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Understanding the protective function of pain: An EEG study of the influence of pain on motor preparation

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Summary

1 Abstract / Zusammenfassung

Pain is mostly conceptualized as a perceptual phenomenon. However, pain serves vital protective functions. It signals potentials harm and drives behavioral responses to avoid injury and promote recovery. Thus, pain does not only include a perceptual component but crucially depends on appropriate motor responses. Moreover, the modulation of motor behavior and motor processing in the brain can alleviate pain and are therefore harnessed for pain therapy. However, how pain and motor processing interact in the human brain is largely unknown. In the present research project, we used electroencephalography (EEG) to investigate how the preparation of an adaptive motor response functionally interacts with pain processing in the human brain. Participants performed button presses to interrupt increasingly painful thermal stimuli and comparable movements without concurrent stimulation. The results show that protective motor responses were associated with reduced amplitude of the preparatory readiness potential as compared to a similar button press, which did not serve any protective function. In contrast, preparatory event-related desynchronizations at alpha and beta frequencies did not differ between conditions. To test for the specificity of our results, we designed a comparable control experiment where participants were required to perform button press in response to non-painful thermal stimuli. The results indicate that the amplitude of the readiness potential is similarly attenuated when a movement interrupts a non-painful stimulation. Taken together, the findings of our research project suggest that pain influences motor preparation in the human brain, although this effect is not pain specific but rather represent a modality-spanning phenomenon. Specific interactions between pain and motor process remain to be demonstrated. Further insights are needed to better understand the protective function of pain and motor-based treatment strategies.

Schmerz wird oft auf die ihm inhärente unangenehme Sinneswahrnehmung reduziert, dient jedoch überlebenswichtigen protektiven Funktionen. Schmerz signalisiert potenziellen Schaden und löst Reaktionen aus, die Verletzungen vermeiden und Heilungsprozesse fördern sollen. Schmerz enthält somit ebenfalls eine motorische Komponente. Darüber hinaus können Veränderungen der Motorik und die Beeinflussung neuronaler motorischer Prozesse Schmerzwahrnehmung reduzieren, was sich im Rahmen der Schmerztherapie zu Nutze gemacht wird. Bis heute ist jedoch noch weitestgehend unklar, wie Schmerz und Motorik im menschlichen Gehirn interagieren. Im vorliegenden Forschungsprojekt wurde deshalb mittels Elektroenzephalographie (EEG) untersucht, wie die Vorbereitung adaptiver motorischer Reaktionen funktionell mit der Verarbeitung von Schmerz im menschlichen Gehirn interagiert. Eine erste Studie untersuchte neuronale Prozesse im Zusammenhang mit Tastendrücken, die entweder dazu dienten, zunehmend schmerzhafte thermale Reize zu beenden, oder ohne zeitgleiche Stimulation durchgeführt wurden. Die Ergebnisse zeigen, dass protektives motorisches Verhalten im Vergleich zu nicht protektivem Verhalten mit einer reduzierten Amplitude des motorischen Bereitschaftspotenzial einhergeht. Desynchronisationen in Alpha- und Beta-Frequenzen unterschieden sich dagegen nicht zwischen den beiden Bedingungen. Um die Spezifität dieser Ergebnisse für Schmerz zu untersuchen, wurde in einer zweiten Studie ein Kontrollexperiment mit vergleichbarem Aufbau durchgeführt, in dem die Probanden auf nicht-schmerzhafte thermale Reize reagierten. Wieder zeigte sich eine reduzierte Amplitude des Bereitschaftspotentials, wenn Tastendrücke die, diesmal nicht-schmerzhafte, Stimulation beendeten. Zusammenfassend deuten diese Befunde an, dass Schmerz die motorische Vorbereitung im Gehirn beeinflusst, dieser Effekt jedoch nicht schmerz-spezifisch, sondern ein modalitätsübergreifendes Phänomen ist. Spezifische Interaktionen zwischen Schmerz und neuronalen motorischen Prozesse müssen daher noch gezeigt werden. Ein tieferes Verständnis dieser Prozesse ist notwendig, um die protektive Funktion von Schmerz und Behandlungsstrategien, die sich motorische Prozesse zu Nutze machen, besser zu verstehen.

2 Introduction

Pain is commonly defined as an unpleasant sensory and emotional experience, associated with actual or potential tissue damage, or described in terms of such damage (IASP, 1994). It represents a fundamental signaling mechanism for imminent harm, which primes the organism for protective behavior, intended to avoid the source of danger, minimize further injury and promote recovery. Through its intrinsic aversive nature, pain serves vital functions aimed at preserving the integrity of the individual (Seymour & Dolan, 2013)

The pain experience is typically driven by nociception, which is the neural process of encoding noxious stimuli (Meyer et al. 2006). For a long time, pain has been conceptualized as a mostly sensory phenomenon, and nociception has been the crucial focus of scientific investigation. However, the magnitude of the painful experience is highly subjective and can be influenced by several variables, including prior experiences, emotional states, attention, expectations, socio-cultural background, contextual factors and individual characteristics (Baliki & Apkarian, 2015; Bushnell et al., 2013). Therefore, pain is an extremely complex phenomenon which not only includes sensory, but also cognitive, affective and motivational components (Kucyi & Davis, 2016). Above all, the protective and adaptive function of pain relies not only on sensory processes, but rather on appropriate behavioral responses to reduce harm and promote healing. Without appropriate behavioral responses, pain would lose any adaptive function (Sullivan, 2008).

In the present chapter, we will discuss the importance of behavior as a central feature of pain. We will then review current knowledge about pain and motor processing in the human brain. Finally, we will present neuroanatomical and neurophysiological evidence for the mutual influence between pain and motor processes in the human brain, which have been scarcely investigated so far.

2.1 The behavioral component of pain

Pain has been historically characterized as a perceptual phenomenon. This view has been inherited from Descartes' model of pain perception (Descartes, 1662), according to which physical injury stimulates the transmission of signals along a direct pathway from the peripheral nervous system to a pain center in the brain, where the experience of pain arises (see Figure 1).

Figure 1: Descartes' model of pain.
A heat stimulus activates fibres which project via the spinal cord to the brain, where the painful message is elaborated. The model assumes a linear relationship between a stimulus and pain, which is conceptualized as a perceptual phenomenon (from Descartes, 1662).

This model laid the foundation of a theorization of pain entirely focused on pain sensation, which dominated for decades clinical and research approaches (Moayedi & Davis, 2013; Sullivan, 2008). However, to consider pain as an exclusively sensory phenomenon is a rather simplistic approach. In fact, pain interferes with ongoing behavior and prompts actions aimed at the individual's safety. Therefore, the behavioral component of pain should not be neglected (Melzack & Casey, 1968). According to the seminal Gate Control Theory of pain developed by Melzack and Wall (Melzack & Casey, 1968; Melzack & Wall, 1965), cognitive and affective variables influence sensory processes by means of a gating mechanism in the dorsal horn of the spinal cord. This theory provided a more complete picture of pain processes, describing pain as a complex and multidimensional phenomenon, where sensory, cognitive and affective

processes result in behavioral responses (Bolles & Fanselow, 1980; Wall, 1979). A merit of the model was, thus, to acknowledge the profound connection of pain with action and to include the behavioral component as an essential dimension of pain. Correspondingly, current concepts of pain acknowledge that the ultimate function of nociceptive signals is to “present the brain with the question “what is to be done?””. (Morrison et al., 2013). Accordingly, brief and acute pain modifies movement to ensure appropriate protective responses (Bank et al., 2013; Butera et al. 2016; Hodges & Smeets, 2015; Hodges & Tucker, 2011). Ranging from simple withdrawal reflexes to more complex movement patterns, such as holding or rubbing the injured body part, altering the posture or the gait, these behavioral responses are directed at minimizing further injury and promoting recovery (Sullivan, 2008). In experimental studies, these effects manifested as pain-induced modulations of force (Novembre et al., 2018) and reaction times (Babiloni et al., 2010; Misra and Coombes, 2014; Misra et al., 2017). Moreover, behavioral responses to pain do not simply represent a direct consequence of a noxious stimulation, but rather mediate the effect of stimulus intensity on pain perception, independently of perceptual processes (May et al., 2017).

In the management of chronic pain, restoring the patient’s capability of action is often a recommended treatment to alleviate pain and facilitate recovery (Hodges & Smeets, 2015; Sullivan & Vowles, 2017), as physical exercise has been reported to have an analgesic effect (Koltyn, 2002; Naugle et al., 2012). Moreover, both invasive and non-invasive stimulation of cerebral motor areas can be used to treat chronic pain resistant to drug treatment (Lefaucheur et al., 2008; Mylius et al., 2012; Nguyen, et al., 2011). However, when persistent and inadequate, alterations of movements in chronic pain can be associated with poor recovery and disability, likely contributing to the pathogenesis of chronic pain (Butera et al., 2016; Hodges & Smeets, 2015).

Thus, although pain is often defined as a mostly perceptual process, behavior constitutes a central component of pain. However, a clear understanding of the interaction between pain and motor processes in the human brain is lacking so far.

2.2 Pain processing in the human brain

In the last decades, the development of neuroimaging techniques allowed to unravel a network of brain regions and processes underlying pain in health and disease. Techniques such as functional magnetic resonance imaging (fMRI) or positron emission tomography (PET) assess non-invasively brain hemodynamics or metabolism, which can be used as an indirect measure of pain-related neural activity with a high spatial resolution (Morton & Jones, 2016). Electroencephalography (EEG) and magnetoencephalography (MEG) are direct measures of neural activity which have a limited spatial resolution but a very high temporal resolution which can capture the exact timing of pain-related neuronal events (Apkarian et al., 2005).

2.2.1 Overview of neural pathways of pain

Nociception allows the organisms to be aware of potential threat in the surrounding environment, providing information to the central nervous system about the location and the intensity of noxious stimuli. Intense thermal, chemical, or mechanical stimuli excite the peripheral nerve fibers, named nociceptors. The fast conducting myelinated Aδ fibers (> 2 m/s) convey the first painful sensation, which is acute, well-localized, pricking and sharp, while the slower unmyelinated C fibers (< 2 m/s) mediate a second, poorly localized and burning painful sensation (Meyer et al., 2006; Basbaum et al., 2009).

Nociceptive afferent axons terminate mainly in the dorsal horn of the spinal cord, and then ascend in the spinothalamic tract (STT), one of the main routes carrying information about noxious stimulation to the thalamus (see Figure 2A). Neurons of the STT project either to medial or lateral thalamic nuclei, which in turn project to somatosensory or limbic regions, respectively. This diversification of the STT projections is reflected in the separation between a lateral and a medial pain system, implicated in discriminative and affective functions, respectively (Basbaum et al., 2009; Bromm & Lorenz, 1998; Dostrovsky & Craig, 2006).

The incoming information from the ascending pathways can be modulated by descending projections, which originate mainly in the hypothalamus, the periaqueductal gray (PAG) and the rostral ventromedial medulla (RVM) (Heinricher & Fields, 2006). Both the PAG and the RVM have strong connections with higher brain regions, such as medial and frontal cortical areas, and limbic regions, including the amygdala, the anterior cingulate cortex, and portions of the insula, representing thus a key network through which cognitive and emotional factors can modulate pain processing (see Figure 2B) (Bingel & Tracey, 2008; Heinricher & Fields, 2006).

