. dorsal anterior cingulate cortex (dACC) and somatosensory cortex) and mediating pain suppression (pregenual ACC) (Vanneste & De Ridder, 2021). Such imbalance is captured in the human resting-state wake EEG by a slow-down of alpha from 8-16 Hz to 4-8 Hz, that is accompanied by an increase in beta-gamma (> 20 Hz) activity. This electrophysiological phenotype, called thalamo-cortical dysrhythmia, is present in patients with chronic pain of different etiologies (Llinás et al., 1999; Vanneste et al., 2018). While increases in theta seem to drive negative psychiatric symptoms (i.e. depression), increases in gamma promote positive symptoms (i.e. enhanced pain perception) (Vanneste et al., 2018).

It is clear that chronic pain perturbs cortical network activity, including increases in cortical arousal (Byers et al., 2016). Given that sleep disturbances affect nearly 90% of chronic pain patients (Breivik et al., 2005; Campbell et al., 2013; Finan et al., 2013; Smith & Haythornthwaite, 2004), it is surprising that the impact of chronic pain on neural network dynamics during sleep remains to be characterized.

Chronic pain has been extensively studied in humans using imaging techniques (Garcia-Larrea & Peyron, 2013; Legrain et al., 2011), but the lack of direct access to the brain has hindered the interrogation of the underlying neural mechanisms. Therefore, animal models, particularly mice, have been widely used to study the neurophysiology of pain and sleep. A recent study in mice (Cardis et al., 2021) revealed that chronic pain increases the number of cortical arousals in the somatosensory cortex but not in the prefrontal cortex or EEG, indicating that chronic pain-induced changes may be brain region specific and not detectable in the EEG. Our own research (Sandoval et al. in preparation) supports this conclusion by demonstrating that intracranial neural activity following noxious stimulation cannot be fully captured by EEG recordings.

Given the well-established relationship between sleep and pain (Finan et al., 2013; Koffel et al., 2016; S. Mazza et al., 2012; Priebe et al., 2020; Sivertsen et al., 2015), understanding the

network changes that occur during sleep in chronic pain may provide a basis for developing interventions aimed at improving sleep quality and, therefore, reducing pain.

To gain a more comprehensive understanding of the effects of chronic pain on sleep at the neural population level, we conducted intracranial recordings in the ACC and the hindlimb area of the S1 (S1HL) in a mouse model of chronic pain using the spared nerve injury (SNI) model. The ACC plays a key role in the emotional processing of pain and the generation of coping strategies, while the S1HL is responsible for the processing of sensory information from the body.

Our findings showed that chronic pain disrupted sleep by increasing the time spent awake and decreasing the time spent in NREM sleep. Although animals recovered from this sleep disturbance, the pain-induced disruption of ACC-S1HL network dynamics persisted over time, suggesting the development of compensatory mechanisms to stabilize the sleep phenotype. Additionally, changes in the autonomous nervous system, as measured by variations in heart rate, induced by chronic pain confirm that pain persists despite the normalization of the sleep phenotype.

Methods

Animals

Male C57BL/6J mice aged 6 weeks were grouped together, caged with food and water *ad libitum*, in a 12:12 light-dark cycle with lights on at 06:30 (corresponding at ZT0) and lights off at 18:30. At 12-14 weeks of age, 8 animals were implanted in the ACC and the S1HL and randomly assigned to a chronic pain group (SNI) or a control group (sham). Misplaced tetrodes were excluded post-hoc. Following implantation, animals were kept housed together. Recordings started 14-16 days after the implantation surgery. All experiments were conducted after the approval of the cantonal veterinary office of Bern, Switzerland.

Surgery for chronic neural recordings

Anesthesia was initially induced with 5% isoflurane at a flow of 2l/min of O2 and analgesia was achieved with an initial injection of 2.5 mg/kg, 0,5 mg/ml of carprofen. During surgery animals were maintained at 1.5 – 2% isoflurane at a flow of 1.5l/min of O2. Animals were fixed on a stereotaxic frame and 0.3 mm craniotomies were performed to implant tetrodes bilaterally in the ACC (AP: + 0.6, ML: +/- 0.3, DV: - 1.4 from brain surface) and the S1HL (AP: -0.1, ML: +/- 1.9; DV: -0.4). Craniotomies of 0.9 mm were used to insert stainless steel screws to measure the frontal electroencephalogram (EEG) (AP: +2.5, ML: - 1.4), the parietal EEG (AP: -2.6, ML: - 2.5) and the ground (GND; AP: -5.2, ML: +/- 1.5). Finally, two bare-ended electromyogram (EMG) wires were sutured to the epaxial neck muscles to record muscle tone and two additional bare-ended EMG wires were positioned at the lower back to record the electrocardiogram (ECG). The GND screws on the cerebellum were used as reference.

Data acquisition

Neural signals were acquired and amplified using RHD2000 amplifier boards and digitized at a rate of 20'000 Hz by the Intan RHD USB Interface board (Intan Technologies, Los Angeles, CA). Animals underwent two 24h habituation sessions to the setup. In the first session, animals were familiarized to the setup. In the second session, animals were connected to the Interfaceboard to get habituated to the SPI cables. The day before the recording session, animals were placed in the recording setup and tethered to the Interface board. Recording sessions started at ZT3 and lasted between 6 and 7 hours. The recording sessions were performed in individual cages were animals had bedding, nesting material and food and water *ad libitum*.

Histological verification of intracranial recording sites

At the end of the experiments, animals were deeply anesthetized with 5% isoflurane at a flow of 2l/min of O2 followed by an intraperitoneal injection of 80/10 mg/kg Ketamine/Xylosine mixture. To confirm the tetrodes location, a 2 seconds long 30 µA current was applied5 times to each electrode. After transcardial perfusion of 4% paraformaldehyde (PFA), heads were kept in PFA at 4ºC for 4 days. Brains were retrieved on day 5 and further post-fixed in PFA for 12h at 4ºC. Brains were washed in PBS and sliced at 70 µm. Brain slices were stored in PBS. For imaging, brain slices were mounted with Mowiol ® 4-88 mounting medium and imaged to confirm the location of the tetrodes.

Analysis

Sleep scoring

The signals used for sleep scoring were the two EMG of the neck, the frontal EEG and the parietal EEG. The scoring of the different arousal states was done manually using a custom-made software written in Python. This software was designed to score without a scoring window, allowing for high temporal precision and precise marking of transition periods, microarousals and quick state transitions. Artifacts and movement-related noise were marked during the scoring and discarded *a posteriori*.

Data preparation

EEG and local field potentials (LFP) were low-pass filtered at 300 Hz. EMG was bandpass filtered between 100 Hz and 500 Hz. LFP, EEG, EMG and ECG were all down-sampled to 1000 Hz. Filters for EEG, LFP and EMG were applied before down-sampling. ECG filters were applied after down-sampling. To process the ECG, first, slow oscillations were removed, then high frequency muscle activity smoothed with the *scipy.signal.wiener* filter. Channels with excessive noise or that broke over the period of the recording were manually discarded after the experiments were completed.

Before initiating the LFP and EEG analysis, bouts shorter than 2 seconds (for PSD) and 3 seconds (for DMA and synchrony analysis) were embedded within the circumventing arousal state, unless the short bout was marked as noise. Then, signals were z-scored for the PSD analysis to ensure the same voltage values distribution for all animals.

Power Spectral Density profiles

Only bouts longer than 20 seconds were included in the analysis. Bouts longer than 900 seconds were split into bouts of a maximum duration of 900 seconds. Therefore, processed bouts had lengths between 20 and 900 seconds.

Extraction of spectral features was performed using a custom-written Complex Wavelet Transform (CWT) function (Cohen, 2014). For each bout, we computed the power of 150 logarithmically spaced frequencies between 0.5 and 160 Hz, by performing wavelet convolution with the raw signal, extracting the power time series, and averaging the power values over time. This method was chosen over an FFT of each bout to preserve the spectral resolution, which allowed for cross-bout averaging. The power spectral density (PSD) profiles served to detect outliers within each animal. The outlier detection method consisted on two steps and was applied per animal. First, a z-score for each bout was computed and bouts with a z-score above or below 3 were rejected. On the accepted bouts, we applied a threshold consisting of mean + 6 * STD to eliminate any outlier not found in the z-scoring procedure.

Synchronization Analysis

In order to study the strength and temporal dynamics of the synchronization of slow oscillations we selected pairs of intracranial electrodes. For each pair of electrodes, we collected the phase angle time series of 0.5 Hz in bouts longer than 30 seconds. Then, a sliding time series of phase synchronization (inter-site phase synchronization; Cohen 2014) was computed in a window of 2 cycles per frequency with a step of 1 data point. This resulted in a time series of inter-regional synchronization from which we performed time series analysis. To quantify synchronization temporal dynamics, we computed the median of the synchrony time series to use as a threshold. Contiguously supra-threshold segments were considered an epoch, and for each epoch, the duration and the strength, measured as the mean synchrony in that epoch, were computed.

Heart rate analysis

We used the function *scipy.signal.find_peaks* to detect the R peaks in the ECG. Using the peak times, we created a binary signal to compute the heart rate (HR). The positive values in the binary signal were substituted by the distance between R peaks to compute the heart rate variability (HRV). Convolution of the newly created signals with a unit kernel of 4 seconds generated the HR and HRV signals.
Outlier detection was done per animal and recording session using the z-scoring method on the HR and HRV signals. Any bout with a z-score value above 3 or below -3 in either the HR or the HRV was discarded.

Statistics

All data are presented as mean ± SEM unless otherwise specified.

These results were obtained from four animals in each group. Post-hoc data cleaning and identification of misplaced electrodes in addition to damage to the connectors over time resulted in the loss of electrodes and an imbalanced data set. We performed a t-test when both groups contained at least three data points throughout the entire duration of the study.

Statistical inference is difficult with such a small sample size and, therefore, the results presented here should be taken as proof of principle for future research.

If one of the groups consisted of two data points at any recording time point, statistics were not performed and the results were qualitatively described.

Results

Chronic pain changes the sleep phenotype only for a period of time

In order to study the effect of chronic pain on sleep over time, animals underwent four sleep recording sessions at -1 (pre), 3, 5 and 7 weeks relative to induction of chronic pain (**Fig 1A**). The mean duration of the recordings was 7h 39min ± 13min. We used the spared nerve injury model (SNI) to induce chronic pain (**Fig 1B**) and avoid possible spontaneous recovery. In this model of chronic pain, the peroneal and tibial nerves are first ligated and then cut. Animals that underwent the same surgical procedure but which sciatic nerves were left intact were assigned to the "sham" group. Animals were implanted in the hind limb area of the somatosensory cortex (S1HL) as well as in the anterior cingulate cortex (ACC) (**Fig 1C,D**) with the aim to evaluate how the receiving cortices of the lateral and the medial pain pathways, respectively, change their activity following induction of chronic pain.

During the recordings, animals underwent their natural sleep cycle (**Fig. 1E**). We used EEG and EMG to identify three sleep stages: wake, non-rapid eye movement sleep (NREM) and rapid eye movement sleep (REM). In addition, we recorded the electrocardiogram (ECG) to evaluate whether chronic pain caused changes in the state of the autonomous nervous system. Peak detection of the ECG signal (**Fig. 1E, bottom**) allowed us to compute the heart rate (HR) and heart rate variability (HRV).

After induction of chronic pain, sham and SNI animals diverged in the percent of total time they spent in wake and NREM sleep (**Fig 2A**). Given the small sample size per group, differences were numerically as predicted, although did not always reach traditional statistical significance (w3: $T(6)=-1.6$, $p=0.1$; w5: $T(6)=-2.3$, $p=0.06$, w7: $T(5)=-0.7$, $p=0.5$) or NREM (w3: $T(6)=1.6$, $p=0.1$; w5: $T(6)=2.3$, $p=0.05$, w7: $T(5)=0.08$, $p=0.9$). Qualitatively, on weeks 3 and 5, SNI animals spent more time awake and less time in NREM compared to sham. Interestingly, on week 7, both groups showed no difference in the time they spent awake and in NREM. This suggests that a compensatory mechanism developed between weeks 5 and 7 to reestablish the sleep phenotype. The time spent in REM did not differ between the two groups (w3: $T(6)=1.2$, $p=0.2$; w5: $T(6)=1.8$, $p=0.1$, w7: $T(5)=1.9$, $p=0.1$).

Sleep fragmentation is a type of sleep disturbance present in patients with chronic pain (Keilani et al., 2018). This measure could explain the differences between sham and SNI observed in the percent time spent in wake and NREM. Therefore, we measured the number of bouts of each arousal state (**Fig 2B**). We found no differences between groups in wake (w3: $T(6)=1.2$, $p=0.2$; w5: $T(6)=1.8$, $p=0.1$, w7: $T(5)=1.9$, $p=0.1$) or NREM (w3: $T(6)=-0.3$, $p=0.7$; w5: $T(6)=-0.08$, $p=0.9$, w7: $T(5)=-1.5$, $p=0.1$). In REM, however, on week 5 the percent of the

number of bouts was somewhat lower in SNI (6.5 ± 0.2) compared to sham (7.3± 0.2) (w3: T(6)=-0.6, p=0.5; w5: T(6)=2.5, p=0.04, w7: T(5)=0.7, p=0.5). Overall, however, sleep architecture in mice was maintained after induction of chronic pain. We also evaluated the mean duration of bouts (**Fig 2C**). While the mean duration of NREM and REM was similar between groups, in week 3 and 5, the SNI group experienced longer wake bouts compared to sham (w3: SNI= 57±10, sham=37±5; w5: SNI=47±3 , sham=35±4; w7: SNI=33±8, sham=39±9). With the current data set, significance is not achieved for wake (w3: T(6)=-1.4, p=0.1; w5: T(6)=-1.8, p=0.1, w7: T(5)=0.4, p=0.6) or NREM (w3: T(6)=-0.2, p=0.7; w5: T(6)=-0.1, p=0.8, w7: T(5)=1, p=0.3). Nevertheless, qualitatively, this result may explain the larger percent time that SNI spent in wake compared to sham (**Fig 2A**).

Because the duration and percentage of bouts, as well as the percent of time spent in each arousal state, capture changes over time, we evaluated the co-evolution of these measures together with the variance of the duration of bouts (**Fig 2D**). We found that when using these parameters, sham and SNI could be better differentiated in wake. In wake, the SNI group (bottom, left) experienced an increase in the mean and the variance of the duration of bouts in weeks 3 and 5. Interestingly, in week 7, the values receded to levels below pre-chronic pain induction. Similarly, in NREM, the mean duration of bouts increased in weeks 3 and 5 in the SNI group, and reestablished to baseline levels in week 7. Sham animals did not show the fluctuations in these parameters as SNI did. These measures showed no fluctuations neither in SNI nor sham animals in REM sleep.

We additionally evaluated transitions from NREM to wake (**Fig 2E**) and from NREM to REM (**Fig 2F**) given that increased awakening is another sleep disturbance commonly encountered in patients with chronic pain (Finan et al., 2013). On week 5, we found that sham animals (82±0.3%) woke up less often than SNI animals (84±0.4%) (w3: T(6)=1.4, p=0.2; w5: T(6)=-2.6, p=0.03, w7: T(5)=-0.7 p=0.4).

Changes in the distribution of the power spectrum are specific of the brain area

After showing that the sleep phenotype is disturbed upon induction of chronic pain, we evaluated how chronic pain affected brain activity in each arousal state. For each recording site and arousal state, we computed the power spectral density (PSD) profiles (**Fig 3A**). Changes in the PSD could be an index of the pain-induced changes in the brain that produced the sleep disturbances.

We observed that chronic pain disturbed the PSD in all arousal states, particularly in wake and NREM sleep. In the ACC, changes induced by chronic pain were found selectively in the hemisphere contralateral to the injured paw. In contrast, in the S1HL, the modulations were bilateral, with a stronger influence in the ipsilateral hemisphere. In addition, the PSD of these two cortical areas in sham animals varied more strongly in S1HL than in ACC, indicating that aging had an effect on the distribution of frequencies in the S1HL. Despite the local changes in PSD, the EEG did not capture any variation, suggesting that the impact of chronic pain in fronto-parietal cortical areas may vary, highlighting the complexity of chronic pain.

Based on findings that oscillations slow down in chronic pain in humans (Llinás et al., 1999; Vanneste et al., 2018), we quantified the evolution of the peak frequencies and their power over time (**Fig 3B, C**). In wake, chronic pain evoked a left shift of the peak frequencies in the EEG (pre=6.7±0.8Hz, w7=5.3±1Hz) that became qualitatively significant after week 5 (**Fig 3B**), indicating a slow-down of the global network activity. Yet, the power remained constant (pre=0.03±0.9x10^{-3} a.u., w7=0.03±0.2x10^{-2} a.u) (**Fig 3C**). The change in the EEG peak frequency was not elicited by neither the ACC nor the S1HL, as neither of these two brain regions followed the same pattern as the EEG (**Fig 3B**). In fact, the peak frequency in both the ipsilateral and contralateral hemispheres to the injured paw in the S1HL shifted towards faster frequencies on week 7 (pre=4±0.1Hz, w7=4.9±1Hz). In S1HL, the peak frequencies in both NREM (pre=3.4±0.09Hz, w7=3±0.05Hz) and REM (pre=4.1±0.9z, w7=4.3±1.1Hz) sleep did not show significant changes over time nor were differences between sham and SNI found. However, in REM, the peak frequency power of the S1HL contralateral to the injured paw in week 5 and 7 was higher in SNI animals compared to sham (w5: SNI=0.06±0.9x10^{-2} a.u, sham=0.04±0.6x10^{-2} a.u; w7: SNI=0.06±0.5x10^{-2} a.u , sham=0.03±0.1x10^{-1} a.u).

In the ACC, while chronic pain did not influence the peak frequencies (**Fig 3B**), it did alter the power of the ACC contralateral to the injured paw (**Fig 3C**). In wake and REM, the peak frequencies showed an increase in power on week 3 that remained heightened until the end of the experiments (wake: pre= 0.03±0.3x10^{-2} a.u, w7=0.04±0.2x10^{-2} a.u; REM: pre=0.02±0.2x10^{-2} a.u, w7=0.03±0.5x10^{-5} a.u). In NREM, the power of the peak frequency progressively decreased over time (pre=0.07±0.4x10^{-2} a.u, w7=0.05±0.3x10^{-2} a.u). These effects could be interpreted as an intrusion of sleep-like features in wake, and of wake-like features in NREM.