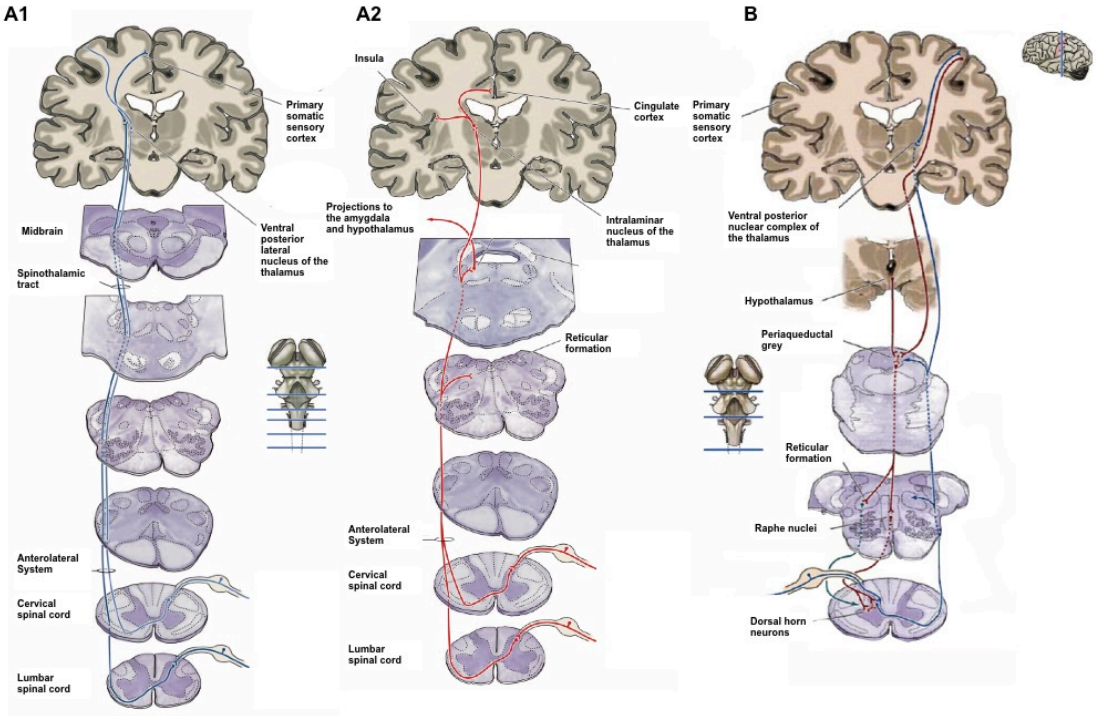

Figure 2: Ascending and descending pain pathways.

A: The spinothalamic tract conveys information from the periphery to different subcortical and cortical areas of the brain. A1: The lateral pain system is involved in the processing of sensory and discriminative components of pain. A2: The medial pain system is responsible for the emotional and motivational aspects of pain. B: The descending modulatory system modulates pain processing at the level of the spinal cord. The periaqueductal grey (PAG) and the rostral ventromedial medulla (RVM) are the core regions involved in descending modulation. (Modified from Purves et al., 2012).

2.2.2 Neuroanatomy of pain processing in the human brain

Nociceptive information is conveyed via different pathways from the spinal cord to the thalamus, and from there they are directed to several cortical and subcortical areas of the brain, giving rise to the conscious experience of pain. Human brain imaging studies revealed an extended network of brain regions

consistently activated by painful stimuli, commonly referred to as the “pain matrix”. This network encompasses diverse areas implicated in sensation, motor control, autonomic responses, affect and attention (see Figure 3: The pain matrix.) (Apkarian et al., 2006; Apkarian et al., 2005; Coghill et al., 1999; Duerden & Albanese, 2013; Morton & Jones, 2016; Schnitzler & Ploner, 2000).

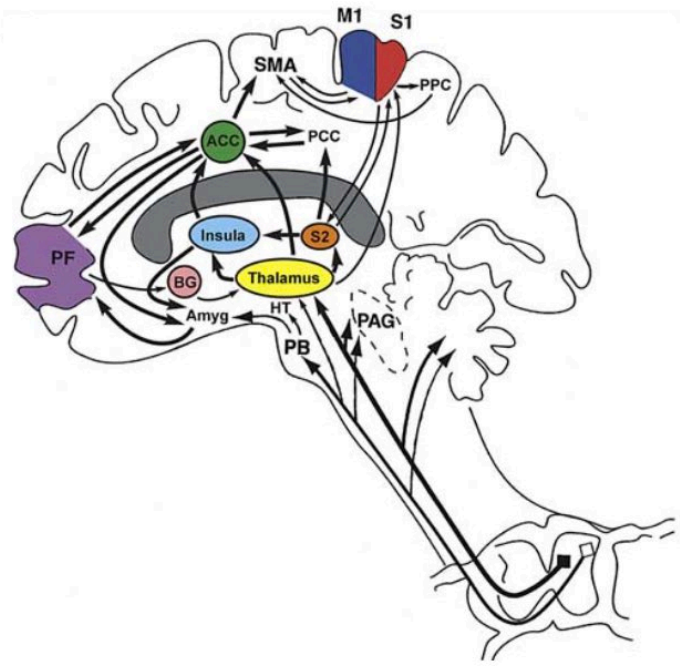

Figure 3: The pain matrix.

A network of regions subserves the processing of the pain experience in the human brain. It includes the primary and secondary somatosensory areas, the prefrontal cortex, the anterior cingulate cortex and the insula, the thalamus and the cerebellum (from Apkarian et al., 2005).

The sensory cortices constitute the neural substrate of the sensory dimension of pain. The primary somatosensory cortex has a specific role in the processing of sensory and discriminative features of the noxious stimulus such as location and duration (Apkarian et al., 2005; Peyron et al., 2000; Schnitzler & Ploner, 2000). The secondary somatosensory cortex is very consistently activated in pain studies (Peyron et al., 2000). Through its connections with the limbic system, this area is implicated in pain-related learning and memory processes, which are essential to promptly recognize the adverse nature of a stimulus (Schnitzler & Ploner, 2000)

The insula and the anterior cingulate cortex (ACC) contribute to the affective and motivational processing of pain (Casey, 1999). The insula is not a nociceptive specific structure, but it represents a fundamental hub for the integration of multimodal inputs with contextual information and autonomic responses. The

insula, moreover, mediates affective and mnemonic pain-related processes via its connection with temporal limbic structures, such as the amygdala and the hippocampus (Peyron et al., 2000; Starr et al., 2009). The rostral part of the ACC mediates mainly the affective reactions to pain, whereas the medial part plays a critical role in pain-related behavior, as response selection or motor inhibition (Vogt, 2005; Vogt & Sikes, 2009). Primate studies indicate that nociceptive spinothalamic input is specifically directed to cingulate motor areas, which in turn project to the primary motor cortex, representing therefore a potential mechanism that regulates motor responses to painful stimuli (Dum et al., 2009). In addition, regions of the motor network are consistently activated by noxious stimuli, reinforcing thus the view that motor responses are a fundamental component of the complex pain experience (Farrell et al., 2005; Gelnar et al., 1999).

2.2.3 Neurophysiology of pain processing in the human brain

Electroencephalography allows to obtain insights into the temporal dynamics of cortical processes. Applied to pain, it can disentangle the specific neural responses associated with the transformation of noxious stimuli into perception and behavior.

Electrical brain activity can be analyzed in the time domain, to capture responses which are phase-locked to the event of interest, and in the time-frequency domain, to analyze phase-locked and non-phase-locked responses at different frequencies (David et al., 2006). The following sections will provide an overview of the most investigated brain responses to pain.

2.2.3.1 Pain-related evoked potentials

Event-related potentials (ERP) represent a well-established measure of neuronal events underlying perceptual, cognitive and motor processes (Roach & Mathalon, 2008). ERPs reflect the synchronized activity of neurons during preparation or in response to exogenous or endogenous events. They are extracted from the raw signal by averaging several epochs time-locked to the event of interest, in order to discriminate event-related from random brain activity (i.e., noise) (Fabiani et al., 2000).

Pain-related evoked potentials are investigated in experimental settings employing painful electrical or heat stimuli, as these stimuli are brief and can be repeatedly applied to increase the signal-to-noise ratio (Bromm & Lorenz, 1998; Kakigi et al., 2005; Plaghki & Mouraux, 2005). Among these, laser stimuli are most popular, as they activate exclusively nociceptive fibers (Bromm & Lorenz, 1998; Kakigi et al., 2000; Mor & Carmon, 1975; Plaghki & Mouraux, 2003; Plaghki & Mouraux, 2005). The typical pattern of the resulting laser-evoked potentials (LEPs) is depicted in Figure 4A. A small early deflection termed N1 is usually detected over contralateral temporal electrodes, and is followed by a negative-positive complex N2-P2 (200-350) which shows maximal amplitude at the vertex electrode (Christmann et al., 2007; Plaghki & Mouraux, 2005). The sources for the earliest laser-evoked component have been localized mainly in the primary somatosensory cortex, whereas the generator of the biphasic complex is thought to be located in the insula and ACC (Apkarian et al., 2005; Garcia-Larrea et al., 2003). These observations support the idea that early components reflect sensory-discriminative processes related to physical properties of the stimulus, whereas later ones are more closely linked with subjective perceived painzintensity (Apkarian et al., 2005; Bromm & Lorenz, 1998).

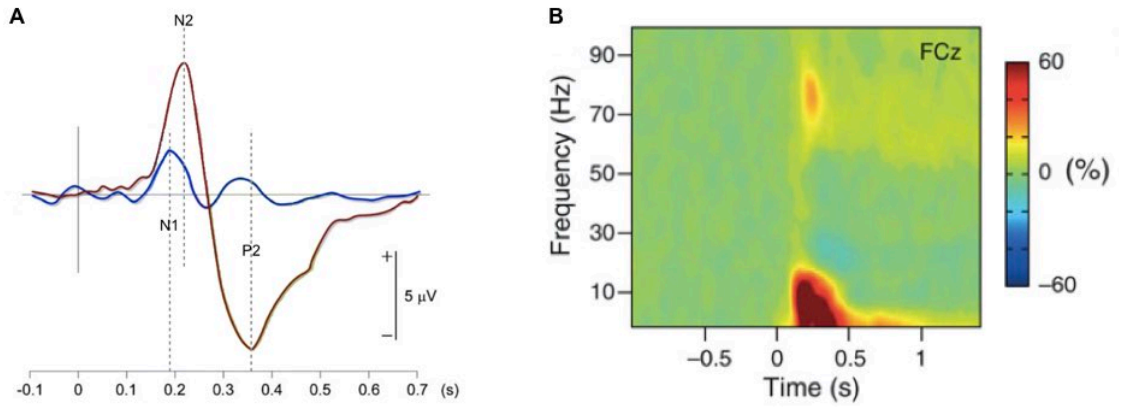

Figure 4: Electrophysiological responses to pain analyzed in the time and time-frequency domain.

A: The typical pattern of laser evoked potentials (LEPs) includes a small negative deflection termed N1 which probably reflects sensory-discriminative processes, and by a biphasic complex termed N2-P2 which is thought to be related to the processing of subjective pain intensity (modified from Legrain et al., 2011). **B**: Phasic pain induces specific changes in the EEG spectrum. Power initially increases in the theta frequency range (< 7 Hz), and decreases in the alpha (8-13 Hz) and the beta (14-30 Hz) range at about 500-1500 ms after the stimulus. Lastly, pain-induced oscillations are detected in the gamma range (40-100 Hz) (from Schulz et al., 2015).

The amplitude and the latency of the potentials can be modulated by features of the stimulus, such as intensity or repetition (Ohara et al., 2004;

Valeriani et al., 2003), as well as by contextual factors, such as stimulus predictability (Wang et al., 2010) placebo manipulation (Colloca et al., 2009; Wager et al., 2006), attentional processes (Beydoun et al., 1993; Kakigi et al., 2000; Lorenz & Garcia-Larrea, 2003) or emotional states (Ring et al., 2013).

2.2.3.2 Pain-induced oscillatory activity

Analyses in the time-frequency domain capture not only phase-locked neural activity (e.g. ERPs), but also non-phase-locked neural oscillations (Hauck et al., 2008; Mouraux et al., 2003). Neural oscillations reflect synchronized rhythmic activity of neuronal assemblies, which serve the flow and integration of information across neuronal assemblies. These processes represent the substrate for complex cognitive functions, which require high flexibility in the transmission of neural signals (Buzsáki & Draguhn, 2004; Fries, 2005). Fourier analysis allows to characterize neuronal oscillations at different frequencies, and their power changes over time in response to specific events (Pizzagalli, 2007).

Neuronal oscillations might play a crucial role the processing of nociceptive information, subserving the flexible and dynamic integration of sensory, emotional and contextual information, which ultimately contribute to shaping the individual experience of pain (Ploner et al., 2017).

Phasic pain stimuli induce a typical pattern of changes in the EEG spectrum, as shown in Figure 4B. An early increase of power in the theta frequency range (< 7 Hz), spread over contralateral S1 and S2, is thought to reflect the evoked response (Ploner et al., 2000; Timmermann et al., 2001). Significant power suppression is observed in the alpha (8-13 Hz) and the beta (14-30 Hz) range, occurring usually 500-1500 ms after the onset of the stimulus at bilateral sensorimotor cortices and occipital cortex (Mouraux et al., 2003; Ploner et al., 2006). Functionally, power desynchronization over the sensorimotor cortices has been considered reflecting the activation of such areas and their involvement in sensory and motor tasks (Hauck et al., 2008; Stancak et al., 2005). Such suppressions of cortical rhythms in the alpha and beta range are presumably related to the alerting function of pain, as they can support thalamocortical gating processes, which allow the cortex to receive information about relevant external stimuli and effectively process them, preparing the individual for fast and

adequate reactions (Hauck et al., 2008; Ploner et al., 2006). Finally, nociceptive stimuli elicit increases of power in the gamma (40-100 Hz) band within the primary sensorimotor cortex (Gross et al., 2007), which increase in amplitude in relation to both objective stimulus intensity and subjective pain perception. It has been proposed that gamma band oscillations might be linked to processes integrating physical stimulus properties and higher-order cognitive processes, like attention, which prepare the individual to preferentially process relevant information from the surrounding environment (Gross et al., 2007; Schulz et al., 2011; Zhang, et al., 2012, Tiemann et al., 2015).

Although neural oscillations in response to pain have been widely investigated by using short phasic noxious stimuli, these stimuli do not necessarily reflect clinically relevant longer-lasting pain which often persists over months and years. Sustained painful stimulation represents, therefore, a more appropriate choice to experimentally characterize clinical pain (Giehl et al., 2014; Nir et al., 2010). Functional imaging studies demonstrated that longer pain duration was associated with a shift away from neural networks involved in the processing of sensory information to networks underlying emotional and motivational processes (Hashmi et al., 2017). Specifically, longer-lasting pain is associated with sustained activation in the medial prefrontal cortex, a key region in the processing and modulation of emotional responses (Baliki et al., 2006; Baliki et al., 2011). Comparatively few studies examined the neurophysiological encoding of ongoing pain. Generally, oscillatory activity in response to tonic painful stimulation has been found to decrease in the alpha and beta band (Giehl et al., 2014; Huber et al., 2006; Nir et al., 2010, Schulz et al., 2015, Nickel et al., 2017) and to increase in the gamma band (Peng et al., 2014, Schulz et al., 2015, Nickel et al., 2017). We recently demonstrated that increased activity in the gamma band over medial prefrontal areas encodes the subjective perception of longer-lasting pain (Schulz et al., 2015, Nickel et al., 2017). This evidence reinforces the view that chronic pain states are no longer reflecting physical properties of the noxious stimulation, but that they rather emerge from complex emotional states, which are built on learning processes and on memories of previous and persistent painful experiences (Hashmi et al., 2017).

2.3 Motor processing in the human brain

The sensory systems, including the nociceptive system, provide an internal representation of the outer world, by conveying sensory information to the cerebral cortex. There, information is integrated across several brain regions. These processes serve as the basis for appropriate motor responses.

Movements are controlled by a complex interplay between neural subsystems, which are depicted in Figure 5. Each subsystem of the motor system regulates specific motor functions and feedback and feedforward allow to integrate information across the different subsystems, in order to produce adaptive responses and to correct the ongoing movement (Kandel et al., 2013; Purves et al., 2012).

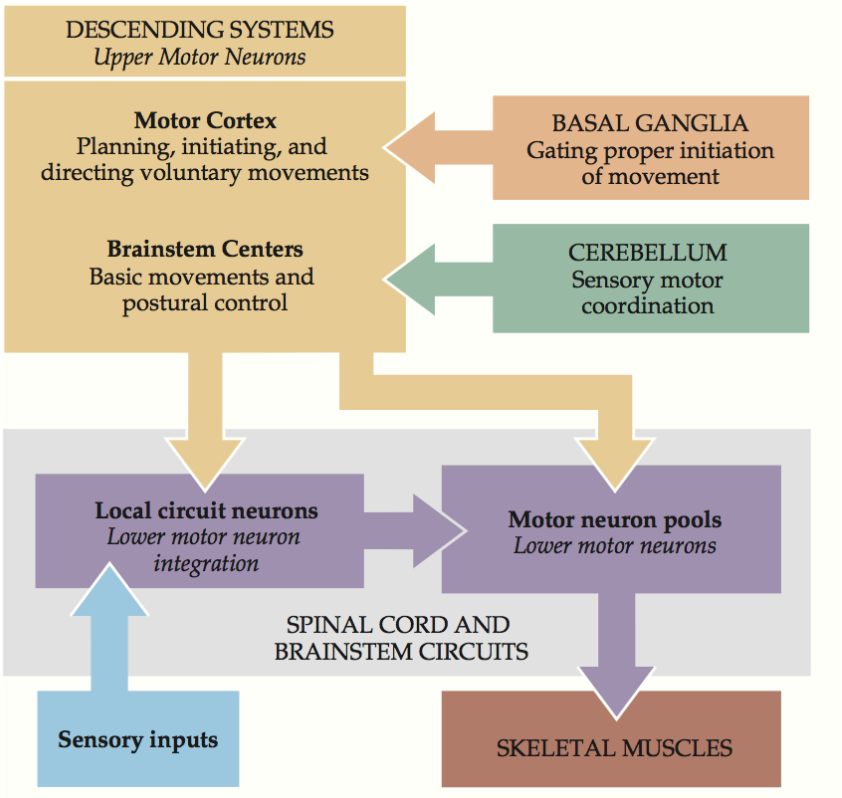

Figure 5: Neural components of the motor system and their organization.
Movements are initiated and controlled by a complex interaction between four systems: spinal cord and brainstem circuits, descending pathways, the cerebellum, and the basal ganglia (from Purves et al., 2012).

Lower motor neurons are responsible for simple reflexes, but they can also be regulated by upper motor neurons in higher cortical centers to implement more complex sequences of movements. Two additional subsystems, the cerebellum and the basal ganglia, do not influence directly the activity of lower motor

neurons, but rather interact with descending motor systems to regulate specific parameters of action, like coordination, synchronization, initiation and suppression of internally generated movements (Purves et al., 2012).

The following section will briefly illustrate the neuroanatomy of motor processing in the human brain, with a particular focus on the contribution of cortical areas to the control of voluntary movements. Next, we will discuss neurophysiological processes related to movements, that is motor related potentials, and desynchronization of the EEG spectrum in the alpha and beta band.

2.3.1 Neuroanatomy of motor processing in the human brain

Each movement, whether voluntary or involuntary, is the consequence of the contraction of skeletal muscles triggered by lower motor neurons located in the ventral horn of the spinal cord gray matter (Mentis, 2013; Purves et al., 2012). Lower motor neurons receive descending projections from higher motor centers, representing therefore the “final common pathway” by which all motor activities are mediated (Liddell & Sherrington, 1925). Upper motor neurons can be found both in the motor regions of the cerebral cortex and in several nuclei of the brain stem. Those neurons provide descending input which influence the activity of the lower motor neurons in order to implement complex motor responses (Purves et al., 2012). Motor neurons in the brainstem centers regulate various involuntary motor activities underlying more complex motor patterns, like walking or swimming, and play a role in adjusting the posture and the position of the body in space (Kandel et al., 2013).

However, action is not merely the implementation of stereotyped responses, but it rather represents the way to effectively interact with the external world and to reach our objectives. Therefore, movements need to be planned to reach a particular goal, and then continuously controlled and adjusted to the characteristics of the environment (Grafton et al., 2000). Such processes are regulated by a complex network of motor areas, which are mostly located in the frontal lobe (Geyer et al., 2012; Grafton et al., 2000; Rizzolatti et al., 2001; Schieber & Baker, 2013). From there, motor commands are conveyed to the lower motor neurons in the spinal cord via the corticospinal tracts, a descending

pathway essentially involved in the voluntary control of the body and the limbs (Dum & Strick, 1991; Mentis, 2013).

The execution of a voluntary movement is associated with increased activity in the primary motor cortex, which regulates the recruitment of lower motor neurons that initiate and control movements parameters such as movement force and trajectories (Schieber & Baker, 2013). Non-primary motor areas are particularly involved in planning and controlling goal directed actions, integrating information from different cortical areas to select movements which respond to the environmental demands (Geyer et al., 2012). Specifically, the premotor cortex, which is functionally divided in a lateral and a medial portion (also known as supplementary motor areas), plays a crucial role in the planning of actions based on external or internal contingencies (Cunnington et al., 2002; Grafton et al., 2000) and in coordinating sequences of movements (Geyer et al., 2012; Tanji, 2001). In addition to frontal areas, other cortical regions are activated in association with a movement. Superior parietal regions are essential for spatial representation, sensorimotor transformation and motor imagery (Gerardin et al., 2000; Geyer et al., 2012; Grafton et al., 2000). Motor areas on the cingulate sulcus receive input from limbic and prefrontal regions on the internal and motivational state of the individual. Through their connections with the primary motor area, they play a key role in the adaptive control of voluntary movements (Picard & Strick, 1996; Shima & Tanji, 1998). Interestingly, cingulate motor areas are the main target of nociceptive information carried by the spinothalamic tract (Dum et al., 2009), and these regions were consistently activated by pain and motor processes occurring simultaneously (Misra & Coombes, 2014; Perini et al., 2013). This evidence suggests that cingulate motor areas may be involved in processing the motor dimension of pain.

2.3.2 Neurophysiology of motor processing in the human brain

Electrophysiological techniques allow to gain an insight on the exact timing of changes in electrical brain activity during voluntary movements. As outlined in 2.2.3.1 and 2.2.3.2, electrical brain activity can be characterized both in the time domain, to capture processes phase-locked to the event of interest, and in the time-frequency domain, to describe non-phase-locked changes in oscillatory power. Movement-related potentials (MRPs) reflect cortical activity exactly timed with the preparation and the execution of voluntary movement. A typical sequence of MRPs is depicted in Figure 6A.

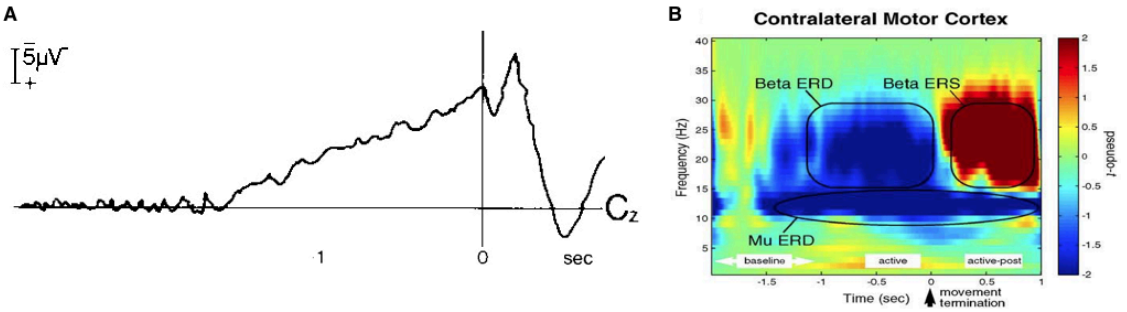

Figure 6: Electrophysiological correlates of motor preparation and execution investigated in the time and in the time-frequency domain.