Furthermore, we investigated the power changes in the theta, alpha and low gamma bands to elucidate whether animals recapitulated the thalamo-cortical dysrhythmia described in humans (Llinás et al., 1999) in both wake and sleep (**Fig 4**). Chronic pain induced an increase in the theta power of the ACC contralateral to the injured paw in wake (pre=0.02±0.2x10^{-2} a.u, w7=0.03±0.2x10^{-2} a.u) and REM (pre=0.01±0.1x10^{-2} a.u, w7=0.02±0.9x10^{-3} a.u), but not in NREM (pre=0.04±0.4x10^{-2} a.u, w7=0.04±0.1x10^{-2} a.u) (**Fig 4A**). In S1HL and the EEG, theta power did not change upon induction of chronic pain. Alpha power, as theta, was also increased in the ACC contralateral to the injured paw (**Fig 4B**) in all three arousal states (wake: pre=0.011±0.6x10^{-3} a.u, w7=0.013±0.7x10^{-3} a.u; NREM: pre=0.02±0.1x10^{-2} a.u, w7=0.03±0.9x10^{-3} a.u; REM: pre=0.011±0.7x10^{-3} a.u, w7=0.013±0.3x10^{-3} a.u). Neither the S1HL nor the EEG showed power changes in the theta band upon chronic pain induction. Numerical increases in the low gamma band after chronic pain induction took place at week 7 exclusively in the contralateral ACC (wake: pre=0.004±0.1x10^{-3} a.u, w7=0.005±0.1x10^{-3} a.u; NREM: pre=0.004±0.6x10^{-3} a.u, w7=0.005±0.2x10^{-3} a.u; REM: pre=0.004±0.6x10^{-3} a.u, w7=0.005±0.3x10^{-5} a.u) (**Fig 4C**).

Neither of the chronic pain-induced changes in the ACC and the S1HL observed in the peak frequencies and the frequency bands that define the thalamo-cortical dysrhythmia were recapitulated in the EEG. This is important because it suggests that the chronic pain modulations of neural circuitry are local.

Inter and intra hemispheric synchrony

We have shown that chronic pain alters the power spectral distribution in all arousal states, indicating that brain areas are working and communicating in a different manner compared to sham animals. Long-range effective communication is promoted by slow oscillations (SO, < 1 Hz) (Niknazar et al., 2022) and changes in the phase synchrony of oscillations indicate aberrant interactions between distant brain areas (Bowyer, 2016; Uhlhaas & Singer, 2006). Therefore, we aimed at measuring the synchrony of SO between hemispheres (interhemispheric synchrony: ACC_{right} – ACC_{left} and $S1HL_{right}$ – $S1HL_{left}$) and within each hemisphere (intrahemispheric synchrony: contralateral and ipsilateral to the injured paw).

In order to analyze the strength and duration of the synchronization between the areas of interest, we computed a time series of windowed phase synchronization (**Fig 5A**). We found that aging had an effect on the strength of the phase synchrony of SO (**Fig 5B**). In sham animals, SO synchrony strength of both intra- and inter-hemispheric connectivity increased over time in wake and NREM sleep. In REM, however, only S1HL interhemispheric connectivity steadily increased over time and ACC interhemispheric connectivity slightly decreased. Induction of chronic pain hindered the natural increase in the synchronization of neural activity, by causing a decrease instead. In the interhemispheric synchrony of both the S1HL and ACC, chronic pain induced a progressive decrease in the strength in all arousal states. The synchrony strength within the contralateral and the ipsilateral hemispheres to the injured paw in wake decreased in week 5 and then remained stable. In NREM, the synchrony strength did not change, and in REM, it decreased in weeks 3 and 5. In the contralateral hemisphere, the strength of the synchrony recovered to baseline levels on week 7, while in the ipsilateral hemisphere, it remained decreased.

Aging also increased the duration of the epochs in synchrony (**Fig 5C**). In wake, the increase was slight and transient as it was only observed in week 5. In NREM, the duration of both intra- and interhemispheric synchrony increased over time, and in REM, increases were exclusive to the interhemispheric synchrony of S1HL. In wake, induction of chronic pain decreased the synchrony duration in the ipsilateral and contralateral hemispheres to the injured paw as well as in the S1HL. However, the duration of the bouts in synchrony at week 7 was equal between the sham and the SNI group. Interhemispheric synchrony in the ACC preserved the same duration over time. In NREM, synchrony duration remained stable within both ipsilateral and contralateral hemispheres in SNI animals. Synchrony duration between both ACC and both S1HL decreased on week 3, and while in the S1HL it progressively decreased over time, in the ACC synchrony duration was similar between sham and SNI at week 7. In REM, induction of chronic pain generally decreased the duration of the epochs in synchrony, except for the ipsilateral hemisphere.

In summary, these results show that chronic pain halts the natural increase in the strength of SO phase synchrony during aging and, by decreasing it. These results suggest that chronic pain disrupts and diminishes effective connectivity between areas implicated in the processing of pain.

Heart rate and heart rate variability changes to chronic pain

Given that we have shown that chronic pain changes neural activity in the ACC throughout time, and that the ACC has been involved in autonomic control (Beissner et al., 2013; Cechetto, 2014; Critchley et al., 2003), we evaluated whether chronic pain also induced changes in two measures of autonomous system activation: the HR and the HRV.

In awake sham animals, the HR steeply decreased over time (pre=562±17, w7=434±11) (**Fig 6A**), supporting previous research in awake humans showing that aging slows down the HR (Birnbaumer et al., 2020; KoSTIS et al., n.d.; Santos et al., 2013). We additionally showed that this phenomenon is preserved in both NREM (pre=492±26, w7=367±17) and REM sleep (pre=482±22, w7=376±23). Induction of chronic pain exacerbated the HR decrease, particularly in wake, from week 5 onwards. In NREM and REM sleep, SNI animals qualitatively showed lower HR than sham animals in week 5 only.

Contrary to the HR, the HRV showed an increase in both sham (wake: pre=15.5±1.5, w7=25.8±1.3; NREM: pre=14.8±2, w7=25.2±3.3; REM: pre=14.4±1.9, w7=19.0±2.4) and SNI (wake: pre=17.6±1.1, w7=22.8±1.9; NREM: pre=19.2±2.2, w7=24±3.1; REM: pre=16.3±1.2, w7=17.7±2.7). Nevertheless, no qualitative differences between groups appeared (**Fig 6B**).

HRV depends on HR and these two measures are inversely correlated (Kazmi et al., 2016). We therefore evaluated the effect of chronic pain in the HR-HRV relation (**Fig 6C**). We found that chronic pain disturbed the HR-HRV in wake, but not in NREM or REM.

Discussion

In this study, we investigated the effects of chronic pain over time on the neural dynamics of sleep and wake in a mouse model of chronic pain. Our results showed that chronic pain temporarily changed the sleep phenotype, probably due to the development of a compensatory mechanism by week 7 (**Fig 2**). We also evaluated sleep fragmentation but found no differences between the experimental and control groups at any recording time point, further corroborating that chronic pain does not alter sleep architecture in mice (Cardis et al., 2021).

Chronic pain-induced changes in the power spectral domain were specific to the recording site and arousal state. As a global measure of activity in fronto-parietal regions, the EEG captured a leftward shift in the peak frequency during wakefulness (**Fig 3A**), but not in NREM or REM. This change is indicative of a slowing of the oscillations of the global fronto-parietal network, potentially indicating a progressive intrusion of sleep-like features in wake as chronic pain becomes consolidated. The decrease observed in the EEG was not driven by either the ACC or the S1HL as we did not find any changes in the peak frequency of the ACC, and the S1HL contralateral to the injured paw showed an increase in the peak frequency by the seventh week. Therefore, the slow down observed in the EEG during wake is likely driven by other fronto-parietal brain areas.

Chronic pain altered the peak frequency power in the ACC contralateral to the injured paw in all arousal states (**Fig 3B**). In wake, we observed an immediate increase in the power of the peak frequency (~ 3.8 Hz), which falls within the delta range (1-4 Hz), typically associated with sleep pressure, but when measured in NREM sleep (Vyazovskiy et al., 2011). The increase of delta activity during wake may represent increased sleep-like activity, and may result from an increased need for sleep. In NREM, we observed the opposite effect. Over the course of the experiment, the peak frequency (~ 3.1 Hz) power progressively decreased, suggesting that the mechanisms that promote restorative sleep are disrupted. Furthermore, the power of the peak frequency in REM increased after induction of chronic pain and remained elevated until the end of the experiment at week 7. REM is important for processing emotions experienced during the day (Vandekerckhove & Wang, 2018), and given that pain is an emotional experience in addition to a physical one, the heightened power in REM may indicate an increased need to process emotions in order to cope with the negative emotional valence of constant pain.

We also evaluated changes in the theta, alpha and low gamma bands to investigate whether the thalamocortical dysrhythmia, as described in humans (Llinás et al., 1999), is replicated in mice (**Fig 4**). We found that global activity in fronto-parietal areas, as measured by EEG, did not change, affirming that intracranial electrodes are necessary for more detailed neural data collection (Sandoval et al., in preparation). Interestingly, we did not observe any changes in the frequency bands in the S1 region of the brain during chronic pain, but observed increases in all three bands in the ACC contralateral to the injured paw across all arousal states. Theta and alpha bands were increased by week 3, while gamma only increased by week 7. These results partially matched the thalamocortical dysrhythmia phenotype described in humans as we observed the generalized slowdown of the network in the EEG (**Fig 3**) and an increase in the theta and gamma bands on week 3 and 7, respectively, that were localized to the ACC contralateral to the injured paw (**Fig 4**). It would be very insightful if future studies would extend the time of the recordings as 7 weeks of recordings might not allow the full thalamo-cortical dyshythmia phenotype to develop. Nevertheless, here we have shown that power spectral changes evoked by chronic pain in the ACC are kept in NREM and REM, indicating a faulty functioning of the network during sleep.

The changes in the power spectrum suggest that chronic pain affects the brain network's functionality and communication patterns. To investigate this further, we assessed inter- and intrahemispheric synchronization of slow oscillations (SO) between the ACC and S1, as these oscillations play a crucial role in promoting effective communication between distant brain areas, particularly during sleep (Niknazar et al., 2022). In sham animals, we observed a general increase in SO synchrony strength across all arousal states over time, indicating that aging leads to an increase in SO synchrony between the recorded brain regions. Chronic pain not only stopped this increase but also promoted the opposite effect, particularly in inter-hemispheric communication (**Fig 5B**). These results suggest that chronic pain halts and impairs the age-related increase of effective communication between distant brain areas. SO are crucial for memory consolidation during sleep (Guo et al., 2019; Hickey & Race, 2021; S. Y. Kim et al., 2017; Niknazar et al., 2022). Hence, the observed decrease in SO synchrony during chronic pain may be an indication of impaired memory consolidation. This aligns with studies

showing that patients with chronic pain can develop memory impairments (Stéphanie Mazza et al., 2018) and have an increased risk of developing dementia (Whitlock et al., 2017). Additionally, studies in rodents have further confirmed that chronic pain impairs memory consolidation (Phelps et al., 2021).

Our results showed that chronic pain leads to changes in the power spectrum and synchrony of the ACC across all arousal states. As the ACC plays a role in modulating the autonomic system, including cardiac responses (Beissner et al., 2013; Cechetto, 2014; Critchley et al., 2003), we examined the impact of chronic pain on heart rate. Awake control animals exhibited a decline in heart rate over time (**Fig 6A**), which is consistent with the effects of aging (Birnbaumer et al., 2020; KoSTIS et al., n.d.; Santos et al., 2013). Additionally, this decrease was observed during both NREM and REM sleep. Chronic pain aggravated the decrease in heart rate on week 5 onwards, particularly when animals were awake. These results replicated clinical research (Danilin et al., 2022) and demonstrated the usefulness of heart rate measurements for assessing pain in animal models. Decreases in heart rate are associated with increased mortality (Chen et al., 2019) and patients in chronic pain are at a heightened risk of major adverse cardiac effects (Chung et al., 2020). Given the bidirectional communication of the ACC and the autonomic nervous system, future research should explore whether interventions aimed at stabilizing heart rate may also stabilize neural activity in the ACC.

It is important to note that a limitation of this study is the suboptimal number of data points per group. As such, the conclusions of this study should be interpreted with caution and considered as preliminary evidence. As the sample size is expanded in future studies, the findings may be subject to revision. Despite this limitation, the concordance of our results with previous literature and the low variability in our data in most analysis suggest that the effects observed in this study are likely robust.

Overall, our study indicates that chronic pain affects brain activity and autonomic nervous activity in all arousal states, which may contribute to the development of sleep disturbances. Given the bidirectional interaction between pain and sleep (Koffel et al., 2016; Priebe et al., 2020; Sivertsen et al., 2015), and that sleep manipulations can change pain ratings, therapies targeting sleep or regulating brain activity during sleep harvest a great potential to improve pain in patients with chronic pain.

Figure legends

Figure 1. Experimental setup. A) Timeline of the experiments from implantation surgery to euthanization of animals. **B)** Spared Nerve Injury (SNI) model used to induce chronic pain in mice. **C)** Implant locations of intracranial electrodes, EEG, EMG and ECG. **D)** Pictograms of electrolytic lesions in the ACC (top) and S1HL (bottom). **E)** Example recording of 15 minutes showing the sleep cycle. From top to bottom: hypnogram, EEG, EMG and ECG. Magnifications of segments of the recording show REM, NREM and Wake (from left to right). For each arousal state, a 1 second magnification of the ECG is shown to illustrate peak detection (green circles) and the differences in ECG between arousal states.

Fig 2. Evolution of sleep characteristics in the chronification of pain. A) Percent of time spent in each arousal state. **B)** Percent of bouts of each arousal state. **C)** Mean duration of bouts. **D)** Radar plots illustrating the co-evolution of the sleep bout feature (quadrant) over different time points (line color). For better visualization, the variance was multiplied by 5. **E)** Percent of awakenings from NREM. Each dot represents an animal. **F)** Percent of transitions from NREM to REM sleep. Data are presented as mean±SEM. T-tests were used to compare groups within each recording time point.

Figure 3. Changes in the distribution of frequency power. A) Power spectral profiles of each recording time point for all arousal states in both hemispheres in SNI animals and the contralateral hemisphere in sham animals. **B)** Evolution of the peak frequency over the four recording time points. **C)** Evolution of the power of the peak frequency. Data are represented as mean ± SEM.

Fig 4. Frequencies of the thalamocortical dysrhythmia. A) Power evolution of theta. **B)** Power evolution of alpha. **C)** Power evolution of low gamma. Data are represented as mean ± SEM.

Figure 5. Inter- and intra-hemispheric phase synchrony of SO. A) Steps of the method used to measure synchrony duration and strength between a pair of electrodes of an example NREM bout. **B, C)** Percent change of the strength **(B)** and duration (**C)** of synchrony of pairs of electrodes that measure interhemispheric synchrony (ipsilateral and contralateral to the injured paw) and intrahemispheric synchrony ($ACC_{right} – ACC_{left}$ and $S1HL_{right} – S1HL_{left}$). Data are represented as mean ± SEM.

Figure 6. Heart rate and heart rate variability. A) Evolution of the heart rate over the recording times. **B)** Evolution of the heart rate variability over the recording times. **C)** Simultaneous changes of the heart rate and the heart rate variability over time. Data are represented as mean ± SEM.

Figures

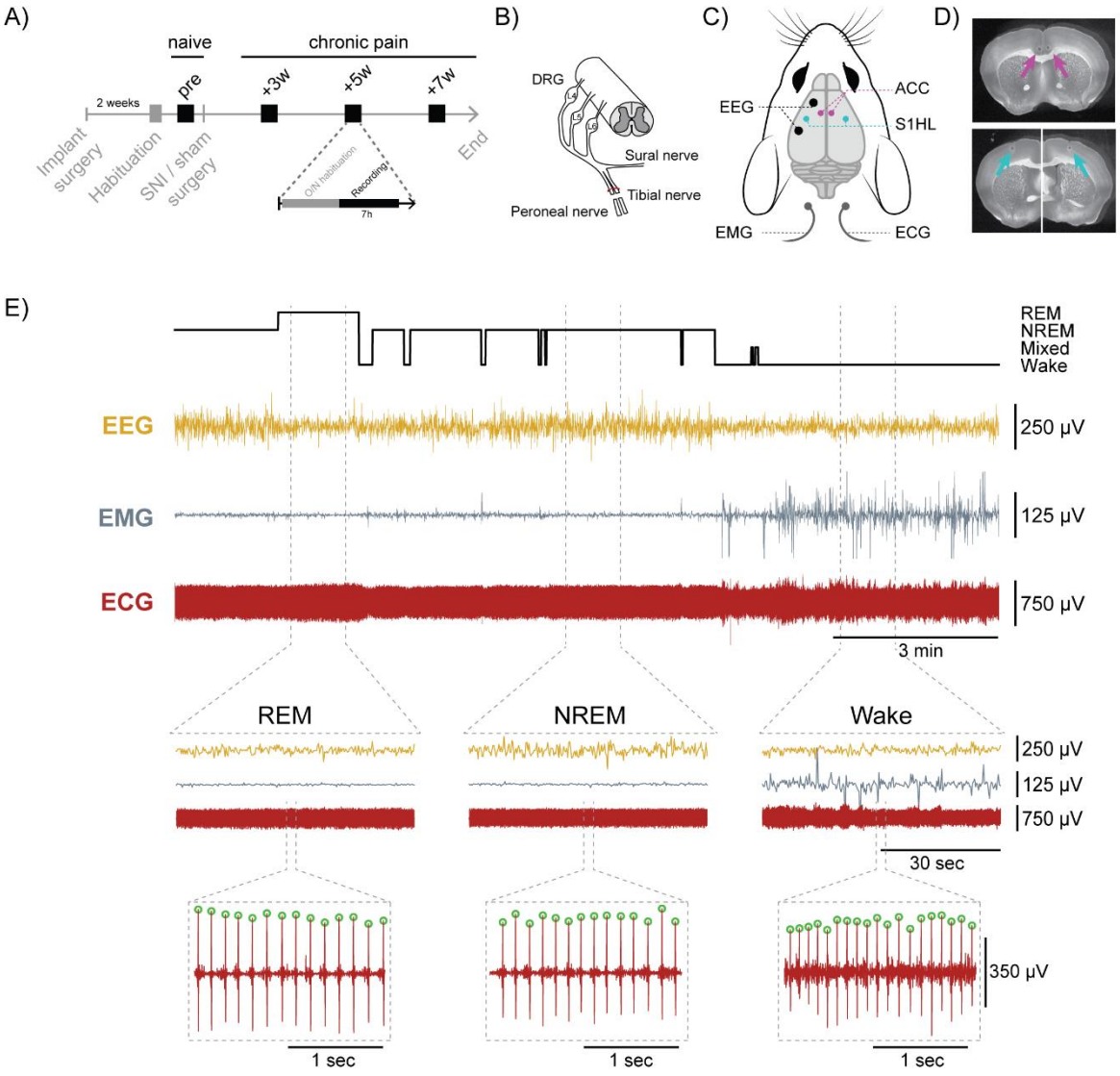
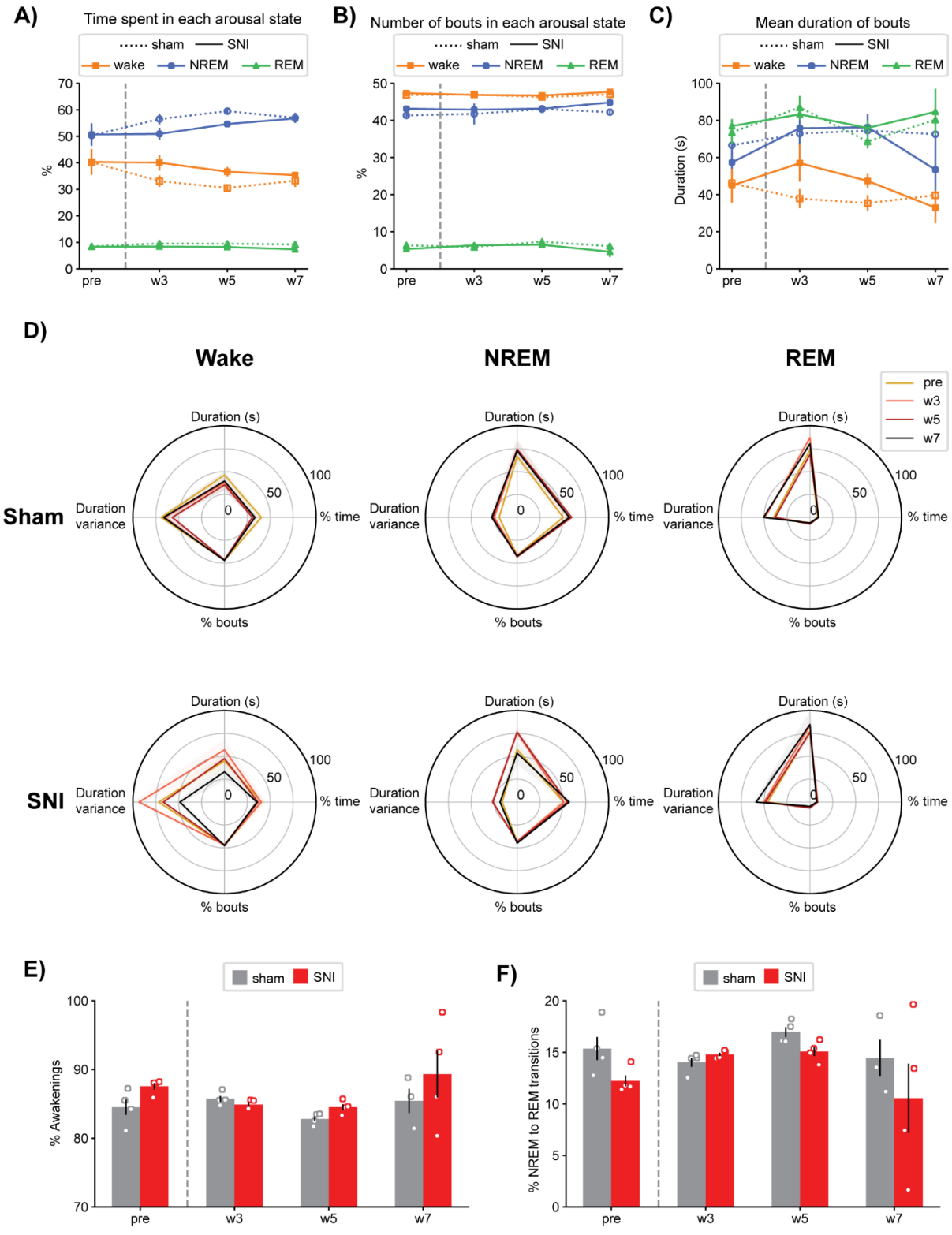


Figure 1. Experimental setup.



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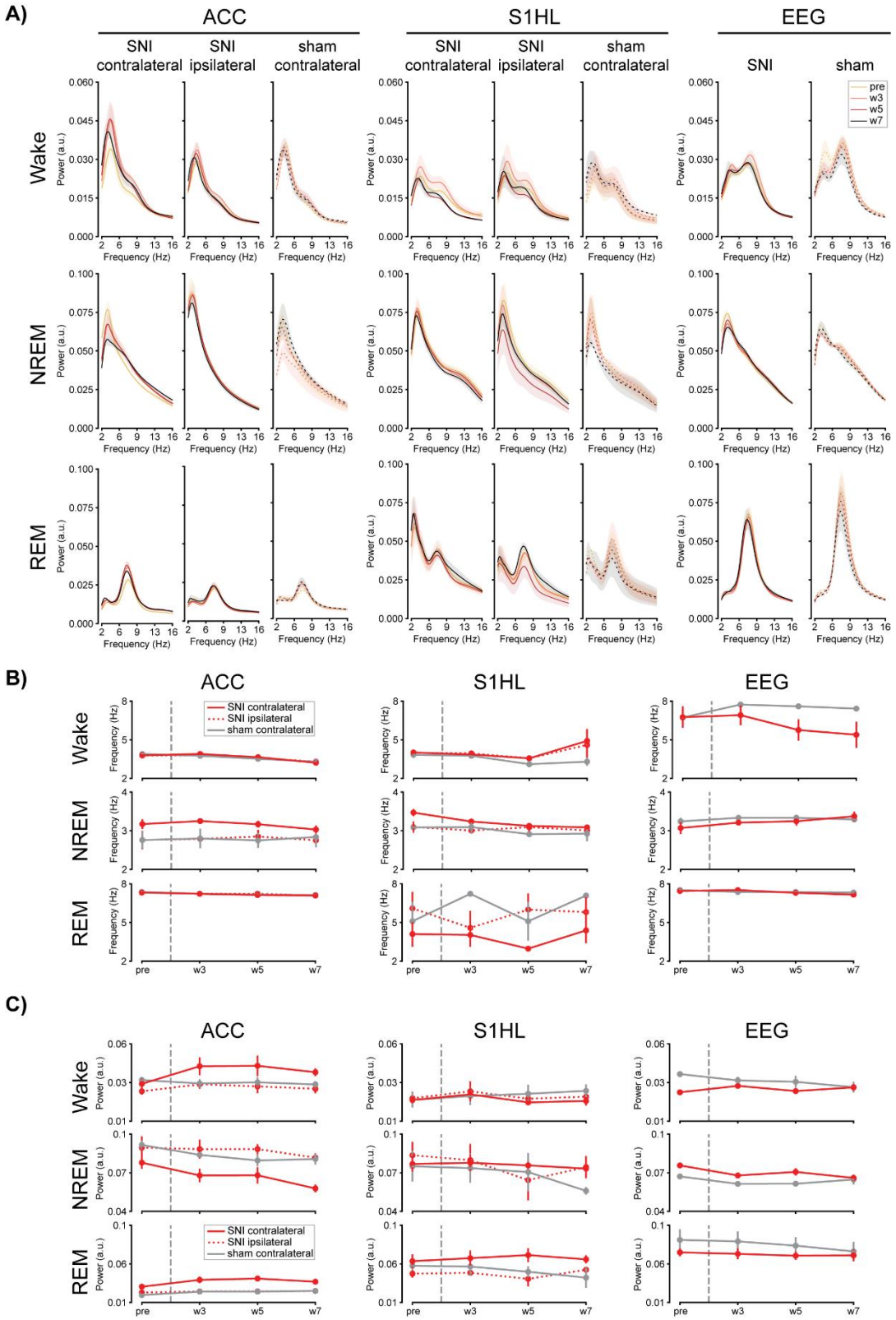


Figure 3. Changes in the distribution of frequency power.

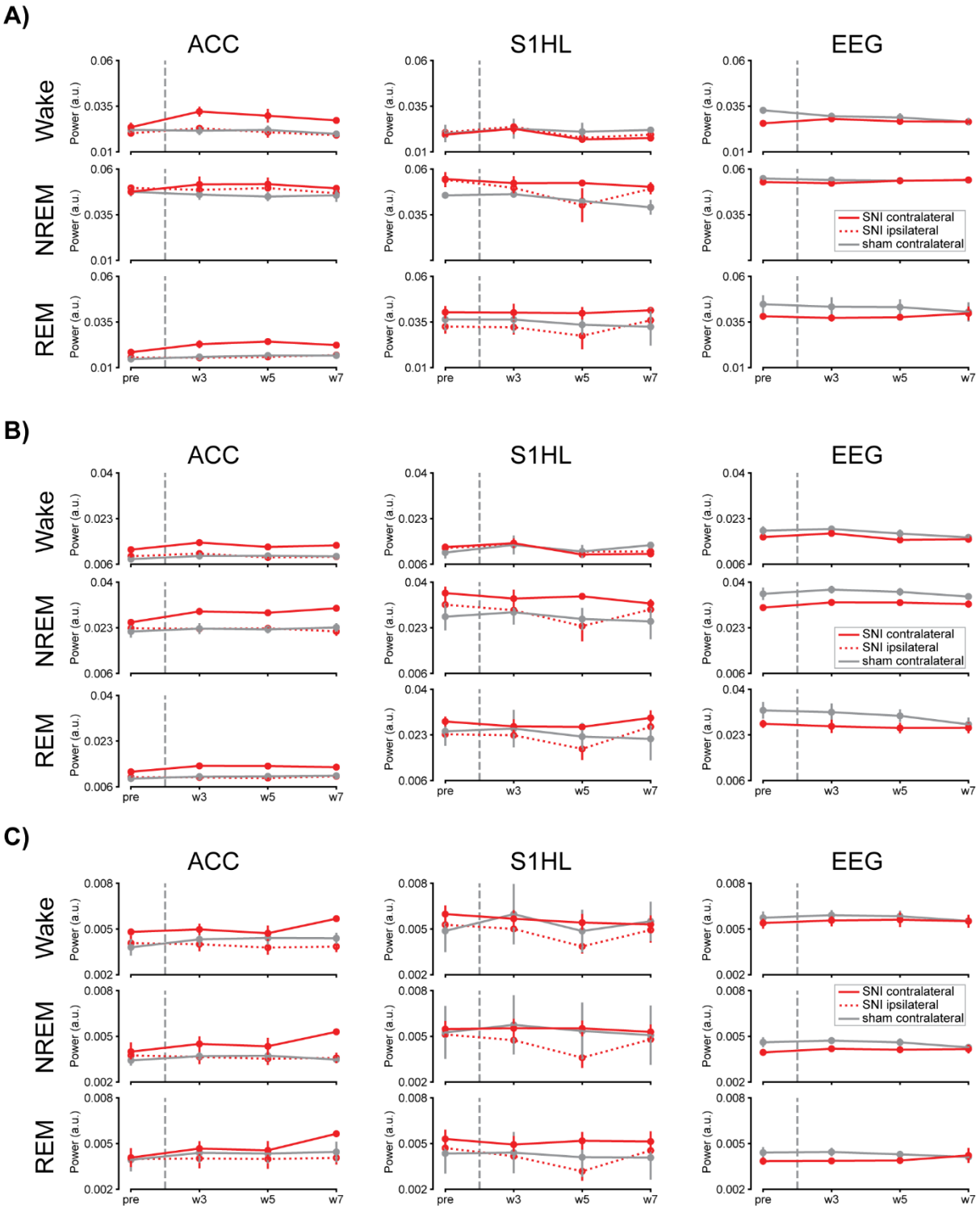


Fig 4. Frequencies of the thalamocortical dysrhythmia.

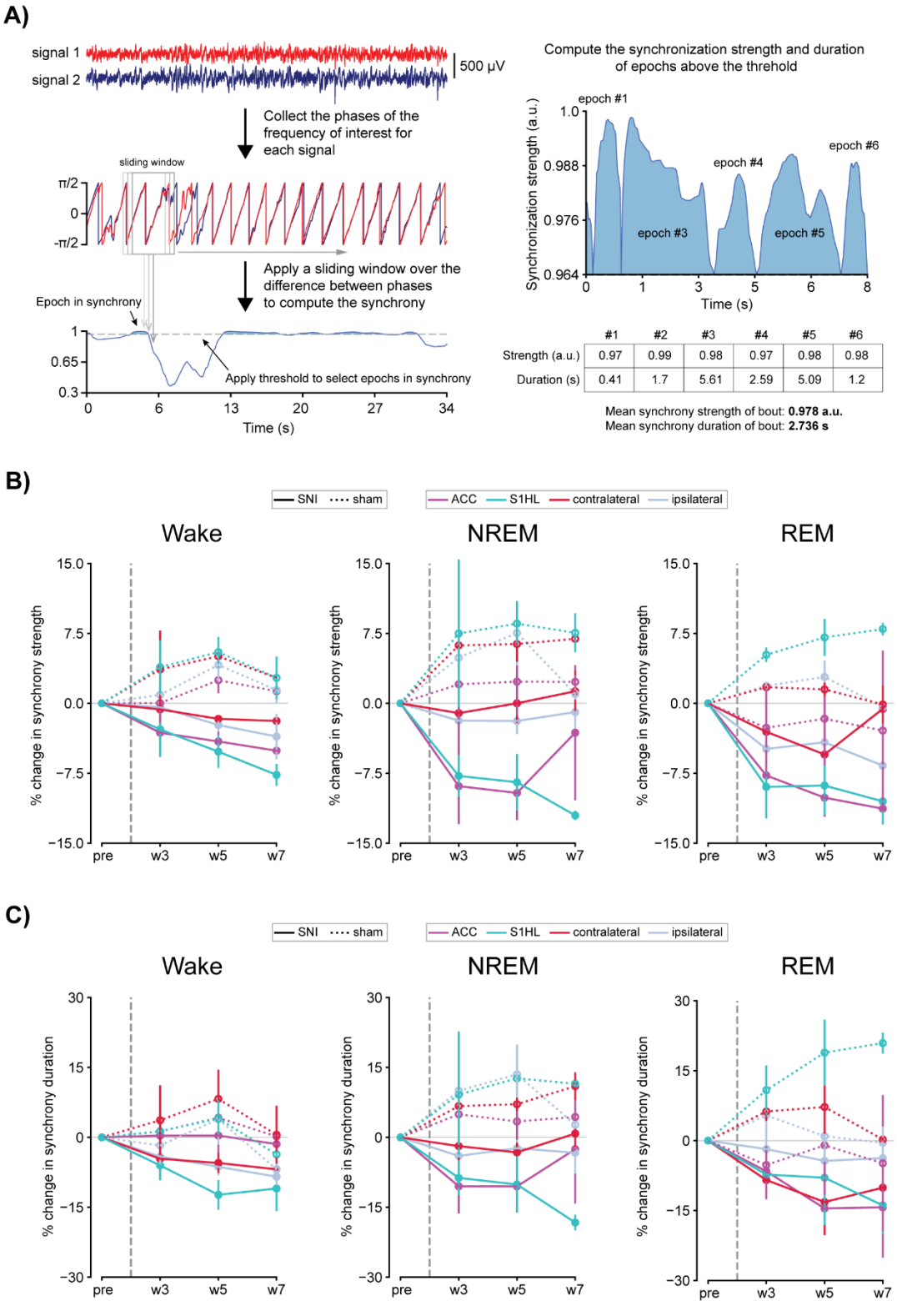


Figure 5. Inter- and intra-hemispheric phase synchrony of SO.

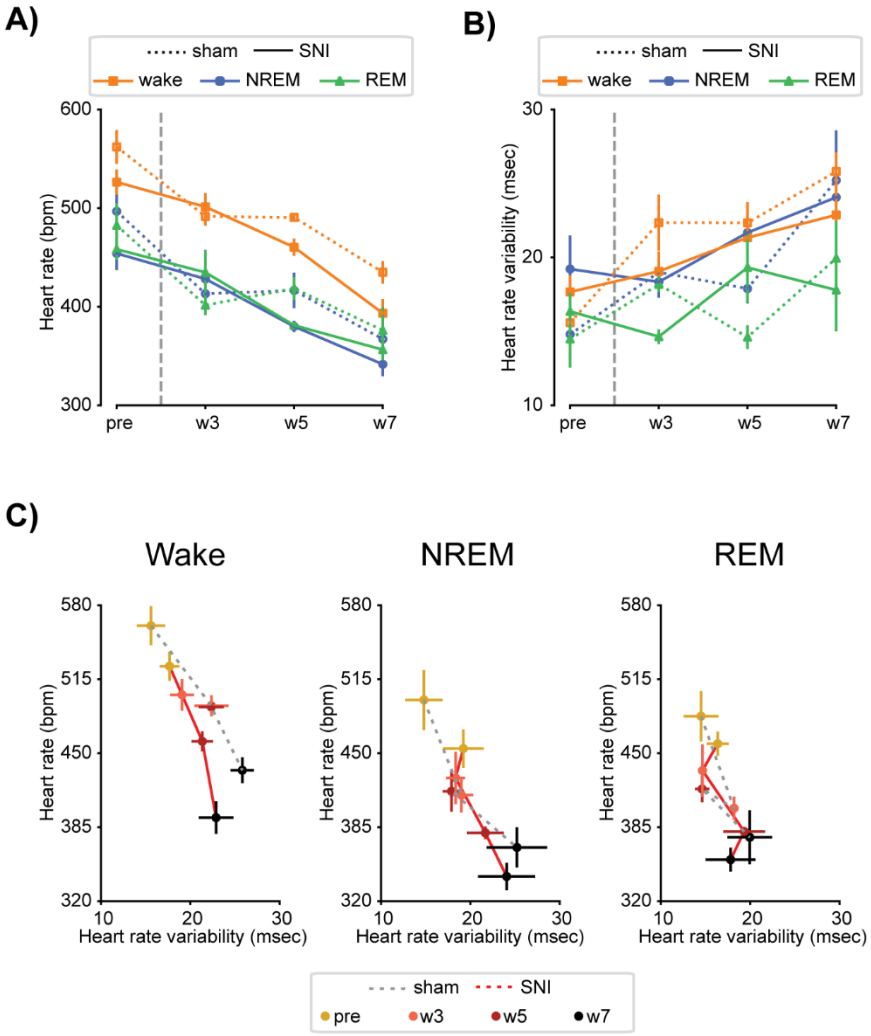


Figure 6. Heart rate and heart rate variability.