A: The preparation of a voluntary movement is reflected by an increasing negativity, named readiness potential, detected mainly over the central electrodes at about 1.5 – 2s before the onset of the movement. The sharp negativity reflect the onset of the movement, and it is followed by positive reafferent activity (modified from Deecke et al., 1998). **B:** Specific changes of the EEG spectrum associated with movement preparation and execution are represented by a desynchronization of power in the alpha (8-13 Hz) and in the beta band (14-30 Hz), which is likely reflecting the engagement of sensorimotor areas during the motor task. The post-movement beta increase probably indicates the processing of somatosensory feedback (Jurkiewicz et al., 2006).

A slowly rising negative deflection, termed readiness potential, reflects the preparation of a voluntary movement (Brunia et al., 2012; Colebatch, 2007; Deecke et al., 1969; Shibasaki & Hallett, 2006). It appears at about 2 s before movement over the midline, and it becomes steeper shortly preceding (about 500 ms) the onset of the movement over central electrodes contralateral to the movement (Colebatch, 2007; Shakeel et al., 2015; Shibasaki & Hallett, 2006). Source modelling and intracranial recordings revealed that the readiness potential is mostly generated in the supplementary motor area, cingulate cortex and primary motor cortex (Ball et al., 1999; Eagleman, 2004; Praamstra et al.,1996; Yazawa et al., 2000), reflecting the cortical facilitation of these areas during the preparation, initiation and execution of a voluntary movement (Cui &

Deecke, 1999; Shibasaki & Hallett, 2006). The amplitude of the readiness potential can be modulated by several features of the movement, as level of intention, precision, force, speed, complexity of the movement (Lang, 2003). A sharp negative potential, i.e. the motor potential, shortly precedes the execution of the movement, likely representing activity in the primary motor cortex conveying the motor signal to the spinal cord (Cui & Deecke, 1999), and it is followed by a positive potential, which is thought to reflect reafferent activity from the somatosensory areas (Bötzel et al., 1997).

The influence of motor processes on brain rhythms has been largely investigated with respect to lower frequency bands. The most consistently observed pattern consists of a decrease in alpha (8–13Hz) and beta (14–30 Hz) bands during movement preparation and execution (Cheyne, 2013; Crone et al., 1998; Neuper & Pfurtscheller, 2001; Ohara et al., 2004; Pfurtscheller & Lopes da Silva, 1999; van Wijk et al., 2012), followed by a post-movement rebound particularly pronounced in the beta band (Figure 6B). Alpha and beta decrease starts at around 1.5 – 2 s before the onset of the movement over the central region contralateral to the movement and extends bilaterally during and after movement execution (Alegre et al., 2003; Leocani et al., 1997; van Wijk et al., 2012), suggesting activation of sensorimotor areas. The beta rebound begins approximately 300 to 400 ms after the onset of the movement and it is localized over bilateral central regions, and it is thought to reflect the processing of somatosensory feedback (Cassim et al., 2001; Cheyne, 2013).

Recently, several studies focused on changes in high frequency (> 30 Hz) rhythms within the human motor system (Ball et al., 2008; Cheyne et al., 2008; Crone et al. 1998; Muthukumaraswamy, 2010; van Wijk et al., 2012). Brief bursts of gamma activity are usually detected over the contralateral sensorimotor areas at about movement onset or shortly after it (Ball et al., 2008; Cheyne et al., 2008; Muthukumaraswamy, 2010; van Wijk et al., 2012). Although their functional significance has not been clarified yet, they presumably play a central role in binding and integrative processes, facilitating the communication between sensory and motor areas during complex motor tasks (Ball et al., 2008; Szurhaj et al., 2005), as well as in the neural computation of detailed movement parameters (Rickert et al., 2005).

2.4 Overlaps and interactions between pain and motor processing in the human brain

Corresponding to the increasing awareness of the intricate relationship between pain and behavior, interactions between pain and motor processes are increasingly attracting attention (Morrison et al. 2013; Sullivan 2008; Vogt & Sikes 2009; Wiech & Tracey 2013).

Neuroanatomical evidences from primate studies indicate that the major afferent pathway of nociception information, that is the spinothalamic tract, significantly project to cingulate motor areas, which are involved in the generation and control of movements by virtue of their projections to the primary motor cortex and the spinal cord (Dum et al., 2009; Picard & Strick, 1996) (see 2.3.1). Moreover, nociceptive neurons in anterior cingulate cortex are particularly responsive during escape behavior from noxious stimuli (Iwata et al., 2005). Correspondingly, functional neuroimaging studies in humans described that brain activations related to pain and motor processing significantly overlap in the anterior midcingulate cortex (aMCC) and supplementary motor area (SMA). Moreover, pain and motor processes did not only overlap but also interact in these regions, providing direct evidence for a convergence of motor control and pain processing in frontal midline areas (Duerden & Albanese, 2013; Misra & Coombes, 2014; Perini et al., 2013; Shackman et al., 2011). We verified these findings by visualizing common brain networks underlying pain and motor processing by means of Neurosynth (Yarkoni et al., 2011). The tool synthesizes neuroimaging literature associated with specific key terms and generates corresponding meta-analysis maps. We used the key terms “pain” and “motor” to generate maps of neuroimaging studies investigating both solely pain or motor process, and pain and motor process occurring simultaneously (Figure 7). We observed that both pain- and motor-related processes are associated with extended activation of sensorimotor areas (Figure 7A). Interestingly, when the two processes occur simultaneously, functional activity converges in regions of the medial wall (Figure 7B), confirming the crucial role of this area as an hub where sensory information are integrated and redirected to motor centers to regulate behaviors.

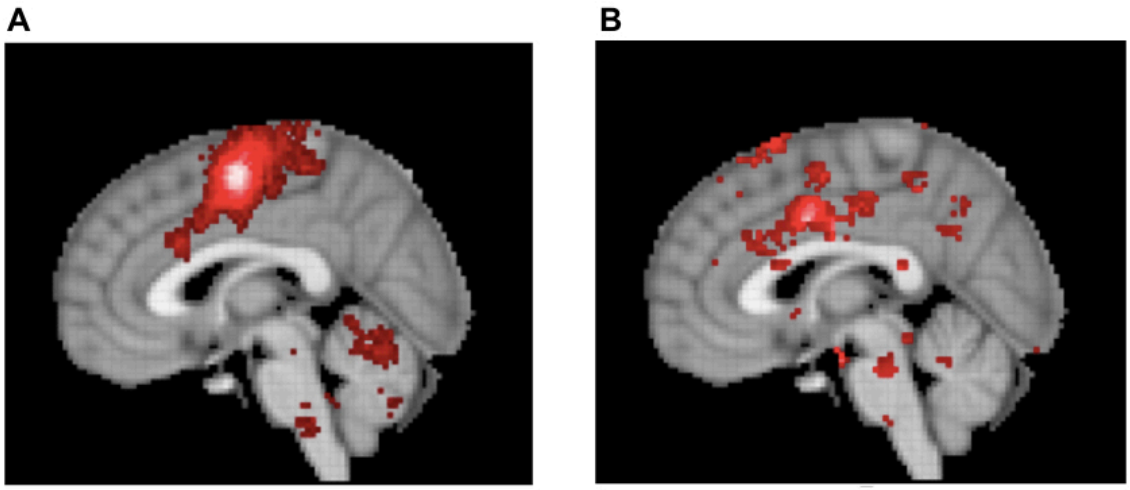

Figure 7: Brain activation maps of pain and motor processes occurring simultaneously.

Neurosynth allows to synthesize the results of several different neuroimaging studies and generate meta-analysis maps for a wide variety of psychological concepts. In order to visualize common networks underlying pain and motor processing in the human brain, we generated meta-analysis maps for the key terms "pain" and "motor". **A**: fMRI meta-analysis maps generated for the keywords "pain" and "motor" showing the results of 950 studies, which separately investigate the brain correlates either of pain or of motor processes. Beyond the expected activation over sensorimotor regions, pain and motor processes induce activation of regions of the medial wall. **B**: fMRI meta-analysis maps generated for the keyword "pain and motor" showing the results of 22 studies, which investigate functional brain responses of pain and motor processes occurring simultaneously. The results show a convergence of functional activation in regions of the medial wall (from http://neurosynth.org).

Evidence from neurophysiological studies corroborate mutual interaction between pain and motor processes in the human brain. Previous works disclosed that the execution of a movement in response to a painful stimulus was associated with a reduction of pain unpleasantness and an attenuation of the pain-related vertex potential. These results might be due to the inhibitory effect of motor projections on pain-related activity in the ACC, where the vertex potential is generated. (Le Pera et al., 2007; Nakata et al., 2009, 2004; Stancak et al., 2012). Interestingly, recent studies showed that pain-related vertex potential is closely related to the execution of defensive motor responses rather than the perception or salience of threatening stimuli, strengthening thus the view that pain-related cortical activity strongly modulates the preparation of defensive responses in the motor system. (Moayedi et al., 2015; Novembre et al., 2018). As regards oscillatory brain activity, painful stimuli and motor tasks occurring simultaneously enhance activation of the sensorimotor areas as reflected by an increase of movement-preparatory alpha and beta desynchronization (Babiloni et al., 2010, 2014; Misra et al., 2017).

On the whole, these results support the notion that pain plays a facilitating effect on the motor system, and provide evidence of a significant interplay between pain and motor processes in the human brain. However, the majority of these studies assessed interaction between pain and motor processes occurring primarily at the moment of the execution of the movement, whereas how the preparation of a motor response can be affected by concomitant pain is largely unknown yet. Most importantly, in none of the previous works the motor tasks had a functional relationship to the painful stimulus, that is movements were generally performed simply in reaction to the noxious stimuli. In light of the utmost relevance of protective responses for pain, we consider fundamental the implementation of an ecologically valid motor task in which movements have a functional role on the concomitant painful stimulation.

3 Aims and hypothesis

The crucial protective function of pain depends on appropriate motor responses. However, despite this intricate relationship between pain and motor processes, interactions between pain and motor processing in the human brain are not fully understood yet. In the present project, we therefore used electroencephalography (EEG) to characterize interactions between pain and motor processing. As a first step, we specifically investigated whether and how pain influences motor preparation in the human brain. Specifically, we tested the following hypothesis:

1. Pain is inherently linked to motor preparation. This inherent link might manifest as an influence of pain on movement preparatory brain activity.

We designed an experiment in which participants performed button presses to interrupt a painful thermal stimulation. In two control conditions, the participants performed similar movements without concomitant stimulation and passively perceived the stimulation without any task. During the experiment, we recorded brain activity using EEG and analyzed neural activity related to movement preparation. In particular, we analyzed phase-locked event-related potentials and non-phase-locked neural oscillations during movement preparation. We expected that pain significantly influences brain activity related to movement preparation. If so, this would provide physiological evidence for an intricate relationship between pain and motor preparation in the human brain.

2. The inherent link between pain and motor preparation is pain-specific.

We tested this hypothesis in a second study. In this study, we assessed whether changes of movement preparatory brain activity were specific to pain or were rather reflecting a modality-spanning phenomenon. We applied non-painful thermal stimulation and participants performed button presses to interrupt the stimulation. The other experimental conditions, recordings and analyses were similar to the first study.

4 Materials and methods

Our research project aimed to characterize whether and how pain impacts motor-related brain responses. Specifically, we assumed that motor processes are an inherent component of pain, which might be reflected by pain-induced changes of movement preparatory brain activity.

We therefore conducted a first experiment, which required participants to perform button presses in response to painful thermal stimuli. EEG was simultaneously recorded. A second study was designed in a different sample to investigate whether our results could be specifically attributed to pain.

The two studies differed exclusively regarding the participant samples and the stimulation parameters, as described below. Apart from that, the recordings and the analysis of the data were similar.

4.1 Study 1

4.1.1 Participants

21 healthy human participants (9 male; age 27 ± 6 ys, mean ± SD) were included in the study, during which they were required to interrupt a painful stimulation by pressing a button. Data from one participant were excluded due to technical problems during the recording. Therefore, in the final sample we included 20 participants (9 male; age 27.3 ± 6.3 ys). The participants were recruited via leaflets and reimbursed for their participation with a monetary reward. Exclusion criteria for participation in the study were the presence of neurological or psychiatric diseases, presence of chronic or acute pain, medication consumption or skin lesions or diseases. All participants were tested for handedness according to the Edinburgh Handedness Inventory (Oldfield, 1971) and only right-handed participants took part in the study. At the beginning of the experiment, all participants provided written informed consent. The study was performed in accordance with the Declaration of Helsinki and approved by the local ethics committee.