General Discussion

"As always in life, people want a simple answer…
and it's always wrong."
Susan Greenfield

In the present thesis, entitled “The Neural Correlates of Pain and Sleep in Health and Disease”, I aimed to contribute to the understanding of the bidirectional interaction of pain and sleep using advanced analysis methods on simultaneous *in vivo* electrophysiological recordings from the S1 and ACC.

Contextualization of Manuscript #1

The first part of this thesis evaluated the effect of the arousal state on neural responses to noxious stimulation.

Results indicated that somatosensory information, regardless of its noxious nature, can reach the cortex in both wake and sleep states. The S1 was recruited earlier than the ACC, suggesting that information flows from primary sensory cortices to higher cortical areas. The spectral dynamics of the signal were also examined as this approach allows for the identification of oscillatory patterns associated with brain processes. Increases in gamma confirmed that the stimulation was processed. However, due to the nature of the stimulation and the ubiquity of this phenomenon, it was unclear how much of these responses were due to sensory processing and how much to salience. Using the heart rate, it was possible to disentangle saliency from pain, as correlations between gamma activity and heart rate were observed only for noxious stimuli.

The spectral dynamics in NREM revealed a particularly strong and long-lasting decrease of frequencies between 2 and 20 Hz. This frequency range is involved in multiple processes. In particular, increased attention to the source of a stimulation modulates alpha (8-16 Hz) power, decreasing it on the contralateral side and increasing it in areas not related to the sensory modality (Bacigalupo & Luck, 2019; Händel et al., 2011; Ikkai et al., 2016; Klimesch, 2012; van Ede et al., 2011). Furthermore, peripheral stimuli activate the locus coeruleous, which releases norepinephrine to the thalamus, in turn stopping the generation of sleep spindles (11-16 Hz) (Devilbiss & Waterhouse, 2011; Osorio-Forero et al., 2021; Vazey et al., 2018), and consequently decreasing alpha power. Given that attention drives changes in the autonomic nervous system (Billings & Shepard, 1910; Laumann et al., 2003), I correlated the heart rate with the alpha band in an early and a late window to differentiate rapid direction of attention from sustained changes in the brain state. A negative correlation between heart rate and alpha activity in the contralateral S1 in NREM, but not in the ACC, was found in instances when animals did not overtly react to the stimulation.

The later finding made me speculate that a decrease in norepinephrine tone in the sensory thalamus selectively altered the state of the S1, potentially resulting in a localized awakening along with increased attention within NREM sleep. The opposite phenomenon, in which sleep features are localized in a specific cortical area, while overall behavior and EEG activity correspond to wakefulness, has been described in humans and rodents, and is termed “local sleep”. Therefore, it is plausible that local wakefulness or increased alertness could emerge following a stimulation during sleep. Particularly, given the high risk that sensory disconnection from the environment entails to an individual – which cannot see, hear nor smell an approaching danger. In my opinion, exploring the concept of local wakefulness and

increases in attention upon stimulation during sleep could provide valuable insights into the levels of sensory disconnection throughout the sleep cycle.

In summary, the first part of the thesis showed that peripheral somatosensation reaches the cortex and is processed during sleep, and that correlating brain activity with physiological measures is a powerful tool to disentangle closely related brain processes such as pain from salience.

Contextualization of Manuscript #2

The second part of this thesis evaluated the impact of pain on sleep behavior and the neural correlates of the different arousal states. My motivation was to push the envelope of understanding the interaction between pain and sleep research through multisite recordings in combination with sophisticated LFP analysis. Preliminary data showed that chronic pain temporarily disturbed sleep but had long-lasting effects on brain activity, indicating that compensatory mechanisms developed over the course of the experiment.

One of the goals of this chapter was to determine whether mice would develop the thalamocortical dysrhythmia observed in humans (Llinás et al., 1999; Vanneste et al., 2018), and whether this phenotype extended to sleep. Our results partially replicated the phenotype of thalamocortical dysrhythmia. The chronic pain-induced slowdown of global neural activity in frontoparietal areas, as measured by the EEG, was similar to what has been described in human studies. Additionally, the ACC in wake recapitulated the increases in theta and gamma power, but not in alpha power, which increased in our experiments, opposite to the decrease described in chronic pain patients. These changes were also observed in NREM and REM, suggesting that the effect of chronic pain on neural activity is not exclusive of wakefulness. The discrepancies between the results of this study and previous studies in humans may be attributed to the duration of chronic pain. Although our study used a relatively long timeline of nearly two months following the induction of chronic pain, patients in human studies have typically been suffering from chronic pain for a minimum of six months, and some for several years. As compensatory mechanisms to cope with persistent pain may take longer to develop, it is possible that our study did not capture these mechanisms. However, our study provides valuable insights into the early stages of chronic pain development, which are difficult to investigate in human populations due to the long-term nature of the condition.

Performing such a long study in mice, we observed striking effects of aging in the synchrony of slow oscillations (SO) between the recorded brain areas as well as in the heart rate.

As mice aged, there was an overall increase in SO synchrony. This is consistent with the known changes that occur during healthy aging: reduction in the number of neurons and strengthening of existing connections. Chronic pain stopped the increase in SO synchrony and even caused a decrease. Given that chronic pain is often accompanied by memory impairments (Mazza et al., 2018; Phelps et al., 2021), and that SO disruptions, particularly in NREM, disrupt memory consolidation (Hickey & Race, 2021; Kim et al., 2017; Niknazar et al.,

2022), it would be worth evaluating the correlations between changes in SO synchronization and memory function in future experiments involving mouse models of chronic pain.

Consistent with the literature, the heart rate decreased as mice aged (Birnbaumer et al., 2020; Chen et al., 2019; Santos et al., 2013). Induction of chronic pain exacerbated the decrease.

Overall, the present thesis aims to advance the field of neuroscience by investigating the neural signatures of the interaction between pain and sleep. In particular, it demonstrates that S1 and ACC, two brain cortices implicated in the processing of pain, exhibit distinct responses and adaptations to pain that are specific to the arousal state. Furthermore, it highlights that the simultaneous analysis of both physiological and neural data provides a more comprehensive understanding of mental processes, as it allows for a more nuanced examination of the underlying mechanisms. Moreover, this thesis confirms that the bidirectional communication between pain and sleep can be observed in cortical areas related to pain, such as the ACC and S1. Our results indicate that higher cortical areas may play a key role in facilitating communication between sleep and pain, as greater disturbances were observed in the ACC than in the S1 upon induction of chronic pain. Furthermore, therapies that target sleep, which involve modulation of brain oscillations, have the potential to improve pain ratings in individuals suffering from chronic pain by reestablishing normal oscillations in the affected neural network.

Sleep as a window of opportunity for the establishment of chronic pain

The ability to distinguish words, understand their meaning, and attribute biological significance to them is retained during sleep, as evidenced by research on auditory processing during sleep (Ameen et al., 2022; Andrillon et al., 2016; Fogel et al., 2022; Perrin et al., 1999). This indicates that the brain retains a minimum level of awareness during sleep. A similar phenomenon is described in Chapter 2, where physiological and neural responses strongly suggest that somatosensation is perceived and processed during sleep. Furthermore, neural responses to both types of stimuli in both the time and frequency domain together with the rate of overt behavioral responses strongly support the notion that the stimulus characteristics can be distinguished during sleep. This evidence allows to speculate that the gating of sensory information during sleep[3] (Andrillon & Kouider, 2020; Steriade, 2003) may be dependent on the biological saliency of the stimulation. Thus, stimuli that are more biologically relevant for the individual will reach further down-stream areas than stimuli that are not biologically relevant.

Decades of research on the question of “why do animals sleep?” have revealed sleep’s crucial role on memory consolidation, emotional regulation, and on maintaining synaptic homeostasis (Cirelli & Tononi, 2008; Vandekerckhove & Wang, 2018). Proper sleep and

[3] **Gating of sensory information during sleep.** Mechanism that filters peripheral information during sleep to prevent awakening. Measured through the dampening of the amplitude of sensory evoked potentials from the lower to the higher brain regions that process that sensory modality.

disconnection from sensory information from the environment is essential for these processes.

It is well established that constant auditory stimulation during sleep modulates the oscillatory rhythms recorded in the EEG (Garcia-Molina et al., 2018; Henin et al., 2019; Ngo et al., 2013). Considering that both auditory and noxious stimuli during sleep can be perceived and processed, I theorize that both sensory modalities can evoke similar neural responses. Persistent auditory stimulation is unlikely to be behaviorally relevant, and may consequently, be filtered out without major consequences for the survival of the individual. However, pain is highly behaviorally relevant as it may indicate danger for the integrity and survival of the individual. Thus, it may bypass the sensory gate in sleep and reach further down-stream areas. Hence, the modulation of oscillatory rhythms by persistent pain may evoke complex oscillatory changes related to cope strategies for the persistent influx of nociceptive information.

All animals become acquainted with pain through wake experiences. During this conscious state, both innate neural mechanisms (i.e. top-down modulation of pain – pgACC and endocannabinoids –) as well as learned coping strategies (i.e. distraction) work together to reduce pain and discomfort. During sleep, these consciously used coping mechanisms are likely unavailable, resulting in less resources to dampen the effects of bottom-up noxious inputs during sleep. However, a crucial question remains: is the absence of painful memories while asleep equivalent to lack of pain sensation? Evidence from Chapter 2 hints at preserved processing of the painful sensation during sleep. If pain sensation is processed during sleep, persistent noxious stimulation causing persistent activation of the somatosensory cortex during sleep may disrupt synaptic homeostasis[4], contributing to the increased spine motility and changes in bouton density observed in the somatosensory cortex in chronic pain (Kim et al., 2017).

In light of the aforementioned factors, I hypothesize that sleep is a susceptible state for pain-induced plasticity changes that allow the shift from acute to chronic pain. Furthermore, recent evidence supports this hypothesis as inhibiting hyperactive cholinergic neurons in the parabrachial nucleus projecting to inhibitory neurons in the somatosensory cortex prevented and reversed chronic pain, if and only if inhibition was performed during NREM sleep (Zhou et al., 2023).

The evolution of the pain-sleep research field

Increasing interest in understanding the relationship between sleep and pain is rising among pain research experts. However, both the sleep cycle and pain, along with pathological pain, are intricate physiological processes that share neural mechanisms. Consequently,

[4] **Synaptic homeostasis.** Pruning of new synapsis generated during wake that takes place during deep stages of NREM sleep. This mechanism allows for neural flexibility and for the ability to generate new synaptic connections on the following wake period. Sleep deprivation disrupts synaptic homeostasis. Poor synaptic homeostasis is associated with memory impairments.

studying the relationship between sleep and pain simultaneously adds an additional layer of complexity to research in this field.

Animal studies offer a unique advantage in understanding the relationship between sleep and pain due to the high level of control over research conditions. I believe that these studies will be instrumental in advancing our knowledge of how sleep and pain communicate with each other. Furthermore, technological and methodological advances provide a fantastic opportunity to thoroughly examine the links between pain and sleep. For instance, chronic multisite *in vivo* electrophysiological recordings, as the ones used in my thesis, generate a wealth of data that can be used to draw a variety of inferences about the dynamics of the recorded neuronal populations. In addition, they provide the advantage of performing consecutive data collections, as well as following neural activity before, during and after a task in one same animal over time, given there is no need to terminate the experiments, as in the case of immunohistological analysis or *ex vivo* electrophysiology.

Calcium imaging is another technique with great potential to decipher brain activity, as it even allows to monitor the activity of specific neuronal populations. Measurement methods that enable chronic calcium recordings from freely behaving animals are being widely integrated in the research of pain and sleep. These include:

- **Miniscopes.** These permit the study single cell activity and even track the activity of a particular cell over multiple recording sessions.
- **Fiber photometry.** This technique allows for the measurements of bulk neural activity if using a calcium indicator is expressed in the cells. Even more, viral transfection of GRAB[5] sensors allow tracking the release of neurotransmitters or the levels of a given metabolite.

Future research on the complex relationship between sleep and pain can benefit from experimental designs that simultaneously use multiple *in vivo* methods to record neural activity. For example, one approach that could prove useful is tracking electrophysiological activity alongside changes in neurotransmitter levels. This would provide a more comprehensive view of the neural mechanisms underlying the pain-sleep interaction.

It is quite obvious at this point, that the research field of pain and sleep will eventually move into the era of big data. However, this change requires the shift of the classical skillset of scientists who want to pursue this research path. Keeping the focus on *in vivo* electrophysiology as the method to study the interaction between sleep and pain, a neuroscientist in the field will need to:

1) Learn the basics on electrical engineering to understand and tinker the recording implants and the recording setup according to the needs of the experiment.
2) Be comfortable on small rodent surgery for intracranial implantation of neural probes.
3) Be knowledgeable on animal welfare.
4) Preserve wet lab skills.
5) Develop strong coding skills for data management, processing, curation and analysis.

[5] **GRAB sensor.** Genetically encoded sensors to measure neurotransmitter levels.

6) Consolidate a solid mathematical and statistical background to analyze the data.
7) Acquire a solid basis on neuro-electrophysiology, from the channel composition of neurons to the generation of oscillations, for a correct interpretation of the results.

The new necessary skills are broad and not necessarily related to the biology background of the researchers. Therefore, investing time in proper training is a requirement, given that mistakes or poor choices can cause great time delays and considerable economic loss. While in industry all these steps are assigned to different teams, in academia, the researcher is expected to master each single step of the equation, in which case, progress becomes slower. In my opinion, academic laboratories that take the leap to combine multiple *in vivo* techniques to measure brain activity in living organisms would greatly benefit to conduct these projects in teams and split the tasks.

Current and future challenges

It is essential to acknowledge that there are challenges associated with the aforementioned techniques to ensure their proper incorporation, as well as the correct interpretation of the results.

One significant challenge is the large amount of data generated by these techniques. Laboratories lacking the proper equipment or expertise to handle these datasets may experience delays, data loss, and excessively long computational times.

Additionally, steps that require manual work, such as scoring the different states of the sleep cycle or marking stimuli, can easily become bottlenecks for researchers and laboratories. Particularly in the field of sleep, some efforts have been directed into creating user interfaces, algorithms and programs to automatically score the different sleep stages. Nevertheless, the automated solutions are often over fitted to the developer needs and hard to transfer from laboratory to laboratory. Furthermore, the high variance in the recorded data further exacerbates the difficulty for the widespread automation of such processes. The sources of this variability are many and include:

- Lack of standardized recordings
- Different ways to do sleep scoring that depend on:
    - the research question
    - the school of thought
    - the software used
- Large variability between recorded individuals
- Different recording rigs
- Difference in the electrical noise levels in the signal
- Deficient technical documentation on the algorithm
- Too many technical details that researchers unfamiliar with the coding language find hard to understand

This highlights the need to:

1) Standardize these type of recordings.
2) Generate comprehensive documentation of the newly developed algorithms so that researchers unfamiliar with coding can easily implement the new tools.

Adopting these measures would facilitate the spread of the newly developed analysis tools by other laboratories, hence speeding up this sort of data analysis.

The analysis of these vast and complex datasets poses additional challenges. In the case of local field potentials, such as in this thesis, the signals contain large amounts of information that can be unlocked using advanced analysis methods. However, the analysis of electrophysiological neural time series in animal models is still at its infancy, and mostly focused on evoked potentials analysis and static power spectral profiles. While the analysis of electrophysiological signals in humans has advanced significantly and offers a wide range of methods, transferring these methods to animal studies is facing some obstacles. With questions such as “How local is the local field potential?” or “Are the frequency bands reflecting the same processes in rodents and humans?”, it is clear that there are basic concepts that need to be clarified in the animal research field as these create resistance to use more sophisticated data analysis methods.

Electrophysiological recordings in animals offer exceptional power, but also come with their own set of challenges. Simple methods, like the use of tungsten wires or tetrodes to record neural activity, have already proven to be powerful tools. Yet, the research community is reaching even higher capabilities for recording neural activity with the development of neural probes with hundreds of recording sites. These neural probes allow for simultaneous recording and analysis of neural spikes, local field potentials, and currents, with a specificity that even permits to differentiate the activity at different cortical layers. In addition, the simultaneous acquisition of data from so many recording sites allows for correlations between local field potentials, current source densities, neural spikes and frequency bands between cortical layers or brain regions. However, these new recording capabilities require the development of more complex analysis, which permits better contextualization of neural activity. Nevertheless, complex sophisticated analysis are not exempt of problems, as careful interpretation of the data will be increasingly needed.