4.1.2 Paradigm

The experimental protocol included the assessment of the subjective pain threshold, a training session, the preparation of the EEG recordings during which the subjects completed self-assessment questionnaires and the EEG recordings during three experimental conditions. Together, each experimental session lasted about 2.5 hours. The experiments took place in a dimly lit, temperature-controlled room. Since variations in skin temperature may affect perception of thermal stimuli, an infrared thermometer was used to ensure that the skin temperature at the beginning of the experiment (31 ± 2.2 °C) was in the suggested range for thermal sensory testing (Hagander et al. 2000). During the recording, participants were exposed to white noise through headphones to cancel out ambient noise.

4.1.2.1 Threshold measurement and training session

Subjective thermal pain threshold was measured according to the method of limits (Rolke et al., 2006). Five thermal stimuli of increasing intensity were applied to the dorsum of the left hand. Stimulus temperature was increased at 0.8 ºC/s from a baseline temperature of 32 ºC and the participants were asked to terminate the temperature increase by a button press as soon as the stimulation was perceived as painful. After the button press, stimulus temperature decreased at 8 ºC/s. Individual pain threshold (45.2 ± 2.5 ºC) was computed as the mean of five stimuli.

Next, participants performed a short training session (5 stimuli), which resembled exactly the first condition of the experiment (see below), in order to get familiar with the stimulation and the task of the experiment.

4.1.2.2 Experimental conditions

The study consisted of three experimental conditions (Figure 8). In the *pain & buttonpress* condition, participants were instructed to press a button to interrupt painful heat stimuli when they became intolerable. In the *buttonpress* condition, participants executed button presses in the absence of concomitant

painful stimulation. In the *pain* condition, participants passively perceived painful heat stimuli without being required to interrupt them.

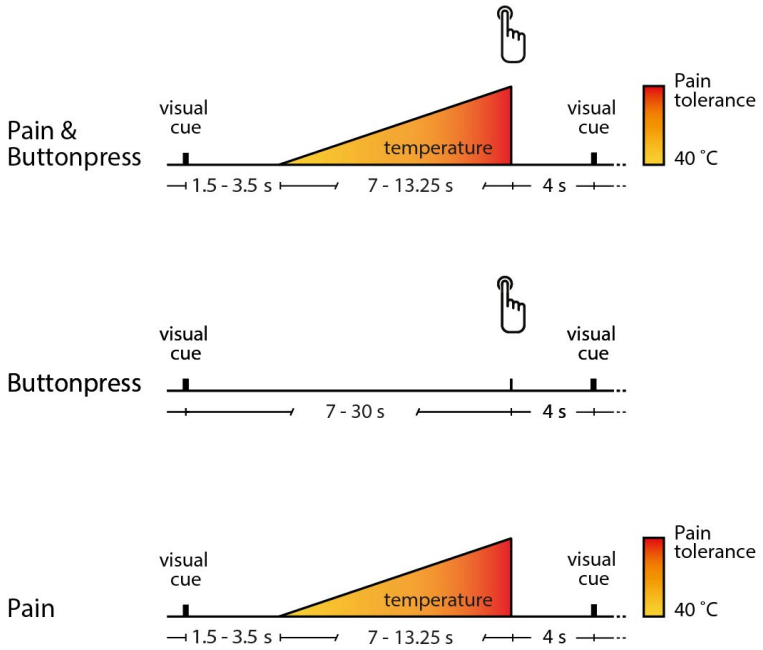

Figure 8: Experimental conditions of Study 1.

In the *pain & buttonpress* condition, we applied 60 heat stimuli of increasing intensity to the dorsum of the left hand. Participants were instructed to interrupt the stimulation at the maximum pain intensity they were willing to tolerate by pressing a button with the right index finger. In the *buttonpress* condition, participants performed button presses at an interval matching the one in the *pain & buttonpress* condition but without simultaneous painful stimulation. In the *pain* condition, 60 stimuli of comparable intensity to the *pain & buttonpress* condition were applied to the dorsum of the left hand, but participants could not interrupt them (modified from Postorino et al., 2017).

Each condition comprised 60 stimuli. Heat stimuli were delivered by a thermode (TSA-II, Medoc, Israel) using a heat probe placed on the dorsum of the left hand (see 4.1.3). The thermode was controlled by a computer using MATLAB (Mathworks, Natick, MA, USA) and the Psychophysics Toolbox (http://psychtoolbox.org/). Participants performed the button presses with the index finger of the right hand. The experimental conditions were presented in a fixed order, as the duration of the stimuli in the *pain* condition matched the duration of stimuli in the *pain & buttonpress* condition, which therefore had to be presented as the first condition, followed by the *buttonpress* and the *pain* conditions. After the first condition, the probe of the thermode was slightly displaced from a lateral to a medial position to prevent skin damage.

The *pain & buttonpress* condition (Figure 8, top row) was designed to assess the influence of pain on motor preparation. Participants were instructed to interrupt increasing heat stimuli by pressing a button at the maximum pain

intensity they were willing to tolerate. A black fixation cross presented at the center of a computer monitor turned green for 1 s to indicate the beginning of a new trial. After an interval randomly varied between 1.5 and 2.5 s, the temperature increased starting from a baseline of 40 °C at a changing rate of 0.8 °C/s. After the button press the temperature returned to baseline with a cooling rate of 8 °C/s and after 4 s a new trial started. During this condition, participants interrupted the stimulation 10.0 ± 1.2 s after the start of the temperature increase at an average temperature of 47.1 ± 0.85 °C. The mean latency between button presses, i.e. the mean duration of trials, was 17.4 ± 1.6 s.

We designed two additional conditions as a control, namely the *buttonpress* and the *pain* condition. However, following statistical analysis were restricted to the comparison between the *pain & buttonpress* and the *buttonpress* conditions, as the *pain* condition does not contribute to answering the core question of the study, i.e. how pain influences motor preparation.

In the *buttonpress* condition (Figure 8, middle row), participants performed a self-paced task during which they pressed a button at an interval comparable to the *pain & buttonpress* condition. As in the previous condition, the appearance of the green cross indicated the beginning of the trial, which ended 4 s after the button press. If the interval between button presses was shorter than 7 s for more than two trials in a row a red cross was displayed and up to 15 additional trials were performed to ensure a number of 60 trials longer than 7 s for each participant. During this condition, mean latency between button presses was 19.0 ± 10.2 s.

Finally, during the *pain* condition (Figure 8, bottom row) participants were instructed to perceive passively heat stimuli. The mean duration and the mean temperature intensity matched exactly the values of the *pain & buttonpress* condition, but trials were presented in a randomized order. In this condition, no motor task was required.

4.1.3 Thermal stimulation

We applied thermal stimuli to the dorsum of the hand using a thermode (TSA-II, Medoc, Israel), a device capable of generating thermal stimuli ranging from cold to hot. The skin of the person is stimulated by a 3x3 cm contact probe

based on Peltier elements, which is composed by semiconductor junctions that can generate a temperature gradient between the upper and the lower layers of the stimulator by means of electric current. The results of the testing are registered in a dedicated software (MEDOC Work Station), which also allows the user to design its own paradigm by setting several parameters, including temperature modality, baseline temperature, ramping rate, cooling down rate, interstimulus interval. TSA is designed for clinical applications to assess the integrity of peripheral somatosensory pathways (Rolke et al., 2006), as well it is employed for research purposes, as it allows to investigate pain-related brain activity.

4.1.4 Questionnaires

Psychological variables like anxiety, depression and individual locus of control can strongly modulate the perception of pain. Therefore, we administered specific questionnaires to assess psychological traits which are known to influence pain perception.

A common measure of trait and state anxiety is the State-Trait Anxiety Inventory (Spielberger, 2010). It consists of 40 self-reporting-items which assess the short and long lasting inclination towards experiencing general anxiety. Depression was assessed by the Beck Depression Inventory (Beck et al., 1961), a 21-items self-report rating scale which quantifies the existence and the severity of depression symptoms. To assess the individual locus of control we used the Internality, Powerful Others, and Chance Scales (Levenson, 1981). The three subscales of the questionnaire investigate the individual propensity to attribute the outcomes of events to their own abilities (internal), other people (powerful others) or external circumstances (chance). Noxious stimulation under condition of perceived control was perceived as less intense and less painful (Wiech et al., 2006).

Correlations between psychological variables and pain perception were tested by means of Pearson's correlation coefficient and using SPSS for Windows (release 22, Chicago, SPSS Inc.). Specifically, we calculated correlation coefficients between anxiety, depression and perceived control scores and the individual pain tolerance threshold. Furthermore, to investigate

whether those variables are related to the influence of pain on motor preparatory brain activity, we correlated the questionnaires scores with the difference in the amplitude of movement preparatory brain activity between experimental conditions.

4.1.5 Electroencephalography

Neurons communicate with each other by transmitting electrical impulses, i.e. action potentials, along their axonal fibers, which elicit excitatory or inhibitory postsynaptic potentials in connected neurons (Pizzagalli, 2007; Speckmann et al., 2012). Spatial summation of voltage gradients in large and synchronously activated clusters of cortical neurons (specifically cortical pyramidal neurons) produces a signal measurable from the scalp by means of electroencephalography (Baillet et al., 2001; Buzsáki, 2006; Buzsáki et al., 2012; Olejniczak, 2006; Speckmann et al., 2012).

4.1.5.1 Recording and preprocessing

During the experiment, electrical brain activity was non-invasively recorded with an electrode cap (Easycap, Herrsching, Germany) and BrainAmp MR plus amplifiers (Brain Products, Munich, Germany) using the BrainVision Recorder software (Brain Products, Munich, Germany). Electrodes were placed on the scalp using an electrically conductive gel or paste to reduce the impedance of the skin (Pizzagalli, 2007). The location of the electrodes were standardized according to the international 10-20 system (Jaspers, 1958). The electrode montage of our study comprised 64 electrodes consisting of all 10-20 system electrodes and the additional electrodes Fpz, FCz, CPz, POz, Oz, Iz, AF3/4, F5/6, FC1/2/3/4/5/6, FT7/8/9/10, C1/2/5/6, CP1/2/3/4/5/6, TP7/8/9/10, P5/6, PO1/2/9/10. Two additional electrodes were placed below the outer canthus of both eyes. All the electrodes were referenced to the FCz electrode and grounded at AFz. Data were digitized at a sampling rate of 1000 Hz, high-pass filtered at 0.015 Hz and digitally stored for subsequent offline analysis. Electrode impedance was kept below 20 kΩ.

The raw EEG data were preprocessed using the BrainVision Analyzer software (Brain Products, Munich, Germany). The data were downsampled to 512 Hz, highpass filtered at 0.5 Hz, and re-referenced to the average of all electrodes (Lei & Liao, 2017; Pizzagalli, 2007). As biological and non-biological artefacts can overlap in frequency and amplitude with the signal of interest and confound therefore the interpretation of the results, we implemented source decomposition techniques such as Independent Component Analysis (Delorme & Makeig, 2004; Jung et al. 2000) to dissect the EEG signal into components reflecting neural activity or artefacts. Once identified, components reflecting artefacts are removed and the signal is further recomposed. Continuous EEG data were then segmented in trials of -7 to 5 s with reference to the button press (*Pain & Buttonpress* and *Buttonpress* conditions) or to the temperature peak (*Pain* condition). An automatic artifact rejection algorithm excluded trials with artifacts exceeding ±100 μV in any channel. After artifact rejection, the number of remaining trials was 51 ± 9 for the *pain & buttonpress*, 54 ± 3 for the *buttonpress* condition and 47 ± 9 for the *pain* condition.