References

"If I have seen further, it is by standing on the shoulders of Giants"

Isaac Newton

Abdus-Saboor, I., Fried, N. T., Lay, M., Burdge, J., Swanson, K., Fischer, R., Jones, J., Dong, P., Cai, W., Guo, X., Tao, Y. X., Bethea, J., Ma, M., Dong, X., Ding, L., & Luo, W. (2019). Development of a Mouse Pain Scale Using Sub-second Behavioral Mapping and Statistical Modeling. *Cell Reports*, *28*(6), 1623-1634.e4. https://doi.org/10.1016/J.CELREP.2019.07.017

Adamantidis, A. R., Gutierrez Herrera, C., & Gent, T. C. (2019). Oscillating circuitries in the sleeping brain. *Nature Reviews Neuroscience 2019 20:12*, *20*(12), 746–762. https://doi.org/10.1038/s41583-019-0223-4

Afolalu, E. F., Ramlee, F., & Tang, N. K. Y. (2018). Effects of sleep changes on pain-related health outcomes in the general population: A systematic review of longitudinal studies with exploratory meta-analysis. *Sleep Medicine Reviews*, *39*, 82–97. https://doi.org/10.1016/J.SMRV.2017.08.001

Ameen, M. S., Heib, D. P. J., Blume, C., & Schabus, M. (2022). The Brain Selectively Tunes to Unfamiliar Voices during Sleep. *Journal of Neuroscience*, *42*(9), 1791–1803. https://doi.org/10.1523/JNEUROSCI.2524-20.2021

Andrillon, T., & Kouider, S. (2020). The vigilant sleeper: neural mechanisms of sensory (de)coupling during sleep. *Current Opinion in Physiology*, *15*, 47–59. https://doi.org/10.1016/J.COPHYS.2019.12.002

Andrillon, T., Poulsen, A. T., Hansen, L. K., Léger, D., & Kouider, S. (2016). Neural Markers of Responsiveness to the Environment in Human Sleep. *The Journal of Neuroscience*, *36*(24), 6583–6596. https://doi.org/10.1523/JNEUROSCI.0902-16.2016

Apkarian, A. V., Bushnell, M. C., Treede, R. D., & Zubieta, J. K. (2005). Human brain mechanisms of pain perception and regulation in health and disease. *European Journal of Pain*, *9*(4), 463–463. https://doi.org/10.1016/J.EJPAIN.2004.11.001

Argoff, C. (2011). Mechanisms of pain transmission and pharmacologic management. *Current Medical Research and Opinion*, *27*(10), 2019–2031. https://doi.org/10.1185/03007995.2011.614934

Bacigalupo, F., & Luck, S. J. (2019). Lateralized Suppression of Alpha-Band EEG Activity As a Mechanism of Target Processing. *The Journal of Neuroscience*, *39*(5), 900. https://doi.org/10.1523/JNEUROSCI.0183-18.2018

Başar, E., Başar-Eroglu, C., Karakaş, S., & Schürmann, M. (2001). Gamma, alpha, delta, and theta oscillations govern cognitive processes. *International Journal of Psychophysiology*, *39*(2–3), 241–248. https://doi.org/10.1016/S0167-8760(00)00145-8

Bastuji, H., Perchet, C., Legrain, V., Montes, C., & Garcia-Larrea, L. (2008). Laser evoked responses to painful stimulation persist during sleep and predict subsequent arousals. *Pain*, *137*(3), 589–599. https://doi.org/10.1016/J.PAIN.2007.10.027

Beissner, F., Meissner, K., Bär, K.-J., & Napadow, V. (2013). *The Autonomic Brain: An Activation Likelihood Estimation Meta-Analysis for Central Processing of Autonomic Function*. https://doi.org/10.1523/JNEUROSCI.1103-13.2013

Bentley, A. J., Newton, S., & Zio, C. D. (2003). Sensitivity of sleep stages to painful thermal stimuli. *Journal of Sleep Research*, *12*(2), 143–147. https://doi.org/10.1046/J.1365-

2869.2003.00352.X

Birnbaumer, P., Traninger, H., Borenich, A., Falgenhauer, M., Modre-Osprian, R., Harpf, H., & Hofmann, P. (2020). Heart Rate Performance Curve Is Dependent on Age, Sex, and Performance. *Frontiers in Public Health*, *8*, 98. https://doi.org/10.3389/FPUBH.2020.00098/BIBTEX

Blom, S. M., Pfister, J.-P., Santello, M., Senn, W., & Nevian, T. (2014). Nerve Injury-Induced Neuropathic Pain Causes Disinhibition of the Anterior Cingulate Cortex. *Journal of Neuroscience*, *34*(17), 5754–5764. https://doi.org/10.1523/JNEUROSCI.3667-13.2014

Bowyer, S. M. (2016). Coherence a measure of the brain networks: past and present. *Neuropsychiatric Electrophysiology 2016 2:1*, *2*(1), 1–12. https://doi.org/10.1186/S40810-015-0015-7

Breivik, H., Collett, B., Ventafridda, V., Cohen, R., & Gallacher, D. (2005). *Survey of chronic pain in Europe: Prevalence, impact on daily life, and treatment*. https://doi.org/10.1016/j.ejpain.2005.06.009

Brown, R. E., Basheer, R., McKenna, J. T., Strecker, R. E., & McCarley, R. W. (2012). Control of sleep and wakefulness. *Physiological Reviews*, *92*(3), 1087–1187. https://doi.org/10.1152/PHYSREV.00032.2011/ASSET/IMAGES/LARGE/Z9J0031226200013.JPEG

Buchheit, T., Van de Ven, T., & Shaw, A. (2012). Epigenetics and the Transition from Acute to Chronic Pain. *Pain Medicine (United States)*, *13*(11), 1474–1490. https://doi.org/10.1111/J.1526-4637.2012.01488.X/2/PME1488_F3.JPEG

Byers, H. D., Lichstein, K. L., & Thorn, B. E. (2016). Cognitive processes in comorbid poor sleep and chronic pain. *Journal of Behavioral Medicine*, *39*(2), 233–240. https://doi.org/10.1007/S10865-015-9687-5/TABLES/3

Campbell, C. M., Bounds, S. C., Kuwabara, H., Edwards, R. R., Campbell, J. N., Haythornthwaite, J. A., & Smith, M. T. (2013). Individual variation in sleep quality and duration is related to cerebral Mu opioid receptor binding potential during tonic laboratory pain in healthy subjects. *Pain Medicine (United States)*, *14*(12), 1882–1892. https://doi.org/10.1111/PME.12231/2/PME12231-FIG-0005.JPEG

Cardis, R., Lecci, S., Fernandez, L. M., Osorio-Forero, A., Chung, P. C. S., Fulda, S., Decosterd, I., & Lüthi, A. (2021). Cortico-autonomic local arousals and heightened somatosensory arousability during NREMS of mice in neuropathic pain. *ELife*, *10*. https://doi.org/10.7554/ELIFE.65835

Cechetto, D. F. (2014). Cortical control of the autonomic nervous system. *Experimental Physiology*, *99*(2), 326–331. https://doi.org/10.1113/EXPPHYSIOL.2013.075192

Chang, P. C., Pollema-Mays, S. L., Centeno, M. V., Procissi, D., Contini, M., Baria, A. T., Martina, M., & Apkarian, A. V. (2014). Role of nucleus accumbens in neuropathic pain: Linked multi-scale evidence in the rat transitioning to neuropathic pain. *PAIN®*, *155*(6), 1128–1139. https://doi.org/10.1016/J.PAIN.2014.02.019

Chen, X. J., Barywani, S. B., Hansson, P. O., Östgärd Thunström, E., Rosengren, A., Ergatoudes, C., Mandalenakis, Z., Caidahl, K., & Fu, M. L. (2019). Impact of changes in heart rate with

age on all-cause death and cardiovascular events in 50-year-old men from the general population. *Open Heart*, *6*(1), e000856. https://doi.org/10.1136/OPENHRT-2018-000856

Chouchou, F., Pichot, V., Perchet, C., Legrain, V., Garcia-Larrea, L., Roche, F., & Bastuji, H. (2011). Autonomic pain responses during sleep: a study of heart rate variability. *European Journal of Pain (London, England)*, *15*(6), 554–560. https://doi.org/10.1016/J.EJPAIN.2010.11.011

Chung, K. M., Ho, C. H., Chen, Y. C., Hsu, C. C., Chiu, C. C., Lin, H. J., Wang, J. J., & Huang, C. C. (2020). Chronic Pain Increases the Risk for Major Adverse Cardiac and Cerebrovascular Events: A Nationwide Population-Based Study in Asia. *Pain Medicine (Malden, Mass.)*, *21*(9), 1985–1990. https://doi.org/10.1093/PM/PNAA107

Cirelli, C., & Tononi, G. (2008). Is Sleep Essential? *PLOS Biology*, *6*(8), e216. https://doi.org/10.1371/JOURNAL.PBIO.0060216

Cohen, M. X. (2014). Analyzing Neural Time Series Data: Theory and Practice. *Analyzing Neural Time Series Data*. https://doi.org/10.7551/MITPRESS/9609.001.0001

Craig, A. D. (2002). How do you feel? Interoception: the sense of the physiological condition of the body. *Nature Reviews Neuroscience 2002 3:8*, *3*(8), 655–666. https://doi.org/10.1038/nrn894

Critchley, H. D., Mathias, C. J., Josephs, O., O'Doherty, J., Zanini, S., Dewar, B. K., Cipolotti, L., Shallice, T., & Dolan, R. J. (2003). Human cingulate cortex and autonomic control: converging neuroimaging and clinical evidence. *Brain*, *126*(10), 2139–2152. https://doi.org/10.1093/BRAIN/AWG216

Crunelli, V., Larincz, M. L., Connelly, W. M., David, F., Hughes, S. W., Lambert, R. C., Leresche, N., & Errington, A. C. (2018). Dual function of thalamic low-vigilance state oscillations: Rhythm-regulation and plasticity. In *Nature Reviews Neuroscience* (Vol. 19, Issue 2, pp. 107–118). Nature Publishing Group. https://doi.org/10.1038/nrn.2017.151

Da Silva, J. T., & Seminowicz, D. A. (2019). Neuroimaging of pain in animal models: a review of recent literature. *Pain Reports*, *4*(4). https://doi.org/10.1097/PR9.0000000000000732

Dalanon, J., Chikahisa, S., Shiuchi, T., Shimizu, N., Chavan, P., Suzuki, Y., Okura, K., Séi, H., & Matsuka, Y. (2021). Pain sensitivity increases with sleep disturbance under predictable chronic mild stress in mice. *Scientific Reports 2021 11:1*, *11*(1), 1–10. https://doi.org/10.1038/s41598-021-93560-7

Danilin, L. K., Spindler, M., Sörös, P., & Bantel, C. (2022). Heart rate and heart rate variability in patients with chronic inflammatory joint disease: the role of pain duration and the insular cortex. *BMC Musculoskeletal Disorders*, *23*(1), 1–12. https://doi.org/10.1186/S12891-022-05009-1/TABLES/6

De Ridder, D., Vanneste, S., Smith, M., & Adhia, D. (2022). Pain and the Triple Network Model. *Frontiers in Neurology*, *13*, 272. https://doi.org/10.3389/FNEUR.2022.757241/BIBTEX

Destexhe, A. (2009). Sleep Oscillations. *Encyclopedia of Neuroscience*, 1027–1034. https://doi.org/10.1016/B978-008045046-9.01417-0

Deuis, J. R., Dvorakova, L. S., & Vetter, I. (2017). Methods used to evaluate pain behaviors in rodents. *Frontiers in Molecular Neuroscience*, *10*, 284. https://doi.org/10.3389/FNMOL.2017.00284/BIBTEX

Di Pietro, F., Macey, P. M., Rae, C. D., Alshelh, Z., Macefield, V. G., Vickers, E. R., & Henderson, L. A. (2018). The relationship between thalamic GABA content and resting cortical rhythm in neuropathic pain. *Human Brain Mapping*, *39*(5), 1945. https://doi.org/10.1002/HBM.23973

Dueñas, M., Ojeda, B., Salazar, A., Mico, J. A., & Failde, I. (2016). A review of chronic pain impact on patients, their social environment and the health care system. *Journal of Pain Research*, *9*, 457–467. https://doi.org/10.2147/JPR.S105892

Eacret, D., Veasey, S. C., & Blendy, J. A. (2020). Bidirectional Relationship between Opioids and Disrupted Sleep: Putative Mechanisms. *Molecular Pharmacology*, *98*(4), 445–453. https://doi.org/10.1124/MOL.119.119107

Eban-Rothschild, A., Appelbaum, L., & De Lecea, L. (2017). Neuronal Mechanisms for Sleep/Wake Regulation and Modulatory Drive. *Neuropsychopharmacology 2018 43:5*, *43*(5), 937–952. https://doi.org/10.1038/npp.2017.294

Eller-Smith, O. C., Nicol, A. L., & Christianson, J. A. (2018). Potential mechanisms underlying centralized pain and emerging therapeutic interventions. *Frontiers in Cellular Neuroscience*, *12*, 35. https://doi.org/10.3389/FNCEL.2018.00035/BIBTEX

Fadda, P., Tortorella, A., & Fratta, W. (1991). Sleep deprivation decreases μ and δ opioid receptor binding in the rat limbic system. *Neuroscience Letters*, *129*(2), 315–317. https://doi.org/10.1016/0304-3940(91)90489-G

Fallon, N., Chiu, Y., Nurmikko, T., & Stancak, A. (2018). Altered theta oscillations in resting EEG of fibromyalgia syndrome patients. *European Journal of Pain (United Kingdom)*, *22*(1), 49–57. https://doi.org/10.1002/ejp.1076

Faraut, B., Léger, D., Medkour, T., Dubois, A., Bayon, V., Chennaoui, M., & Perrot, S. (2015). Napping Reverses Increased Pain Sensitivity Due to Sleep Restriction. *PLOS ONE*, *10*(2), e0117425. https://doi.org/10.1371/JOURNAL.PONE.0117425

Finan, P. H., Goodin, B. R., & Smith, M. T. (2013). The association of sleep and pain: An update and a path forward. *Journal of Pain*, *14*(12), 1539–1552. https://doi.org/10.1016/j.jpain.2013.08.007

Finan, P. H., & Smith, M. T. (2013). The Comorbidity of Insomnia, Chronic Pain, and Depression: Dopamine as a Putative Mechanism. *Sleep Medicine Reviews*, *17*(3), 173. https://doi.org/10.1016/J.SMRV.2012.03.003

Fogel, S., Ray, L., Fang, Z., Silverbrook, M., Naci, L., & Owen, A. M. (2022). While you were sleeping: Evidence for high-level executive processing of an auditory narrative during sleep. *Consciousness and Cognition*, *100*. https://doi.org/10.1016/J.CONCOG.2022.103306

Fried, N. T., Chamessian, A., Zylka, M. J., & Abdus-Saboor, I. (2020). Improving pain assessment in mice and rats with advanced videography and computational approaches. *Pain*, *161*(7), 1420–1424. https://doi.org/10.1097/J.PAIN.0000000000001843

Garcia-Larrea, L., & Peyron, R. (2013). Pain matrices and neuropathic pain matrices: A review. *Pain*, *154*(SUPPL. 1). https://doi.org/10.1016/J.PAIN.2013.09.001

Garcia-Molina, G., Tsoneva, T., Jasko, J., Steele, B., Aquino, A., Baher, K., Pastoor, S., Pfundtner, S., Ostrowski, L., Miller, B., Papas, N., Riedner, B., Tononi, G., & White, D. P. (2018). Closed-loop system to enhance slow-wave activity. *Journal of Neural Engineering*, *15*(6), 066018. https://doi.org/10.1088/1741-2552/AAE18F

Goldman, N., Hablitz, L. M., Mori, Y., & Nedergaard, M. (2020). The Glymphatic System and Pain. *Medical Acupuncture*, *32*(6), 373. https://doi.org/10.1089/ACU.2020.1489

Guo, R., Chen, L. H., Xing, C., & Liu, T. (2019). Pain regulation by gut microbiota: molecular mechanisms and therapeutic potential. *British Journal of Anaesthesia*, *123*(5), 637–654. https://doi.org/10.1016/J.BJA.2019.07.026

Haack, M., Simpson, N., Sethna, N., Kaur, S., & Mullington, J. (2020). Sleep deficiency and chronic pain: potential underlying mechanisms and clinical implications. *Neuropsychopharmacology*, *45*(1), 205. https://doi.org/10.1038/S41386-019-0439-Z

Haefeli, M., & Elfering, A. (2006). Pain assessment. *European Spine Journal*, *15*(Suppl 1), S17. https://doi.org/10.1007/S00586-005-1044-X

Händel, B. F., Haarmeier, T., & Jensen, O. (2011). Alpha Oscillations Correlate with the Successful Inhibition of Unattended Stimuli. *Journal of Cognitive Neuroscience*, *23*(9), 2494–2502. https://doi.org/10.1162/JOCN.2010.21557

Hauck, M., Domnick, C., Lorenz, J., Gerloff, C., & Engel, A. K. (2015). Top-down and bottom-up modulation of pain-induced oscillations. *Frontiers in Human Neuroscience*, *9*(JULY), 375. https://doi.org/10.3389/FNHUM.2015.00375/BIBTEX

Häuser, W., Bernardy, K., Üçeyler, N., & Sommer, C. (2009). Treatment of Fibromyalgia Syndrome With Antidepressants: A Meta-analysis. *JAMA*, *301*(2), 198–209. https://doi.org/10.1001/JAMA.2008.944

Hayashida, K. I., & Obata, H. (2019). Strategies to Treat Chronic Pain and Strengthen Impaired Descending Noradrenergic Inhibitory System. *International Journal of Molecular Sciences 2019, Vol. 20, Page 822*, *20*(4), 822. https://doi.org/10.3390/IJMS20040822

Henin, S., Borges, H., Shankar, A., Sarac, C., Melloni, L., Friedman, D., Flinker, A., Parra, L. C., Buzsaki, G., Devinsky, O., & Liu, A. (2019). Closed-Loop Acoustic Stimulation Enhances Sleep Oscillations But Not Memory Performance. *ENeuro*, *6*(6). https://doi.org/10.1523/ENEURO.0306-19.2019

Hennevin, E., Huetz, C., & Edeline, J. M. (2007). Neural representations during sleep: From sensory processing to memory traces. *Neurobiology of Learning and Memory*, *87*(3), 416–440. https://doi.org/10.1016/J.NLM.2006.10.006

Hickey, P., & Race, E. (2021). Riding the slow wave: Exploring the role of entrained low-frequency oscillations in memory formation. *Neuropsychologia*, *160*, 107962. https://doi.org/10.1016/J.NEUROPSYCHOLOGIA.2021.107962

Higginbotham, J. A., Markovic, T., Massaly, N., & Morón, J. A. (2022). Endogenous opioid systems alterations in pain and opioid use disorder. *Frontiers in Systems Neuroscience*,

16, 118. https://doi.org/10.3389/FNSYS.2022.1014768/BIBTEX

Hiraga, S. ichiro, Itokazu, T., Nishibe, M., & Yamashita, T. (2022). Neuroplasticity related to chronic pain and its modulation by microglia. *Inflammation and Regeneration*, *42*(1), 1–10. https://doi.org/10.1186/S41232-022-00199-6/FIGURES/3

Hogrefe, N., Blom, S. M., Valentinova, K., Ntamati, N. R., Jonker, L. J. E., Nevian, N. E., & Nevian, T. (2022). *Long-Lasting, Pathway-Specific Impairment of a Novel Form of Spike-Timing-Dependent Long-Term Depression by Neuropathic Pain in the Anterior Cingulate Cortex*. https://doi.org/10.1523/JNEUROSCI.0326-21.2022

Hopp, M., Bosse, B., & Dunlop, W. (2014). The Socioeconomic Costs of the Undertreatment of Pain. *Value in Health*, *17*(7), A785. https://doi.org/10.1016/J.JVAL.2014.08.400

Horner, R. L., & Peever, J. H. (2017). Brain Circuitry Controlling Sleep and Wakefulness. *CONTINUUM Lifelong Learning in Neurology*, *23*(4, SleepNeurology), 955–972. https://doi.org/10.1212/CON.0000000000000495

Ikkai, A., Dandekar, S., & Curtis, C. E. (2016). Lateralization in Alpha-Band Oscillations Predicts the Locus and Spatial Distribution of Attention. *PLoS ONE*, *11*(5). https://doi.org/10.1371/JOURNAL.PONE.0154796

Issa, E. B., & Wang, X. (2008). Sensory Responses during Sleep in Primate Primary and Secondary Auditory Cortex. *The Journal of Neuroscience*, *28*(53), 14467. https://doi.org/10.1523/JNEUROSCI.3086-08.2008

Jensen, O., Kaiser, J., & Lachaux, J. P. (2007). Human gamma-frequency oscillations associated with attention and memory. *Trends in Neurosciences*, *30*(7), 317–324. https://doi.org/10.1016/J.TINS.2007.05.001

Ji, M. J., Yang, J., Gao, Z. Q., Zhang, L., & Liu, C. (2021). The Role of the Kappa Opioid System in Comorbid Pain and Psychiatric Disorders: Function and Implications. *Frontiers in Neuroscience*, *15*, 130. https://doi.org/10.3389/FNINS.2021.642493/BIBTEX

Jones, J., Foster, W., Twomey, C., Burdge, J., Ahmed, O., Wojick, J. A., Corder, G., Plotkin, J. B., & Abdus-Saboor, I. (2020). A machine-vision approach for automated pain measurement at millisecond timescales. *BioRxiv*, 2020.02.18.955070. https://doi.org/10.1101/2020.02.18.955070

Kakigi, R., Naka, D., Okusa, T., Wang, X., Inui, K., Qiu, Y., Tran, T. D., Miki, K., Tamura, Y., Nguyen, T. B., Watanabe, S., & Hoshiyama, M. (2003). Sensory perception during sleep in humans: a magnetoencephalograhic study. *Sleep Medicine*, *4*(6), 493–507. https://doi.org/10.1016/S1389-9457(03)00169-2

Kanda, H., Kobayashi, K., Yamanaka, H., & Noguchi, K. (2013). COX-1-dependent prostaglandin D2 in microglia contributes to neuropathic pain via DP2 receptor in spinal neurons. *Glia*, *61*(6), 943–956. https://doi.org/10.1002/GLIA.22487

Karakaş, S., Başar-Eroğlu, C., Özesmi, Ç., Kafadar, H., & Erzengin, Ö. Ü. (2001). Gamma response of the brain: a multifunctional oscillation that represents bottom-up with top-down processing. *International Journal of Psychophysiology*, *39*(2–3), 137–150. https://doi.org/10.1016/S0167-8760(00)00137-9

Kazmi, S. Z. H., Zhang, H., Aziz, W., Monfredi, O., Abbas, S. A., Shah, S. A., Kazmi, S. S. H., & Butt, W. H. (2016). Inverse Correlation between Heart Rate Variability and Heart Rate Demonstrated by Linear and Nonlinear Analysis. *PLoS ONE*, *11*(6). https://doi.org/10.1371/JOURNAL.PONE.0157557

Kędziora, M., Boccella, S., Marabese, I., Mlost, J., Infantino, R., Maione, S., & Starowicz, K. (2023). Inhibition of anandamide breakdown reduces pain and restores LTP and monoamine levels in the rat hippocampus via the CB1 receptor following osteoarthritis. *Neuropharmacology*, *222*, 109304. https://doi.org/10.1016/J.NEUROPHARM.2022.109304

Keilani, M., Crevenna, R., & Dorner, T. E. (2018). Sleep quality in subjects suffering from chronic pain. *Wiener Klinische Wochenschrift*, *130*(1–2), 31–36. https://doi.org/10.1007/S00508-017-1256-1/TABLES/3

Kim, J., Lee, D. A., Lee, H. J., Park, B. S., Ko, J., Park, S. H., Lee, Y. J., Kim, I. H., Park, J. H., & Park, K. M. (2022). Glymphatic system dysfunction in patients with cluster headache. *Brain and Behavior*, *12*(6), e2631. https://doi.org/10.1002/BRB3.2631

Kim, S. Y., Pardilla-Delgado, E., & Alger, S. E. (2017). Enhancing Memory Consolidation through Slow Oscillation and Spindle Synchronization. *The Journal of Neuroscience*, *37*(48), 11517. https://doi.org/10.1523/JNEUROSCI.2512-17.2017

Kim, W., Kim, S. K., & Nabekura, J. (2017). Functional and structural plasticity in the primary somatosensory cortex associated with chronic pain. *Journal of Neurochemistry*, *141*(4), 499–506. https://doi.org/10.1111/JNC.14012

Kitamura, Y., Kakigi, R., Hoshiyama, M., Koyama, S., & Nakamura, A. (1996). Effects of sleep on somatosensory evoked responses in human: a magnetoencephalographic study. *Brain Research. Cognitive Brain Research*, *4*(4), 275–279. https://doi.org/10.1016/S0926-6410(96)00066-3

Klimesch, W. (2012). Alpha-band oscillations, attention, and controlled access to stored information. *Trends in Cognitive Sciences*, *16*(12), 606–617. https://doi.org/10.1016/J.TICS.2012.10.007

Koechlin, H., Kharko, A., Probst, T., Pradela, J., Buechi, S., & Locher, C. (2021). Placebo Responses and Their Clinical Implications in Fibromyalgia: A Meta-Analysis Using SSRI and SNRI Trials. *Frontiers in Pain Research*, *2*, 107. https://doi.org/10.3389/FPAIN.2021.750523

Koffel, E., Kroenke, K., Bair, M. J., Leverty, D., Polusny, M. A., & Krebs, E. E. (2016). The Bidirectional Relationship Between Sleep Complaints and Pain: Analysis of Data from a Randomized Trial. *Health Psychology : Official Journal of the Division of Health Psychology, American Psychological Association*, *35*(1), 41. https://doi.org/10.1037/HEA0000245

KoSTIS, J. B., Moreyra, A. E., Amendo, M. T., Di PIETRO, J., Cosgrove, N., Kuo, P. T., & Kostis, J. B. (n.d.). *The Effect of Age on Heart Rate in Subjects Free of Heart Disease Studies By Ambulatory Electrocardiography and Maximal Exercise Stress Test*. Retrieved January 11, 2023, from http://ahajournals.org

Krause, A. J., Prather, A. A., Wager, T. D., Lindquist, M. A., & Walker, M. P. (2019). The Pain of Sleep Loss: A Brain Characterization in Humans. *Journal of Neuroscience*, *39*(12), 2291–2300. https://doi.org/10.1523/JNEUROSCI.2408-18.2018

Langford, D. J., Bailey, A. L., Chanda, M. L., Clarke, S. E., Drummond, T. E., Echols, S., Glick, S., Ingrao, J., Klassen-Ross, T., Lacroix-Fralish, M. L., Matsumiya, L., Sorge, R. E., Sotocinal, S. G., Tabaka, J. M., Wong, D., Van Den Maagdenberg, A. M. J. M., Ferrari, M. D., Craig, K. D., & Mogil, J. S. (2010). Coding of facial expressions of pain in the laboratory mouse. *Nature Methods 2010 7:6*, *7*(6), 447–449. https://doi.org/10.1038/nmeth.1455

Lascano, A. M., Lalive, P. H., Hardmeier, M., Fuhr, P., & Seeck, M. (2017). Clinical evoked potentials in neurology: a review of techniques and indications. *Journal of Neurology, Neurosurgery & Psychiatry*, *88*(8), 688–696. https://doi.org/10.1136/JNNP-2016-314791

Latremoliere, A., & Woolf, C. J. (2009). Central Sensitization: A Generator of Pain Hypersensitivity by Central Neural Plasticity. *The Journal of Pain*, *10*(9), 895–926. https://doi.org/10.1016/J.JPAIN.2009.06.012

Legrain, V., Iannetti, G. D., Plaghki, L., & Mouraux, A. (2011). The pain matrix reloaded: A salience detection system for the body. *Progress in Neurobiology*, *93*(1), 111–124. https://doi.org/10.1016/J.PNEUROBIO.2010.10.005

Letzen, J. E., Remeniuk, B., Smith, M. T., Irwin, M. R., Finan, P. H., & Seminowicz, D. A. (2020). Individual differences in pain sensitivity are associated with cognitive network functional connectivity following one night of experimental sleep disruption. *Human Brain Mapping*, *41*(3), 581. https://doi.org/10.1002/HBM.24824

Liu, S. S., Pickens, S., Burma, N. E., Ibarra-Lecue, I., Yang, H., Xue, L., Cook, C., Hakimian, J. K., Severino, A. L., Lueptow, L., Komarek, K., Taylor, A. M. W., Olmstead, M. C., Carroll, F. I., Bass, C. E., Andrews, A. M., Walwyn, W., Trang, T., Evans, C. J., … Cahill, C. M. (2019). Kappa Opioid Receptors Drive a Tonic Aversive Component of Chronic Pain. *Journal of Neuroscience*, *39*(21), 4162–4178. https://doi.org/10.1523/JNEUROSCI.0274-19.2019

Llinás, R. R., Ribary, U., Jeanmonod, D., Kronberg, E., & Mitra, P. P. (1999). Thalamocortical dysrhythmia: A neurological and neuropsychiatric syndrome characterized by magnetoencephalography. *Proceedings of the National Academy of Sciences of the United States of America*, *96*(26), 15222–15227. https://doi.org/10.1073/pnas.96.26.15222

Magosso, E., De Crescenzio, F., Ricci, G., Piastra, S., & Ursino, M. (2019). EEG alpha power is modulated by attentional changes during cognitive tasks and virtual reality immersion. *Computational Intelligence and Neuroscience*, *2019*. https://doi.org/10.1155/2019/7051079

Martins, I., Carvalho, P., de Vries, M. G., Teixeira-Pinto, A., Wilson, S. P., Westerink, B. H. C., & Tavares, I. (2015). Increased Noradrenergic Neurotransmission to a Pain Facilitatory Area of the Brain Is Implicated in Facilitation of Chronic Pain. *Anesthesiology*, *123*(3), 642–653. https://doi.org/10.1097/ALN.0000000000000749

Mazza, S., Magnin, M., & Bastuji, H. (2012). Pain and sleep: From reaction to action. *Neurophysiologie Clinique/Clinical Neurophysiology*, *42*(5), 337–344. https://doi.org/10.1016/J.NEUCLI.2012.05.003

Mazza, Stéphanie, Frot, M., & Rey, A. E. (2018). A comprehensive literature review of chronic pain and memory. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *87*(Pt B), 183–192. https://doi.org/10.1016/J.PNPBP.2017.08.006

Menon, J. M. L., Nolten, C., Marijke Achterberg, E. J., Joosten, R. N. J. M. A., Dematteis, M., Feenstra, M. G. P., Drinkenburg, W. H., & Leenaars, C. H. C. (2019). Brain microdialysate monoamines in relation to circadian rhythms, sleep, and sleep deprivation - A systematic review, network meta-analysis, and new primary data. *Journal of Circadian Rhythms*, *17*(1), 1–32. https://doi.org/10.5334/JCR.174/METRICS/

Metz, A. E., Yau, H. J., Centeno, M. V., Apkarian, A. V., & Martina, M. (2009). Morphological and functional reorganization of rat medial prefrontal cortex in neuropathic pain. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(7), 2423–2428. https://doi.org/10.1073/PNAS.0809897106

Muzyka, I. M., & Estephan, B. (2019). Somatosensory evoked potentials. *Handbook of Clinical Neurology*, *160*, 523–540. https://doi.org/10.1016/B978-0-444-64032-1.00035-7

Nagumo, Y., Ueta, Y., Nakayama, H., Osaki, H., Takeuchi, Y., Uesaka, N., Kano, M., & Miyata, M. (2020). Tonic GABAergic Inhibition Is Essential for Nerve Injury-Induced Afferent Remodeling in the Somatosensory Thalamus and Ectopic Sensations. *Cell Reports*, *31*(12), 107797. https://doi.org/10.1016/J.CELREP.2020.107797

Nasseef, M. T., Ma, W., Singh, J. P., Dozono, N., Lançon, K., Séguéla, P., Darcq, E., Ueda, H., & Kieffer, B. L. (2021). Chronic generalized pain disrupts whole brain functional connectivity in mice. *Brain Imaging and Behavior*, *15*(5), 2406–2416. https://doi.org/10.1007/S11682-020-00438-9

Neugebauer, V. (2020). Amygdala physiology in pain. *Handbook of Behavioral Neuroscience*, *26*, 101. https://doi.org/10.1016/B978-0-12-815134-1.00004-0

Ngo, H. V. V., Martinetz, T., Born, J., & Mölle, M. (2013). Auditory Closed-Loop Stimulation of the Sleep Slow Oscillation Enhances Memory. *Neuron*, *78*(3), 545–553. https://doi.org/10.1016/J.NEURON.2013.03.006

Nicholson, B., & Verma, S. (2004). Comorbidities in chronic neuropathic pain. *Pain Medicine*, *5*(SUPPL. 1), S9–S27. https://doi.org/10.1111/J.1526-4637.2004.04019.X/2/PME_4019_F4.JPEG

Niknazar, H., Malerba, P., & Mednick, S. C. (2022). Slow oscillations promote long-range effective communication: The key for memory consolidation in a broken-down network. *Proceedings of the National Academy of Sciences of the United States of America*, *119*(26), e2122515119. https://doi.org/10.1073/PNAS.2122515119/SUPPL_FILE/PNAS.2122515119.SAPP.PDF

Nir, Y., Vyazovskiy, V. V., Cirelli, C., Banks, M. I., & Tononi, G. (2015). Auditory responses and stimulus-specific adaptation in rat auditory cortex are preserved across NREM and REM sleep. *Cerebral Cortex (New York, N.Y. : 1991)*, *25*(5), 1362–1378. https://doi.org/10.1093/CERCOR/BHT328

Obata, H. (2017). Analgesic Mechanisms of Antidepressants for Neuropathic Pain. *International Journal of Molecular Sciences*, *18*(11).

https://doi.org/10.3390/IJMS18112483

Okada, T., Kato, D., Nomura, Y., Obata, N., Quan, X., Morinaga, A., Yano, H., Guo, Z., Aoyama, Y., Tachibana, Y., Moorhouse, A. J., Matoba, O., Takiguchi, T., Mizobuchi, S., & Wake, H. (2021). Pain induces stable, active microcircuits in the somatosensory cortex that provide a therapeutic target. *Science Advances*, *7*(12), 8261. https://doi.org/10.1126/SCIADV.ABD8261

Osorio-Forero, A., Cardis, R., Vantomme, G., Guillaume-Gentil, A., Katsioudi, G., Devenoges, C., Fernandez, L. M. J., & Lüthi, A. (2021). Noradrenergic circuit control of non-REM sleep substates. *Current Biology : CB*, *31*(22), 5009-5023.e7. https://doi.org/10.1016/J.CUB.2021.09.041

Pereira, F. G., França, M. H., Paiva, M. C. A. de, Andrade, L. H., & Viana, M. C. (2017). Prevalence and clinical profile of chronic pain and its association with mental disorders. *Revista de Saúde Pública*, *51*, 96. https://doi.org/10.11606/S1518-8787.2017051007025

Perrin, F., García-Larrea, L., Mauguière, F., & Bastuji, H. (1999). A differential brain response to the subject's own name persists during sleep. *Clinical Neurophysiology*, *110*(12), 2153–2164. https://doi.org/10.1016/S1388-2457(99)00177-7

Phelps, C. E., Navratilova, E., & Porreca, F. (2021). Chronic Pain Produces Reversible Memory Deficits That Depend on Task Difficulty in Rats. *Journal of Pain*, *22*(11), 1467–1476. https://doi.org/10.1016/j.jpain.2021.04.016

Priebe, J. A., Utpadel-Fischler, D., & Toellev, T. R. (2020). Less Pain, Better Sleep? The Effect of a Multidisciplinary Back Pain App on Sleep Quality in Individuals Suffering from Back Pain – a Secondary Analysis of App User Data. *Journal of Pain Research*, *13*, 1121. https://doi.org/10.2147/JPR.S232792

Qi, C., Guo, B., Ren, K., Yao, H., Wang, M., Sun, T., Cai, G., Liu, H., Li, R., Luo, C., Wang, W., & Wu, S. (2018). Chronic inflammatory pain decreases the glutamate vesicles in presynaptic terminals of the nucleus accumbens. *Molecular Pain*, *14*. https://doi.org/10.1177/1744806918781259