4.1.5.2 Analysis

EEG data were exported and analyzed using FieldTrip, an open-source toolbox for Matlab (Oostenveld et al. 2011). We analyzed neural activity related to movement preparation both in the time and in the time-frequency domain, focusing on a period of time preceding the onset of the movement. In fact, as button presses coincided with the end of the painful stimulation in the *pain & buttonpres*s condition, post movement neural activity could not be unequivocally interpreted.

4.1.5.2.1 Statistical analysis

Changes in electrophysiological brain activity were statistically compared between different experimental conditions implementing a cluster-based permutation approach (Maris & Oostenveld, 2007), a non-parametric procedure which is well suited to control for the problem of multiple comparisons. The multiple comparisons problem is caused by the multidimensional structure of EEG data, as they are usually recorded at multiple electrodes and time points.

Significant effects are tested by comparing experimental conditions at several electrodes and time points, and a large number of comparisons increases the probability of the incorrect rejection of a true null hypothesis. The cluster-based permutation approach controls the multiple comparisons problem computing cluster-level statistics. As a first step, the signal amplitudes between experimental conditions is compared at each electrode and/or time point by means of point-by-point t-tests. Secondly, contiguous sensors or time-points where the t statistics exceeds a given threshold ($p = 0.05$) are clustered together and cluster-level statistics is calculated by summing up the t-values within each cluster. To determine statistical significance, the maximum cluster-level statistic is next evaluated against a reference distribution, created by drawing 1000 random permutations of the original dataset. The cluster-based permutation approach represents, therefore, a data-driven procedure which deals with the multiple comparisons problem avoiding extremely conservative corrections and taking physiological plausibility into account (van Ede & Maris, 2016). All the statistical analysis were performed using the Fieldtrip toolbox (Oostenveld et al. 2011).

4.1.5.2.2 Time domain analysis

The event-related potentials technique is a well-established methodology to investigate electrophysiological changes associated with certain events (see 2.2.3.1). We implemented this analysis to examine brain responses related to the button press or the temperature peak.

The segmented data were low-pass filtered at 30 Hz and for each experimental condition trials were averaged time-locked to the button press. As long lasting stimulation is not suited to elicit evoked-related potentials, the *pain* condition was not included in further statistical comparisons.

Previous studies showed that most brain activity related to movement preparation begins at around 2 s before the onset of the movement (Brunia et al. 2012; Colebatch 2007; Shibasaki and Hallett 2006). We therefore focused our statistical analysis on a 2 s time-window preceding the button press. We compared the amplitude of the potentials between the *pain & buttonpress* and *buttonpress* conditions to test the effect of a concomitant thermal stimulation on brain activity related to motor preparation. Firstly, we computed multi-sensor

analysis by clustering across neighboring electrodes and time points. As the readiness potential is most pronounced at electrodes close to the vertex (Colebatch, 2007; Shibasaki and Hallett, 2006), we later focused the statistical analysis on the electrode Cz, clustering exclusively across time points.

All statistical comparisons were performed using the cluster-based permutation approach (see 4.1.5.2.1).

4.1.5.2.3 Time-frequency domain analysis

The raw electrophysiological data can be seen as a mixture of different frequencies. Spectral analysis allows to decompose the data into magnitude and phase information for different frequency bands and to describe their changes over time with reference to a specific event (see 2.2.3.2). In order to characterize how frequency-specific brain activity evolves over time related to motor preparation, we computed time-frequency analysis by applying a Hanning-tapered sliding window Fast Fourier transformation. The window length was 0.25 s and it was shifted in steps of 30 ms over a frequency spectrum ranging from 1 to 100 Hz. We averaged power across theta (4-7 Hz), alpha (8-13 Hz), beta (14-30 Hz) and gamma (40-100 Hz) frequencies to extract frequency-band specific time-courses.

To assess whether power changes were significant over time and how the effect was distributed over the scalp, we averaged for each trial, at each electrode site and for each frequency band, a 1 s baseline time-window at the beginning of the trial (-6 to -5 s) and a 1 s time-window preceding the button press or the temperature peak (-1 to 0 s). The early baseline (-6 to -5 s) and the late (-1 to 0 s) phase of the stimulation were then compared for each experimental condition.

Furthermore, to verify whether power time courses significantly differed between experimental conditions, we compared time courses of each frequency band between the *pain & buttonpress* and the *buttonpress* condition. As a statistical approach, we implemented cluster-based permutation analysis (see 4.1.5.2.1) on a 2 s time window preceding the button press (Cheyne 2013; Pfurtscheller & Lopes da Silva 1999; van Wijk et al. 2012). Firstly, the analysis was performed on a multi-sensor level, clustering across time and neighboring electrodes, and secondly restricted to a selection of electrodes covering the

sensorimotor areas contralateral to the movement (i.e. Cz, CPz, C1, C3, CP1, CP3), as changes in motor-related brain activity mainly occur over these regions (Cheyne 2013; Pfurtscheller & Lopes da Silva 1999; van Wijk et al. 2012).

4.2 Study 2

4.2.1 Participants

24 participants (9 male; age 26 ± 4 ys) took part in the study, in which button presses were performed in response to a non-painful warm stimulation. During the course of the analysis, the data from four participants had to be excluded due to technical problems during the recording and/or to poor data quality, resulting in a final sample of 20 participants (8 male; age 25.8 ± 4.7 ys). We followed a comparable recruitment procedure as in the first study and applied identical exclusion criteria (see 4.1.1). Only right-handed participants were included in the study according to the Edinburgh Handedness Inventory (Oldfield, 1971). The study was approved by the local ethics committee and performed in accordance with the Declaration of Helsinki. Informed consent was obtained before participation.

4.2.2 Paradigm

The experimental procedure was mostly similar to the first study (see 4.1.2). As for the first study, each experimental session lasted approximately 2.5 hours. Skin temperature at the beginning of the experiment was in the suggested range for thermal sensory testing (32 ± 1.8 °C) (Hagander et al. 2000).

4.2.2.1 Threshold and training session

To ensure a comparable sample in terms of pain perception, we assessed subjective pain threshold as in the first study according to the method of limits (Rolke et al., 2006). Number of stimuli and stimulation parameter were comparable to the procedure for the first study. The mean pain threshold was

44.4 ± 3.9 ºC and it did not significantly differ from the mean thresholds of the sample of the first study (independent t-test, p = > 0.05).

A short version of the first condition of the study was executed to train the participants and to individually adjust the ramping of the temperature. Each session started at a changing rate of 0.3 ºC/s, which was later decreased or increased to ensure an average duration of trials longer than 7 s, therefore comparable to the first study. The average changing rate implemented for the experimental condition was 0.4 ± 0.14°C/s.

4.2.2.2 Experimental conditions

Participants underwent three experimental conditions, each including 60 stimuli: *warmth & buttonpress*, *buttonpress* and *warmth* condition (Figure 9).

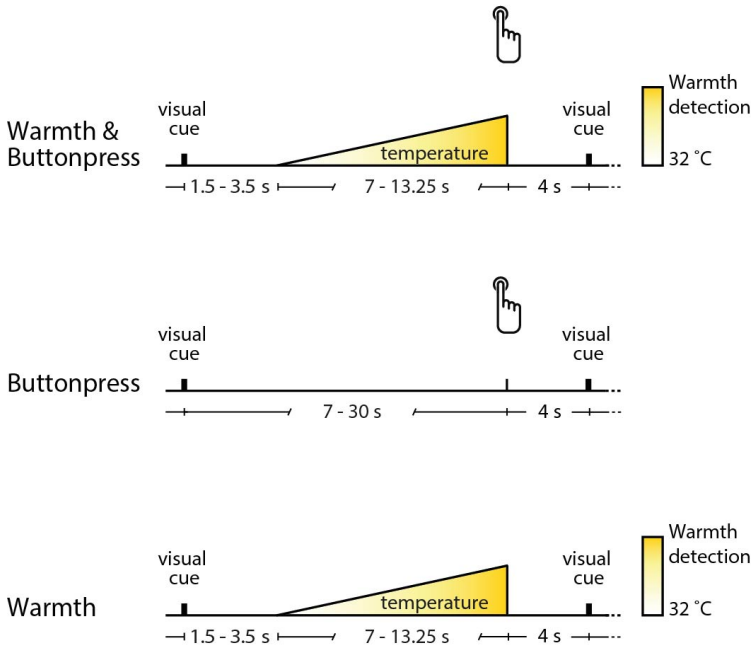

Figure 9: Experimental conditions of Study 2.

In the *warmth & buttonpress* condition, participants were required to interrupt the stimulation when perceived as clearly warm. 60 stimuli of increasing intensity were applied. The *buttonpress* and the *warmth* conditions matched that of the first study (modified from Postorino et al., 2017).

In the *warmth & buttonpress* condition (Figure 9, top row) an increasing non-painful thermal stimulation was applied on the dorsum of the left hand by mean of a thermode (TSA-II, Medoc, Israel) (see 4.1.3), and participants were instructed to interrupt the stimulation as soon as it was perceived as clearly warm. Temperature started from a baseline of 32 ºC and increased at a changing rate which was adjusted to each participant to obtain trials longer than 7 s (0.4 ±

0.14°C/s). If trials were shorter than 7 s, up to 15 additional trials were implemented aiming at a total number of at least 60 trials longer than 7 s for each participant. After the button press, the temperature returned to baseline at a changing rate of 0.8 ºC. On average, participants stopped the stimulation 15.1 ± 5.9 s after the start of the temperature increase, at an average temperature of 37.2 ± 2.7 °C/s. The mean latency between button presses was 22.4 ± 7.5 s.

The task of the *buttonpress* condition (Figure 9, middle row) was identical to the one of the first study (see 4.1.2.2). The mean latency between button presses in this condition was 22.9 ± 11.8 s.

Likewise, the *warmth* condition (Figure 9, bottom row) required the participants to perceive warm stimuli at comparable intensities as the first condition without performing any additional task.

4.2.3 Questionnaires

We assessed by means of questionnaires psychological variables like anxiety, depression and individual locus of control, which can strongly influence the perception of pain, both in everyday life and in experimental settings. Specifically, we administered the State-Trait Anxiety Inventory (Spielberger, 2010), the Beck Depression Inventory (Beck, 1961) and the Internality, Powerful Others, and Chance Scales (Levenson, 1981) (see 4.1.4).

To assess whether psychological traits could influence the warmth detection task, we calculated Pearson's correlation coefficients between anxiety, depression and perceived control scores and maximum average temperature. Questionnaires scores were also correlated with the difference in the amplitude of the readiness potential between experimental conditions, to evaluate the impact of individual traits on electrophysiological responses. All the correlations were performed using SPSS for Windows (release 22, Chicago, SPSS Inc.)

4.2.4 Electroencephalography

4.2.4.1 Recording and preprocessing

EEG data were recorded using an electrode montage of 64 electrodes (Easycap, Herrsching, Germany) and BrainAmp MR plus amplifiers (Brain Products, Munich, Germany) (see 4.1.5.1). All electrodes were referenced to FCz and grounded at AFz. The EEG was sampled at 1000 Hz and high-pass filtered at 0.015 Hz. Impedances were kept below 20 kΩ.

Preprocessing was performed using the BrainVision Analyzer software (Brain Products, Munich, Germany) and it included downsampling to 512 Hz highpass filtering at 0.5 Hz, and recomputation to the average reference Independent component analysis was implemented to correct for eye and muscle artefacts (Jung et al., 2000). Continuous data were segmented in trials of -7 to 5 s with reference to the button press (*warmth & buttonpress* and *buttonpress* conditions) or to the temperature peak (*warmth* condition). Trials with artifacts exceeding ±100 μV in any channel were automatically rejected. The number of remaining trials was 51 ± 6 for the *warmth & buttonpress*, 53 ± 5 for the *buttonpress* condition and 50 ± 7 for the *pain* condition.