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., & Zhao, M. G. (2013). An increase in synaptic NMDA receptors in the insular cortex contributes to neuropathic pain. *Science Signaling*, *6*(275). https://doi.org/10.1126/SCISIGNAL.2003778/SUPPL_FILE/6_RA34_SM.PDF

Radat, F., Margot-Duclot, A., & Attal, N. (2013). Psychiatric co-morbidities in patients with chronic peripheral neuropathic pain: A multicentre cohort study. *European Journal of Pain*, *17*(10), 1547–1557. https://doi.org/10.1002/J.1532-2149.2013.00334.X

Rosseland, R., Pallesen, S., Nordhus, I. H., Matre, D., & Blågestad, T. (2018). Effects of Sleep Fragmentation and Induced Mood on Pain Tolerance and Pain Sensitivity in Young Healthy Adults. *Frontiers in Psychology*, *0*, 2089. https://doi.org/10.3389/FPSYG.2018.02089

Sadler, K. E., Mogil, J. S., & Stucky, C. L. (2022). Innovations and advances in modelling and measuring pain inanimals. *Nature Reviews. Neuroscience*, *23*(2), 70. https://doi.org/10.1038/S41583-021-00536-7

Sahbaie, P., Irvine, K. A., Shi, X. you, & Clark, J. D. (2022). Monoamine control of descending pain modulation after mild traumatic brain injury. *Scientific Reports 2022 12:1*, *12*(1), 1–11. https://doi.org/10.1038/s41598-022-20292-7

Salberg, S., Sgro, M., Brady, R. D., Noel, M., & Mychasiuk, R. (2020). The Development of Adolescent Chronic Pain following Traumatic Brain Injury and Surgery: The Role of Diet and Early Life Stress. *Developmental Neuroscience*, *42*(1), 2–11. https://doi.org/10.1159/000508663

Salomons, T. V., Iannetti, G. D., Liang, M., & Wood, J. N. (2016). The "Pain Matrix" in Pain-Free Individuals. *JAMA Neurology*, *73*(6), 755–756. https://doi.org/10.1001/JAMANEUROL.2016.0653

Santello, M., & Nevian, T. (2015). Dysfunction of Cortical Dendritic Integration in Neuropathic Pain Reversed by Serotoninergic Neuromodulation. *Neuron*, *86*, 233–246. https://doi.org/10.1016/j.neuron.2015.03.003

Santos, M. A. A., Sousa, A. C. S., Reis, F. P., Santos, T. R., Lima, S. O., & Barreto-Filho, J. A. (2013). Does the Aging Process Significantly Modify the Mean HeartRate? *Arquivos Brasileiros de Cardiologia*, *101*(5), 388. https://doi.org/10.5935/ABC.20130188

Sela, Y., Krom, A. J., Bergman, L., Regev, N., & Nir, Y. (2020). Sleep Differentially Affects Early and Late Neuronal Responses to Sounds in Auditory and Perirhinal Cortices. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *40*(14), 2895–2905. https://doi.org/10.1523/JNEUROSCI.1186-19.2020

Sharon, O., & Nir, Y. (2018). Attenuated Fast Steady-State Visual Evoked Potentials During Human Sleep. *Cerebral Cortex*, *28*(4), 1297–1311. https://doi.org/10.1093/cercor/bhx043

Shaw, F. Z., Lee, S. Y., & Chiu, T. H. (2006). Modulation of somatosensory evoked potentials during wake-sleep states and spike-wave discharges in the rat. *Sleep*, *29*(3), 285–293. https://doi.org/10.1093/SLEEP/29.3.285

Sivertsen, B., Lallukka, T., Petrie, K. J., Steingrimsdottir, O. A., Stubhaug, A., & Nielsen, C. S. (2015). Sleep and pain sensitivity in adults. *Pain*, *156*(8), 1433–1439. https://doi.org/10.1097/J.PAIN.0000000000000131

Skarpsno, E. S., Nilsen, T. I. L., Hagen, K., & Mork, P. J. (2021). Long-term changes in self-reported sleep quality and risk of chronic musculoskeletal pain: The HUNT Study. *Journal of Sleep Research*, *30*(6), e13354. https://doi.org/10.1111/JSR.13354

Smith, M. T., & Haythornthwaite, J. A. (2004). How do sleep disturbance and chronic pain inter-relate? Insights from the longitudinal and cognitive-behavioral clinical trials literature. *Sleep Medicine Reviews*, *8*(2), 119–132. https://doi.org/10.1016/S1087-0792(03)00044-3

Smith, M. T., Mun, C. J., Remeniuk, B., Finan, P. H., Campbell, C. M., Buenaver, L. F., Robinson, M., Fulton, B., Tompkins, D. A., Tremblay, J. M., Strain, E. C., & Irwin, M. R. (2020). Experimental sleep disruption attenuates morphine analgesia: findings from a randomized trial and implications for the opioid abuse epidemic. *Scientific Reports 2020 10:1*, *10*(1), 1–12. https://doi.org/10.1038/s41598-020-76934-1

Staffe, A. T., Bech, M. W., Clemmensen, S. L. K., Nielsen, H. T., Larsen, D. B., & Petersen, K. K. (2019). Total sleep deprivation increases pain sensitivity, impairs conditioned pain modulation and facilitates temporal summation of pain in healthy participants. *PLoS ONE*, *14*(12). https://doi.org/10.1371/JOURNAL.PONE.0225849

Steriade, M. (2003). The corticothalamic system in sleep. *Frontiers in Bioscience : A Journal and Virtual Library*, *8*. https://doi.org/10.2741/1043

Sur, S., & Sinha, V. K. (2009). Event-related potential: An overview. *Industrial Psychiatry Journal*, *18*(1), 70. https://doi.org/10.4103/0972-6748.57865

Thakkar, M., & Mallick, B. N. (1993). Effect of rapid eye movement sleep deprivation on rat brain monoamine oxidases. *Neuroscience*, *55*(3), 677–683. https://doi.org/10.1016/0306-4522(93)90433-G

Thompson, S. J., Pitcher, M. H., Stone, L. S., Tarum, F., Niu, G., Chen, X., Kiesewetter, D. O., Schweinhardt, P., & Catherine Bushnell, M. (2018). Chronic neuropathic pain reduces opioid receptor availability with associated anhedonia in rat. *Pain*, *159*(9), 1856–1866. https://doi.org/10.1097/J.PAIN.0000000000001282

Thorpe, R. V., Black, C. J., Borton, D. A., Hu, L., Saab, C. Y., & Jones, S. R. (2021). Distinct neocortical mechanisms underlie human SI responses to median nerve and laser evoked peripheral activation. *BioRxiv*, 2021.10.11.463545. https://doi.org/10.1101/2021.10.11.463545

Topham, L., Gregoire, S., Kang, H., Salmon-Divon, M., Lax, E., Millecamps, M., Szyf, M., & Stone, L. (2020). The Transition from Acute to Chronic Pain: Dynamic Epigenetic Reprogramming of the Mouse Prefrontal Cortex up to One Year Following Nerve Injury. *BioRxiv*, 2020.02.22.956128. https://doi.org/10.1101/2020.02.22.956128

Tsuchiya, K., Toru, M., & Kobayashi, T. (1969). Sleep deprivation: Changes of monoamines and acetylcholine in rat brain. *Life Sciences*, *8*(15), 827–835. https://doi.org/10.1016/0024-3205(69)90143-X

Turner, P. V., Pang, D. S. J., & Lofgren, J. L. S. (2019). A Review of Pain Assessment Methods in Laboratory Rodents. *Comparative Medicine*, *69*(6), 451. https://doi.org/10.30802/AALAS-CM-19-000042

Uhlhaas, P. J., & Singer, W. (2006). Neural Synchrony in Brain Disorders: Relevance for Cognitive Dysfunctions and Pathophysiology. *Neuron*, *52*(1), 155–168. https://doi.org/10.1016/J.NEURON.2006.09.020

Vandekerckhove, M., & Wang, Y. L. (2018). Emotion, emotion regulation and sleep: An intimate relationship. *AIMS Neuroscience*, *5*(1), 1. https://doi.org/10.3934/NEUROSCIENCE.2018.1.1

Vanneste, S., & De Ridder, D. (2021). Chronic pain as a brain imbalance between pain input and pain suppression. *Brain Communications*, *3*(1). https://doi.org/10.1093/braincomms/fcab014

Vanneste, S., Song, J. J., & De Ridder, D. (2018). Thalamocortical dysrhythmia detected by machine learning. *Nature Communications*, *9*(1), 1103. https://doi.org/10.1038/s41467-018-02820-0

Vartiainen, P., Roine, R. P., Kalso, E., & Heiskanen, T. (2022). Worse health-related quality of life, impaired functioning and psychiatric comorbidities are associated with excess mortality in patients with severe chronic pain. *European Journal of Pain*, *26*(5), 1135–1146. https://doi.org/10.1002/EJP.1938

Viguier, F., Michot, B., Hamon, M., & Bourgoin, S. (2013). Multiple roles of serotonin in pain control mechanisms--implications of 5-HT_7 and other 5-HT receptor types. *European Journal of Pharmacology*, *716*(1–3), 8–16. https://doi.org/10.1016/J.EJPHAR.2013.01.074

Vyazovskiy, V. V., Cirelli, C., & Tononi, G. (2011). Electrophysiological correlates of sleep homeostasis in freely behaving rats. *Progress in Brain Research*, *193*, 17. https://doi.org/10.1016/B978-0-444-53839-0.00002-8

Wager, T. D., Atlas, L. Y., Lindquist, M. A., Roy, M., Woo, C.-W., & Kross, E. (2013). An fMRI-Based Neurologic Signature of Physical Pain. *The New England Journal of Medicine*, *368*(15), 1388. https://doi.org/10.1056/NEJMOA1204471

Walitt, B., Urrútia, G., Nishishinya, M. B., Cantrell, S. E., & Häuser, W. (2015). Selective serotonin reuptake inhibitors for fibromyalgia syndrome. *The Cochrane Database of Systematic Reviews*, *2015*(6). https://doi.org/10.1002/14651858.CD011735

Wang, A., Chen, L., Tian, C., Yin, X., Wang, X., Zhao, Y., Zhang, M., Yang, L., & Ye, Z. (2022). Evaluation of the Glymphatic System With Diffusion Tensor Imaging-Along the Perivascular Space in Cancer Pain. *Frontiers in Neuroscience*, *16*, 161. https://doi.org/10.3389/FNINS.2022.823701/BIBTEX

Wang, X., Inui, K., Qiu, Y., & Kakigi, R. (2004). Cortical responses to noxious stimuli during sleep. *Neuroscience*, *128*(1), 177–186. https://doi.org/10.1016/J.NEUROSCIENCE.2004.06.036

Wang, Xiaohong, Inui, K., Qiu, Y., Hoshiyama, M., Tran, T. D., & Kakigi, R. (2003). Effects of sleep on pain-related somatosensory evoked potentials in humans. *Neuroscience Research*, *45*(1), 53–57. https://doi.org/10.1016/S0168-0102(02)00198-0

Wey, H. Y., Catana, C., Hooker, J. M., Dougherty, D. D., Knudsen, G. M., Wang, D. J. J., Chonde, D. B., Rosen, B. R., Gollub, R. L., & Kong, J. (2014). Simultaneous fMRI-PET of the Opioidergic Pain System in Human Brain. *NeuroImage*, *102*(0 2), 275. https://doi.org/10.1016/J.NEUROIMAGE.2014.07.058

Whitlock, E. L., Diaz-Ramirez, L. G., Glymour, M. M., Boscardin, W. J., Covinsky, K. E., & Smith, A. K. (2017). Association Between Persistent Pain and Memory Decline and Dementia in a Longitudinal Cohort of Elders. *JAMA Internal Medicine*, *177*(8), 1146. https://doi.org/10.1001/JAMAINTERNMED.2017.1622

Wilson, K. G., Eriksson, M. Y., D'Eon, J. L., Mikail, S. F., & Emery, P. C. (2002). Major depression and insomnia in chronic pain. *Clinical Journal of Pain*, *18*(2), 77–83. https://doi.org/10.1097/00002508-200203000-00002

Woo, C. W., Schmidt, L., Krishnan, A., Jepma, M., Roy, M., Lindquist, M. A., Atlas, L. Y., & Wager, T. D. (2017). Quantifying cerebral contributions to pain beyond nociception. *Nature Communications*, *8*. https://doi.org/10.1038/NCOMMS14211

Woodman, G. F. (2010). A Brief Introduction to the Use of Event-Related Potentials (ERPs) in Studies of Perception and Attention. *Attention, Perception & Psychophysics*, *72*(8), 2031–2046. https://doi.org/10.3758/APP.72.8.2031

Wöstmann, M., Maess, B., & Obleser, J. (2021). Orienting auditory attention in time: Lateralized alpha power reflects spatio-temporal filtering. *Neuroimage*, *228*. https://doi.org/10.1016/J.NEUROIMAGE.2020.117711

Wu, L. J., Toyoda, H., Zhao, M. G., Lee, Y. S., Tang, J., Ko, S. W., Yong, H. J., Shum, F. W. F., Zerbinatti, C. V., Bu, G., Wei, F., Xu, T. Le, Muglia, L. J., Chen, Z. F., Auberson, Y. P., Kaang, B. K., & Zhuo, M. (2005). Upregulation of Forebrain NMDA NR2B Receptors Contributes to Behavioral Sensitization after Inflammation. *Journal of Neuroscience*, *25*(48), 11107–11116. https://doi.org/10.1523/JNEUROSCI.1678-05.2005

Xu, H., Wu, L. J., Wang, H., Zhang, X., Vadakkan, K. I., Kim, S. S., Steenland, H. W., & Zhuo, M. (2008). Presynaptic and postsynaptic amplifications of neuropathic pain in the anterior cingulate cortex. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *28*(29), 7445–7453. https://doi.org/10.1523/JNEUROSCI.1812-08.2008

Yalcin, I., & Barrot, M. (2014). The anxiodepressive comorbidity in chronic pain. *Current Opinion in Anaesthesiology*, *27*(5), 520–527. https://doi.org/10.1097/ACO.0000000000000116

Yang, J. X., Hua, X., Li, Y. Q., Jiang, Y. Y., Han, D., Liu, H., Tang, Q. Q., Yang, X. N., Yin, C., Hao Yu, L. Y. Le, Wu, P., Shao, C. J., Ding, H. L., Zhang, Y. M., & Cao, J. L. (2015). Caveolin-1 in the anterior cingulate cortex modulates chronic neuropathic pain via regulation of NMDA receptor 2B subunit. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *35*(1), 36–52. https://doi.org/10.1523/JNEUROSCI.1161-14.2015

Yang, S., & Chang, M. C. (2019). Chronic Pain: Structural and Functional Changes in Brain Structures and Associated Negative Affective States. *International Journal of Molecular Sciences 2019, Vol. 20, Page 3130*, *20*(13), 3130. https://doi.org/10.3390/IJMS20133130

Yi, T., Gao, P., Zhu, T., Yin, H., & Jin, S. (2022). Glymphatic System Dysfunction: A Novel Mediator of Sleep Disorders and Headaches. *Frontiers in Neurology*, *13*, 898. https://doi.org/10.3389/FNEUR.2022.885020/BIBTEX

Yong, R. J., Mullins, P. M., & Bhattacharyya, N. (2022). Prevalence of chronic pain among adults in the United States. *Pain*, *163*(2), E328–E332. https://doi.org/10.1097/J.PAIN.0000000000002291

Zhang, C., Chen, R. X., Zhang, Y., Wang, J., Liu, F. Y., Cai, J., Liao, F. F., Xu, F. Q., Yi, M., & Wan, Y. (2017). Reduced GABAergic transmission in the ventrobasal thalamus contributes to thermal hyperalgesia in chronic inflammatory pain. *Scientific Reports 2017 7:1*, *7*(1), 1–19. https://doi.org/10.1038/srep41439

Zhou, H., Li, M., Zhao, R., Sun, L., & Yang, G. (2023). A sleep-active basalocortical pathway crucial for generation and maintenance of chronic pain. *Nature Neuroscience 2023*, 1–12. https://doi.org/10.1038/s41593-022-01250-y

Curriculum Vitae, Acknowledgements and Declaration of Originality

"Let us all be the leaders we wish we had."

Simon Sinek

1. Curriculum vitae

Raquel Adaia Sandoval Ortega

Dept. Physiology, University of Bern, Switzerland
raquel.sandoval@unibe.ch | +41 (0) 787 33 52 92 | sandovalortega.com
Spanish, Catalan, English, German, Swiss German, French

I am a curious and committed scientist, resourceful, creative and willing to put in time and energy for a cause or a goal. I enjoy working in a team and the discussions that derive from such interactions, as I believe this is the way to be dynamic, novel and move forward. From all the skills I have developed, data analysis and interpretation is one of my favorites and I want to continue improving it during my career.

My moto: “Leave it better than you found it.”

In this CV you will find the following sections:

Manuscripts (in preparation)

1) Sandoval Ortega RA, Renard M, de Luna P, Cohen MX, Nevian T. "Interactive Effects of Pain and Arousal State on Heart Rate and Cortical Activity in the Mouse Anterior Cingulate and Somatosensory Cortices"
2) Sandoval Ortega RA, Renard M, de Luna P, Cohen MX, Nevian T. "Chronic Pain Induces Transient Sleep Disturbances and Long-Lasting Changes in Neural Dynamics in Mice"

Scientific Communications (Since 2020)

Posters

2022 FENS – Paris, FR

Sandoval Ortega RA, Renard M, de Luna P, Cohen MX, Nevian T. "The Neural Correlates of Acute and Chronic Pain in Sleep and Wake"

SSN – Fribourg, CH

1) Sandoval Ortega RA, Renard M, de Luna P, Cohen MX, Nevian T. "Sleep-dependent Modulation of Somatosensation in the Anterior Cingulate and Somatosensory Cortices in Mice"

2) Renard M, Sandoval Ortega RA, Nevian T "Optogenetic Manipulation of the Thalamocortical Circuits of Pain"

DNM – Tiel, NL

Sandoval Ortega RA, Renard M, de Luna P, Cohen MX, Nevian T. "The pain-sleep dialog: Interactive effects of pain and sleep state on behavior and cortical activity in the anterior cingulate and somatosensory cortices in mice"

ICON – Helsinki, FI

Sandoval Ortega RA, Renard M, de Luna P, Cohen MX, Nevian T. "The pain-sleep dialog: Interactive effects of pain and sleep state on behavior and cortical activity in the anterior cingulate and somatosensory cortices in mice"

GCB – Bern, CH

Sandoval Ortega RA, Renard M, de Luna P, Nevian T. "A Pain during Sleep: The Neural Correlates of Pain during Sleep and Wake"

2021 SfN – online

Sandoval Ortega RA, Renard M, de Luna P, Nevian T. "Neuronal Dynamics of the Sensory-Discriminative and Affective-Motivational Components of Pain during Sleep"

IRC "Decoding Sleep" – Bern, CH

Sandoval Ortega RA, Renard M, de Luna P, Nevian T. "Cortical Processing of Pain and Touch during Sleep"

CNB – Bern, CH

1) Sandoval Ortega RA, de Luna P, Nevian T. "A Dance between the Emotions and the Physicality of Pain: Differential Modulation of Behavioral Responses to Pain in Sleep and Wake depends on the Interplay between the Somatosensory Cortex and the Anterior Cingulate Cortex"

2) Renard M, Sandoval Ortega RA, Nevian T "Optogenetic Manipulation of the Thalamocortical Circuits of Pain"

ERS – online

Sandoval Ortega RA, de Luna P, Nevian T. "The Emotional and Physical Processing of Pain during Sleep"

GCB – online

Sandoval Ortega RA, de Luna P, Nevian T. "Touch and Pain Processing during Sleep"

2020 FENS – online
Sandoval Ortega RA, de Luna P, Nevian T. "Thalamic gating of painful stimuli during sleep and wake"
SSN – Bern, CH
Sandoval Ortega RA, de Luna P, Nevian T. "Thalamic gating of painful stimuli during sleep and wake"
CNB – Bern, CH
Sandoval Ortega RA, de Luna P, Nevian T. "Touch and Pain Processing during Sleep"
GCB – Bern, CH
Sandoval Ortega RA, de Luna P, Nevian T. "Thalamic gating of painful stimuli during sleep and wake"

Talks

2022 ERS – Fribourg, CH
"Sleep-dependent Modulation of Somatosensation in the Anterior Cingulate and Somatosensory Cortices in Mice"

2021 PhD & Postdoc Retreat of the Dept. of Physiology of the University of Bern
"How Pain and Sleep talk to each other: The Neural Dynamics of Pain in Sleep and Wake"
Neuro Meetups Bern – online
"The Processing of Touch and Pain during Sleep"

Invited Talks (Since 2018)

2023 **Seminar**, Leuven University, BE
Invited by Dr. Giulia Liberati
"TBD"

2022 **Seminar**, University of Rochester, USA
Invited by Prof. Dr. Maiken Nedergaard and Dr. Lauren Hablitz
"The Neural Correlates of sleep and Pain: Interactive effects of pain and sleep state on behavior and cortical activity in the S1 and ACC in mice**"**
Seminar, University of Pennsylvania, USA
Invited by Prof. Dr. Gregory Corder
"The Neural Correlates of sleep and Pain: Interactive effects of pain and sleep state on behavior and cortical activity in the S1 and ACC in mice**"**
Seminar, Columbia University, USA
Invited by Prof. Dr. Christopher Makinson
"The Neural Correlates of sleep and Pain: Interactive effects of pain and sleep state on behavior and cortical activity in the S1 and ACC in mice**"**
Seminar on Neuroelectrophysiology, Master of Biotechnology, Polytechnical University of Barcelona, ES
Invited by Dr. Marcel Sorribas
"Ephys: From ions to protheses"
Seminar on Neuroelectrophysiology to the students of the Master of Biotechnology. Last minutes spent on a short overview about career paths.
8x8 Junge Forschende Erzählen, University of Bern, CH
"The conversations of pain and sleep: The modulation of pain processing by sleep and how pain decreases sleep quality"
Awarded to 8 students who have received funds from the Promotion Fund of the University of Bern

2021 **Selected talk** – Clinical Neuroscience Bern – annual meeting, CH
"A Dance between the Emotions and the Physicality of Pain"
I was one of the four selected abstracts to a talk, in addition to the poster I presented at that meeting.
2019 CNB **outreach interview** "Meet our Scientists"

Skills

"Hard" skills

Coding	Electrophysiology	Optogenetics	Small rodents	Wet lab
Python	Implant assembly	Fiber assembly	Surgeries (intracranial & peripheral)	Molecular biol.
MATLAB	Implantation	Implantation	Handling	Cell cultures
Data processing, analysis & interpretation	Recordings	Viral injections	Behavior testing	Histology
	LFP, MUA, ECG, EEG, EMG	Behav. & Circuit manipulation	Perfusion	Immunohistochem.
			Tissue processing	

"Soft" skills

In addition to my experience as PhD student, the extracurricular activities have provided me with **additional competences** and have strengthened those learned as a PhD student.

Being involved in the board of scientific organizations allowed me to develop strong **project and team management** as well as **negotiation** skills. On top of that, and including the scientific presentations I have given, I have also honed my **communication** skills to adapt to the audience. My experience as mentor and supervisor as well as a tutor has improved my **interpersonal** skills, including **mentoring**, **conflict resolution** and diplomacy. Writing grant applications and abstracts has given me the ability for generating targeted and **versatile writing styles**. Lastly, my initiative to create and lead student organizations and my resolution to hire a master student, has endowed me with a caring but firm **leadership** style, what can be seen in the willingness of my master student to prolong her stay one more year, and in being selected PhD representative and President of the ySSN on two consecutive years.

Awarded Grants (Since 2018)

2022 Doc.Mobility of University of Bern
Equivalent to the **Doctoral Mobility grant of the Swiss National Science Foundation**. Conceived to support mobility of PhD students whose projects are not funded by the SNSF.
SSN Travel Fellowship for FENS
GCB Travel Aid for ICON
Promotion Fund for ERS
Promotion Fund: Funds of the University of Bern awarded to projects envisioned to organize scientific events.
2021 FENS IBRO-PERC Travel Grant for SfN
Promotion Fund for the 2nd PhD & Postdoc retreat of the Dept. of Physiology
2020 IBRO-PERC Travel Grant for FENS
Promotion Fund for NMB seminars

Promotion Fund for ERS
2019 Promotion Fund for NMB seminars

ERS, Early-career Researchers Symposium of the young Swiss Society for Neuroscience;
GCB, Graduate School of Cellular Biology of the University of Bern;
ICON, International conference on COgnitive Neuroscience;
NMB, Neuro Meetups Bern; SSN, Swiss Society for Neuroscience

Teaching and Supervision Experience

2021 - present **Supervisor** of master student and later research assistant

I hired a master student to help me with my PhD project. I conducted the interviews and chose a candidate. I trained the candidate on animal handling, electronics, optogenetics, intracranial surgeries, electrophysiological recordings and coding with Python. After graduating from the master, the candidate decided to stay one more year in the lab to continue working on the project. **The candidate has become totally independent on many of the skills mentioned above throughout the time she has spent under my supervision.**

Margot Renard – current: PhD at University of Lausanne, CH

2021 **Tutor** of medicine students

I guided two groups of ~10 students each during the Problem Based Learning sessions of the course "Neurophysiology" of the second semester of the degree of medicine.

Education and Scientific Experience

2018 - present **Ph.D. in Neuroscience**, University of Bern, Switzerland
Prof. Thomas Nevian

"The Thalamic Gating of Painful Stimuli during Sleep and Wake in Health and Disease"

In this project I am interested in studying the neural substrates of the interaction between pain and sleep. Using **intracranial tetrodes**, I recorded the **electrophysiological** activity of pain-related brain areas under multiple conditions.
In addition, **I have established all the necessary techniques and protocols to conduct these new type of experiments in the lab**. These include the design, creation and management of: surgical procedures for intracranial tetrodes implantation, the recording room, the recording setup, the experimental protocol, the data management platform and the data analysis pipeline (**Python**).

Visiting PhD (2022, Doc.Mobility)
Donders Medical Center, Radboud University, The Netherlands
Prof. Mike X Cohen

In 2021 I earned a mobility grant to join Prof. Mike X Cohen lab and learn **advanced analysis methods for neural time series analysis**. Besides to contributing to knowledge transfer during my stay, I am applying my new skills on data analysis to a large dataset I have collected during my PhD.

2014 - 2017 **Joint Master in Neuroscience**
University of Strasbourg (France) in partnership with University of Basel (Switzerland) and Albert-Ludwigs University of Freiburg (Germany)
Master Student and later **Research Assistant** (2015 - 2017)

Brain Mind Institute, EPFL, Switzerland
Prof. Pierre Magistretti
"Regulation of Adult Hippocampal Neurogenesis by Lactate: Relevance to Antidepressant Treatments"
During these two years I developed strong competence on cell cultures, immunohistochemistry and confocal imaging. Furthermore, I developed a **MATLAB script** for automatic counting of hippocampal dendritic projections.

Student lab rotations (2015)
Prof. Robert Schmidt, BNCN, University of Freiburg, Germany
"Modelling of Beta Oscillations in the Basal Ganglia"
Prof. Dominique Bagnard – INCI, University of Strasbourg, France
"Characterization of Glioblastoma Multiforme Stem Cells"

2014 & 2017 **Research Assistant**
Prof. Bruno Weber – University of Zurich, Switzerland (2017)
"Implementation of Fluorescence Lifetime Imaging Microscopy (FLIM)"
Prof. Benoit Zuber – University of Bern, Switzerland (2014)
"Anatomical Characterization of Glycine Receptors"

2007 - 2012 **Licenciatura in Biology**, University of Barcelona, Spain
Equivalent to Bachelor & Master | **Specialization:** Human Biology

Voluntary Internships
Department of Basic Psychology, IDIBELL, Spain (2011 – 2012)
Prof. Antoni Rodriguez Fornells
"Dissociation between Musical and Economic Rewards"
University Hospital Charité , Germany (2011)
Prof. Barbara Steiner
"The Role of Prolactin Receptor in Proliferation and Differentiation of Adult Precursor Cells in the Murine Central Nervous System"
University of Barcelona, Spain (2009)
Prof. Carme Lluís Auladell
"Determination of G coupled Protein Histamine Heteromeric Receptors in the Striatum"
Erasmus (2010 - 2011)
University Denis Diderot Paris VII, France
Master of Molecular Biology

Board Membership & Organization of Scientific Meetings (Since 2018)

Neuro Meetups Bern (2018 – 2022)

I **founded** and **directed** a series of **monthly seminars** to foster networking and to give visibility to the work of young neuroscientists in Bern. Shortly after I started my PhD, I realized that the young neuroscience community of the University of Bern was not connected. Within a few months I gathered a team with whom to share the organization tasks. We acquired enough funding to comfortably cover the travel costs of a second external speaker (from another Swiss University) to share the session with the local speaker. We also provided an apero after the seminar for a more informal interaction with the speakers and to strengthen the local young neuroscience community.

Organization roles:

2018– 2022. **Founder and President.**

I have organized over 20 seminars and chaired at least half in the past 4 years.

young Swiss Society for Neuroscience (2018 – 2022)

During my time as **president I boosted the visibility and the financial resources of the ySSN**. I pushed for the consolidation of the ERS, which has become really appreciated by the Swiss neuroscience community, started the career-oriented talks NeuroRoutes and increased the organization board by bringing new members.

The ySSN aims at creating a strong early-career neuroscience network in Switzerland and providing a space for young neuroscientists to present their research.

Organization roles:

2021 – 2022. Treasurer

2019 – 2021. **President**

2019 – 2019. Social Media Manager

Meetings organized and chaired:

2022. 4th ERS, Fribourg – main organizer

2021. 3rd ERS, online

NeuroRoutes, online

2020. 2nd ERS, Bern

NeuroRoutes, online

2019. 1st ERS, Geneva

Pint of Science Festival (2018 – 2022)

In my role as a City Coordinator I was the liason with the national team and **coordinated the local team (~ 20 people)**. I was the first City Coordinator of Pint of Science in the city of Bern. The Pint of Science Festival is a world-wide event for science outreach. 2019 was the first time Pint of Science took place as a national event in Switzerland.

Organization roles:

2020. Fundraiser & City coordinator

2019. **City coordinator**

PhD & Postdoc Representative (2018 – 2020)

As a representative of the PhD students and Postdocs of the Department of Physiology, **I initiated the PhD & Postdoc retreat** of the department and **secured financial support** from the department for such type of events. Additionally, I attended the board meetings of the institute and brought forward student initiatives. After I left this role, I **coached** the next representative.

Coordinator of the PhD & Postdoc retreat of the department (2021)

I **coordinated** and **overviewed** a team of 10 PhD students and Postdocs. I **set the deadlines**, made sure they were met and was the **mediator** with the institute professors board. I was also involved in **fundraising**.

2. Acknowledgements

As I sit in front of this blank page, I cannot help but to find the acknowledgement section sort of amusing. Where to draw the line? Where to set the limit? There are so many people to thank, so many people who, in their own unique ways, have played a role in bringing me to write this PhD dissertation.

I know I cannot list every single person who has had a meaningful impact on the experiences that have led me to write this thesis in the way I have. To those who read this section and don't see their names, thank you.

I must first thank Prof. Dr. Thomas Nevian for setting the ground for the story of my PhD experience to develop. For which I am deeply thankful. I also would like to acknowledge the fantastic feedback and always enriching conversations of my co-advisor Prof. Dr. Anita Lüthi, as well as the heart-warming support of my mentor Prof. Dr. Hans Rudolf Widmer. Each of you have opened new perspectives and taught me lessons I was not even aware of their existence, setting the path for me to become who I am today.

I am deeply indebted to Margot Renard and Dr. Mike X Cohen, who played a pivotal role in my PhD. I find no good enough words to thank Margot, who joined me in the quest of searching for the correlates of sleep and pain in wiggly lines on a screen coming from mice that sometimes just didn't want to sleep. Thank you, Mike, for all the roles you volunteered to take. Thank you for your supervision, your patience and your knowledge, for your passion for teaching, for teaching me how to structure and write a paper, for helping me put together this dissertation and the two manuscripts, for showing me the fun of linear algebra…

I also would like to thank the Interfaculty Research Cooperation "Decoding Sleep" for funding this project, and, particularly, Prof. Dr. Antoine Adamantidis and Dr. Carolina Gutierrez Herrera as well as Ida Boccalaro, Irene, and Lukas Oesch for their very helpful input, discussions, and for teaching me an essential part of my whole project: sleep scoring.

Needless to say, I would like to thank my lab mates Dr. Liselot Jonker, Federica Franciosa, Dr. Mario Acuna, Dr. Kristina Valentinova, Dr. Niels Ntamati Rwaka, Natalie Nevian, Sergej Kasavica, Conny Raltschev and Dr. Shankar Sachidhanandam, as well as past group members Marta Falkowska, Dr. Paolo de Luna, Norbert Hogreffe and Alberto Bisco. Unforgettable for me are the librarians, my 20 office mates with whom I shared library-contained heatwaves, beers, sweets, poster printing rushes and coffees among other things.

Another very important part of my PhD experience was the Neuro Meetups Bern, which I ran along with Liselot, Federica and Camille Gontier. I really enjoyed organizing the scientific meetings, and of course, the aperos, with you. Furthermore, being a member of the organizing committee of the young Swiss Society of Neuroscience and the Pint of Science festival in Bern was not only fun but very enriching.

Scientifically, I would like to acknowledge the following researchers for their significant contributions to my thesis via discussions, conversations, and poster sessions: Prof. Dr. Giandommenico Iannetti for taking the time to discuss my results and the effect of saliency.

Prof. Dr. Carl Saab for giving me input on my project and discussing the meaning of Evoked Potentials. Prof. Dr. Yuval Nir for a fantastic conversation at the School on Advance Studies on Sleep, as well as for keeping his promise to come to my poster to discuss the alpha desynchronization in my results, even when everyone had left. Dr. Yaniv Sela for always being ready to share his thoughts and Dr. Flavio Schmidig for his suggestions on how to denoise my recording setup at a critical point during my experiments.

There is a lot of work behind the scenes that allowed me to do my job. I'd like to sincerely thank Edi Scheuner for being an incredibly efficient and versatile janitor and always a friendly face to see. I would also like to thank Stefan for IT support, Michael for taking care of my animals, as well as Hans, Christian D. and Christian for their technical assistance on the recording rigs

On a more personal side, I want to thank my family for their unconditional support. I also want to thank The Circle, that although silent, it is always there. Specifically, Dr.Eva Kaulich, great scientist and great person, with an impeccable writing style and a fantastic ability to give feedback. Thank you for all your help, Eva. I additionally would like to acknowledge the warm support of the crew I met at the Donders during my stay at Dr. Cohen's lab, in particular Nina, Lea, Laura, Riccardo and Michael O. Thank you guys for making my stay one of the best times of my PhD! And thank you, Alejo, for your joy, your enthusiasm and your words of encouragement!

And last, but not least, Pascal Dufour. My adventures partner. The person who has always been there for me, in the good, in the bad and in the very bad. Thank you for believing in me, for holding my hand and showing me that you were right, that I can do it. Thank you for celebrating my successes, even when I could not see them. Thank you for encouraging me to always choose the path of adventure. Thank you for teaching me to pause, breathe and think before boldly taking action. Thank you for being there for me. Thank you, Pascal, for keeping me afloat.

And to you reader. Thank you for reading this thesis.

3. Declaration of originality

Last name, first name: Sandoval Ortega, Raquel Adaia

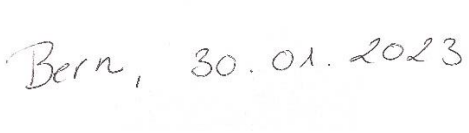
Matriculation number: 18-122-788

I hereby declare that this thesis represents my original work and that I have used no other sources except as noted by citations.

All data, tables, figures and text citations which have been reproduced from any other source, including the internet, have been explicitly acknowledged as such.

I am aware that in case of non-compliance, the Senate is entitled to withdraw the doctorate degree awarded to me on the basis of the present thesis, in accordance with the "Statut der Universität Bern (Universitätsstatut; UniSt)", Art. 69, of 7 June 2011.

Place, date



Signature