4.2.4.2 Analysis

EEG data were exported and analyzed using FieldTrip (Oostenveld et al. 2011). We analyzed neural activity related to movement preparation in the time domain to assess whether the results of the first study could be specifically ascribed to pain. Post movement neural activity was not further analyzed, as it could not be unequivocally interpreted due to the temporal coincidence of button presses and end of the thermal stimulation.

All the statistical comparisons were performed using the cluster-based permutation approach outlined in 4.1.5.2.1.

4.2.4.2.1 Time domain analysis

We investigated electrophysiological responses related to the button press by means of event-related potentials.

The segmented data were low-pass filtered at 30 Hz and for each experimental condition trials were averaged time-locked to the button press. The warmth condition was not further analyzed, as long lasting stimulation is not suited to elicit evoked-related potentials.

We focused our statistical analysis on a 2 s time-window preceding the button press, since previous studies indicated that brain activity related to movement preparation begins essentially at around 2 s before the onset of the movement (Brunia et al. 2012; Colebatch 2007; Shibasaki and Hallett 2006). We contrasted the amplitude of the readiness potential in *warmth & buttonpress* to the *buttonpress* condition, both at a multi-sensor and at a single electrode (Cz) levels (see 4.1.5.2.2). Furthermore, in order to evaluate the differential effect of pain and warmth on motor preparation, we calculated the differences of the amplitude of the readiness potential between the *pain/warmth & buttonpress* and the respective *buttonpress* condition and we contrasted the differences against each other. The analyses were performed again clustering both across time at electrodes and across time only at electrode Cz.

All the statistical comparisons were performed using the cluster-based permutation approach (see 4.1.5.2.1).

5 Results

Pain fulfills fundamental protective functions, priming the organism to react to potential threat. Despite the tight connection between pain and motor processes, few studies have investigated how they interact in the human brain (Misra & Coombes, 2014; Misra et al., 2017; Morrison et al., 2013; Perini et al., 2013). Therefore, we applied electroencephalography to disentangle the possible effect of a painful stimulation on motor preparation. To ensure the specificity of our results, we designed a second study where a non-painful thermal stimulation was implemented. The data were further analyzed to describe changes in movement preparatory brain activity associated to the presence of a painful stimulation.

5.1 Study 1

5.1.1 Behavioral results

Since subjective pain perception can be influenced by psychological variables, we assessed anxiety level, depression level and individual locus of control by means of specific self-reporting questionnaires (see 4.1.4). The scores of the questionnaires were correlated with the individual maximum average temperature which participants were willing to tolerate during the *pain & buttonpress* condition by using the Pearson correlation coefficient. There was no significant correlation between questionnaires scores and subjective pain tolerance ($p>0.05$). Therefore, the results do not provide evidence for an influence of individual variables on pain tolerance.

We next evaluated whether the effect of a painful stimulation on motor related brain activity was modulated by individual locus of control. Previous studies showed that individual perceived control modulates pain perception (Wiech et al., 2006). Specifically, we correlated the questionnaire scores with the difference of the amplitude of the readiness potential in the *pain & buttonpress* condition and in the *buttonpress* condition. We did not detect any significant

correlation ($p>0.05$). The results do not support therefore a role of individual variables like locus of control on the modulation of pain on movement preparatory brain activity.

5.1.2 Time domain analysis: effect of pain on the readiness potential

We investigated brain activity time-locked to an event of interest, that is the button press or the temperature peak. Figure 10 shows event-related potentials averaged across participants at each electrode site. In the *pain & buttonpress* and in the *buttonpress* conditions, a typical pattern of movement-related potentials can be described (see Figure 11). Shortly preceding the onset of the movement, a negative slope (i.e. the readiness potential) is detected in association to movement preparation, showing maximal amplitude at the vertex electrode. The execution of the movement is reflected by the motor potential, a sharp negative peak which is tightly locked to the onset of the movement. A positive wave termed reafferent potential follows the execution of the movement, most probably indicating feedback mechanisms. However, as post-movement activity temporally coincides with the offset of the temperature, it was not possible to disentangle potential results and to attribute them to motor-related or pain-related processes. Therefore, we restricted our statistical comparisons on a 2 s time window preceding the onset of the movement. We did not expect to detect any motor-related potential in the pain condition, as no movement was performed. Moreover, the long-lasting stimulation implemented in our paradigm does not elicit any pain-related potential. Consequently, the pain condition was not included in further statistical analysis.

Being interested in the effect of painful stimulation on motor-related brain processes, we compared the amplitude of the readiness potential in the *pain & buttonpress* and *buttonpress* conditions. Significant differences were detected in a cluster of electrodes covering central and frontal areas with predominant distribution contralateral to the movement side (see Figure 11A).

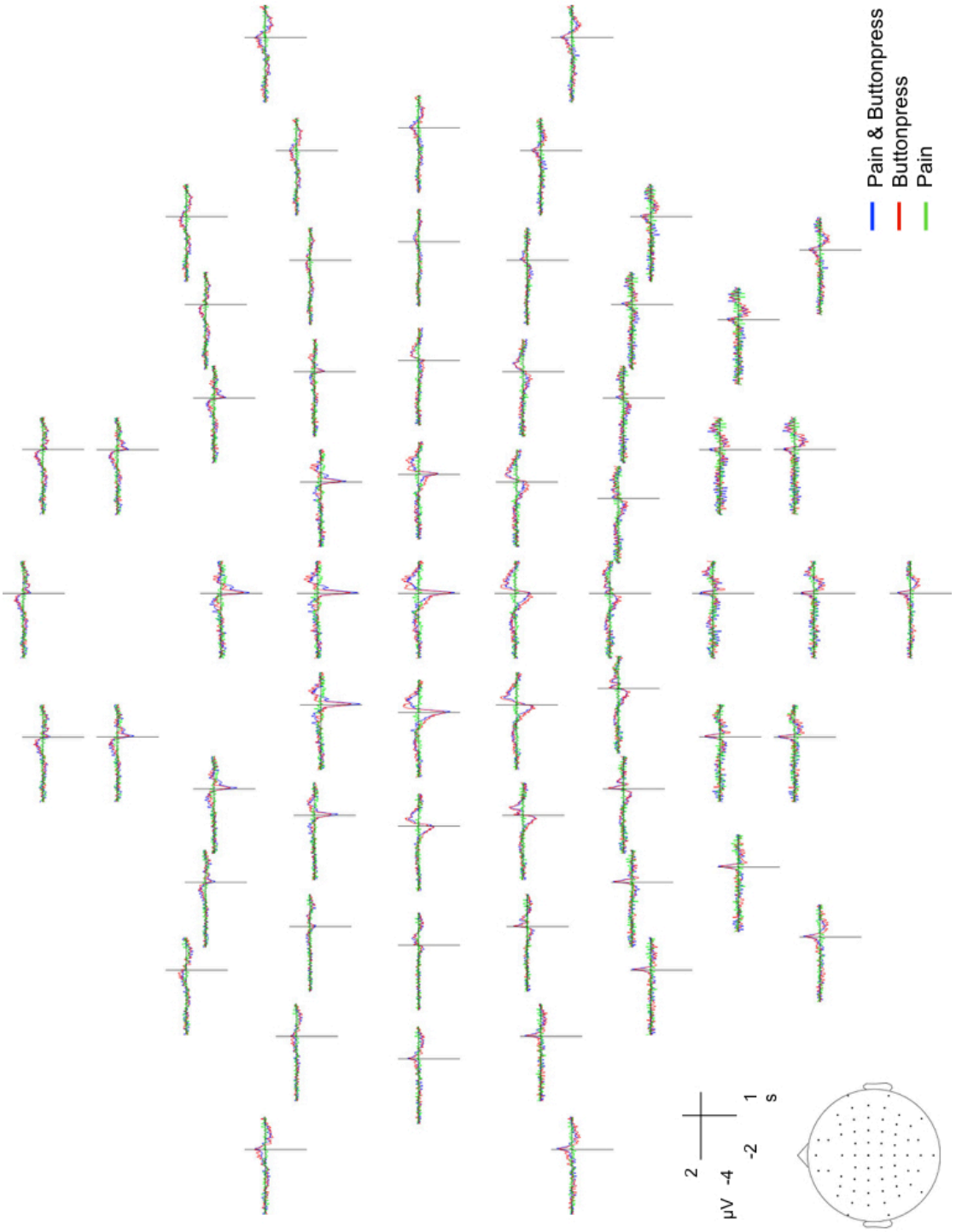

Figure 10: Grand average of the ERP waveforms of the Study 1.
Topographical distribution of the movement-related potentials at each electrode site for each experimental condition. The increasing negativity preceding the onset of the movement, namely the readiness potential, is mainly distributed at fronto-central electrodes. No movement-related potentials were detected in the pain condition, as no movement was performed.

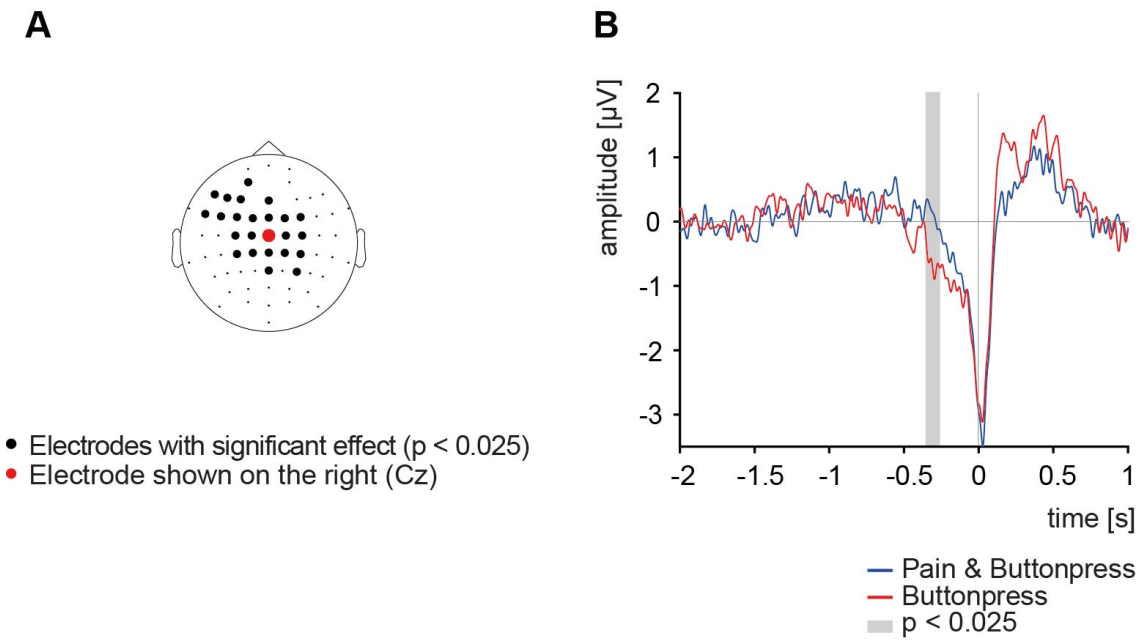

Figure 11: Statistical comparison of movement-related potentials of the Study 1.

A: Electrodes where statistical comparisons between the experimental conditions revealed significant differences are marked by bold black dots. **B:** Movement-related potentials at electrode Cz. During the last 2 s of motor preparation, we statistically compared the amplitude of the movement-related potentials between *pain & buttonpress* and *buttonpress* conditions. Significantly different time periods are highlighted by the grey shaded time window. Statistical analyses were conducted using cluster-based permutation statistics (modified from Postorino et al., 2017).

The results of the cluster-based test revealed a significant reduction of the amplitude of the readiness potential in the *pain & buttonpress* condition ($p<0.025$) between -0.35 s and -0.26 s before the button press (see Figure 11B).

Therefore, we observed a reduction of brain activity related to motor preparation when a movement was performed in response to a painful stimulation.

5.1.3 Time-frequency domain: effect of pain on movement-related oscillatory activity

We next investigated the effects of pain perception and motor preparation on brain activity at different frequency bands. As a first step, we averaged power across frequency bands of interest and analyzed the time course of brain activity in the different experimental conditions. Figure 12-16 show power time courses in a time window ranging from – 4 to 1 s for each experimental condition averaged across participants for theta, alpha, beta and gamma frequency bands,

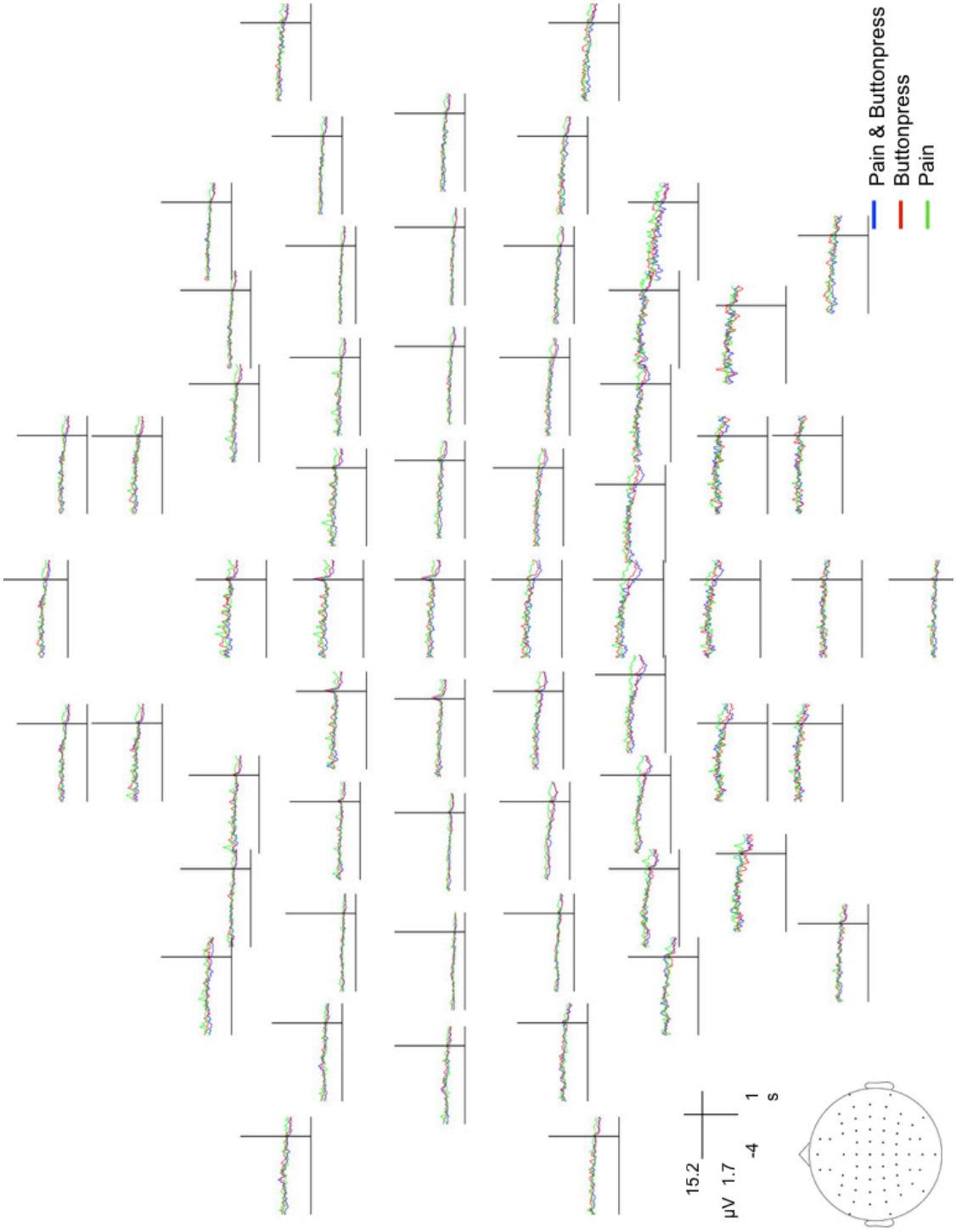

Figure 12: Grand average of theta power (4-7 Hz) time courses.

Power evolution over time at each electrode site and for each experimental condition. No particular changes of power over time are observable.

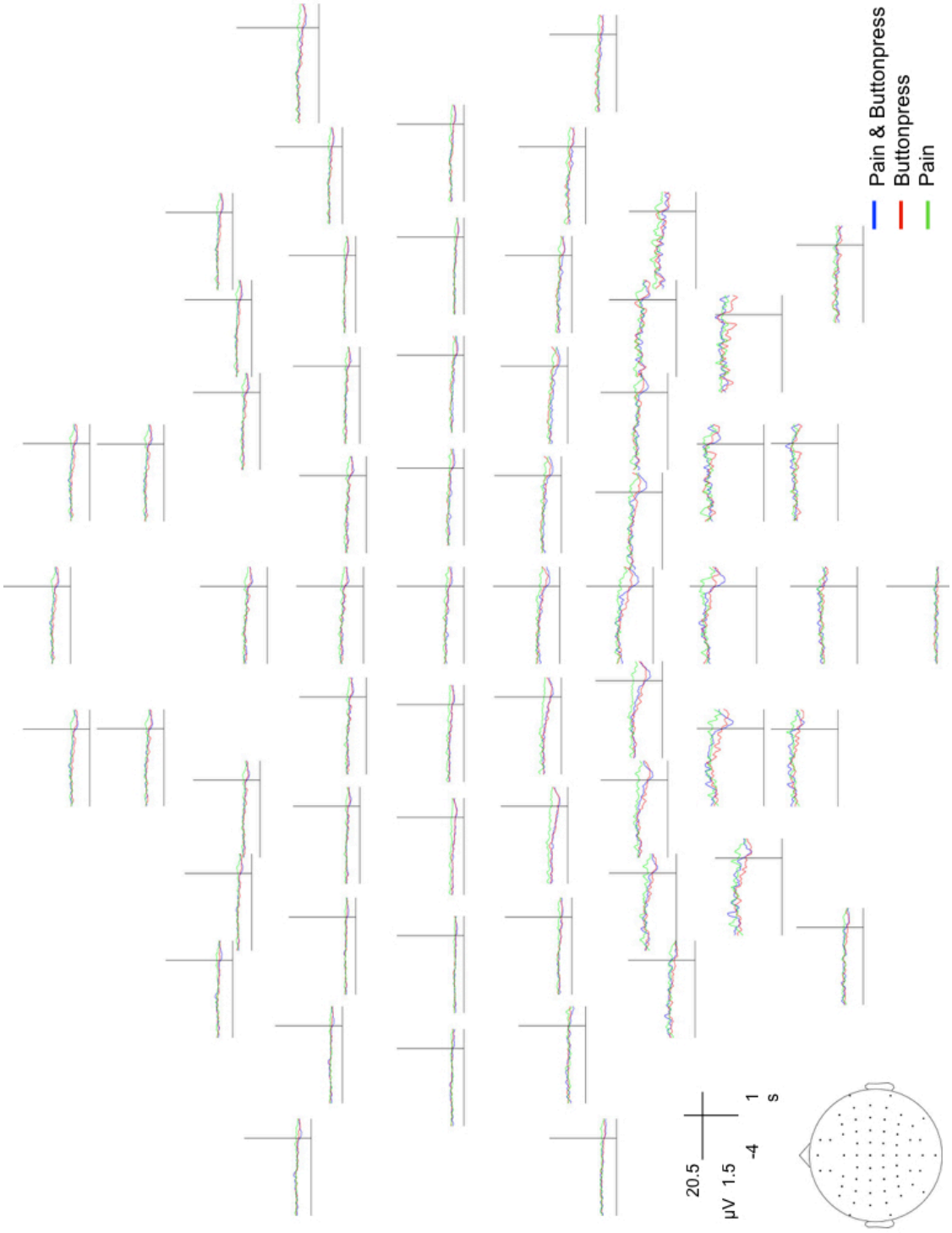

Figure 13: Grand average of alpha power (8-13 Hz) time courses.

Power evolution over time at each electrode site and for each experimental condition. It is possible to detect a decrease of power preceding the onset of the movements at central electrodes covering the sensorimotor areas.

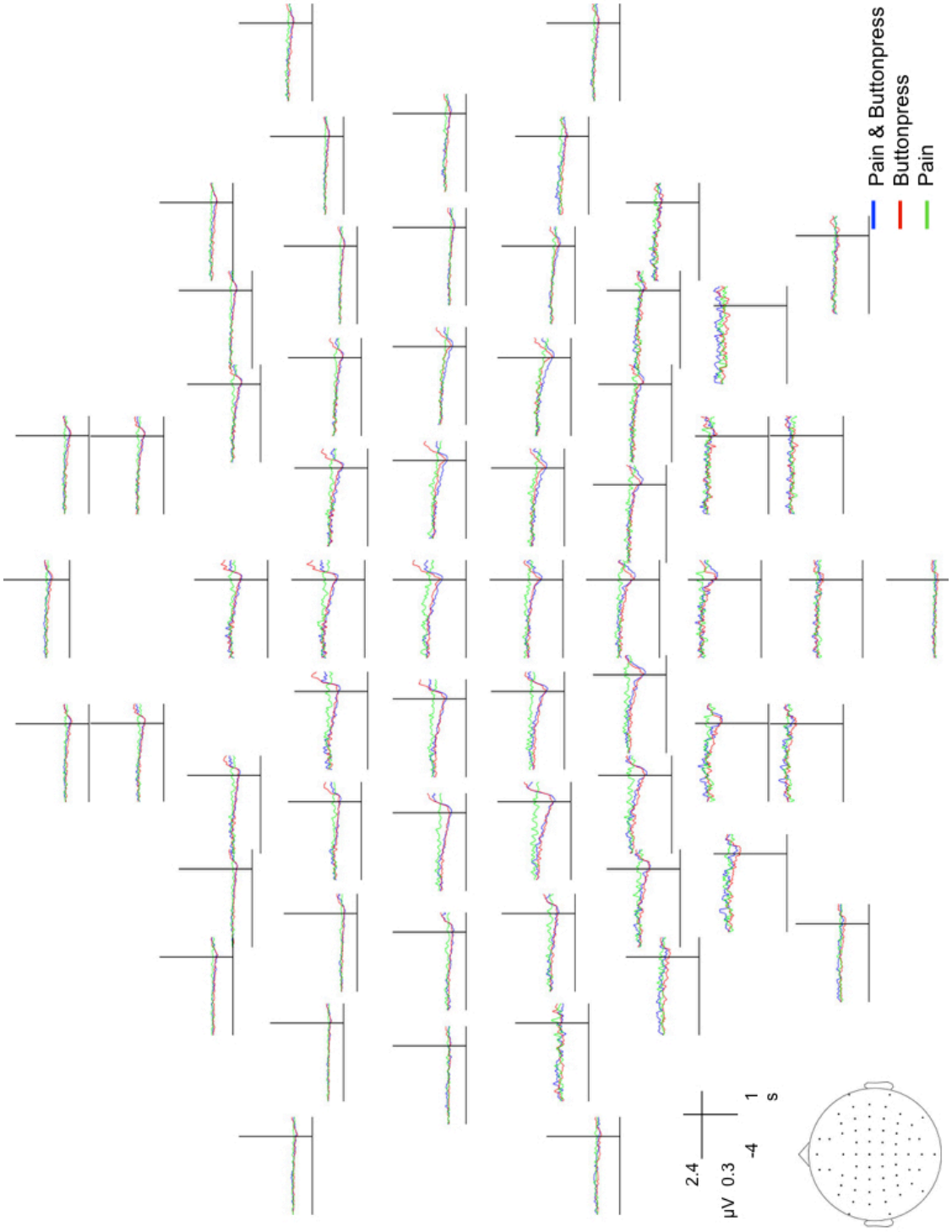

Figure 14: Grand average of beta power (14-30 Hz) time courses.

Power evolution over time at each electrode site and for each experimental condition. Power decreases shortly before the onset of the movements at central electrodes covering the sensorimotor areas.

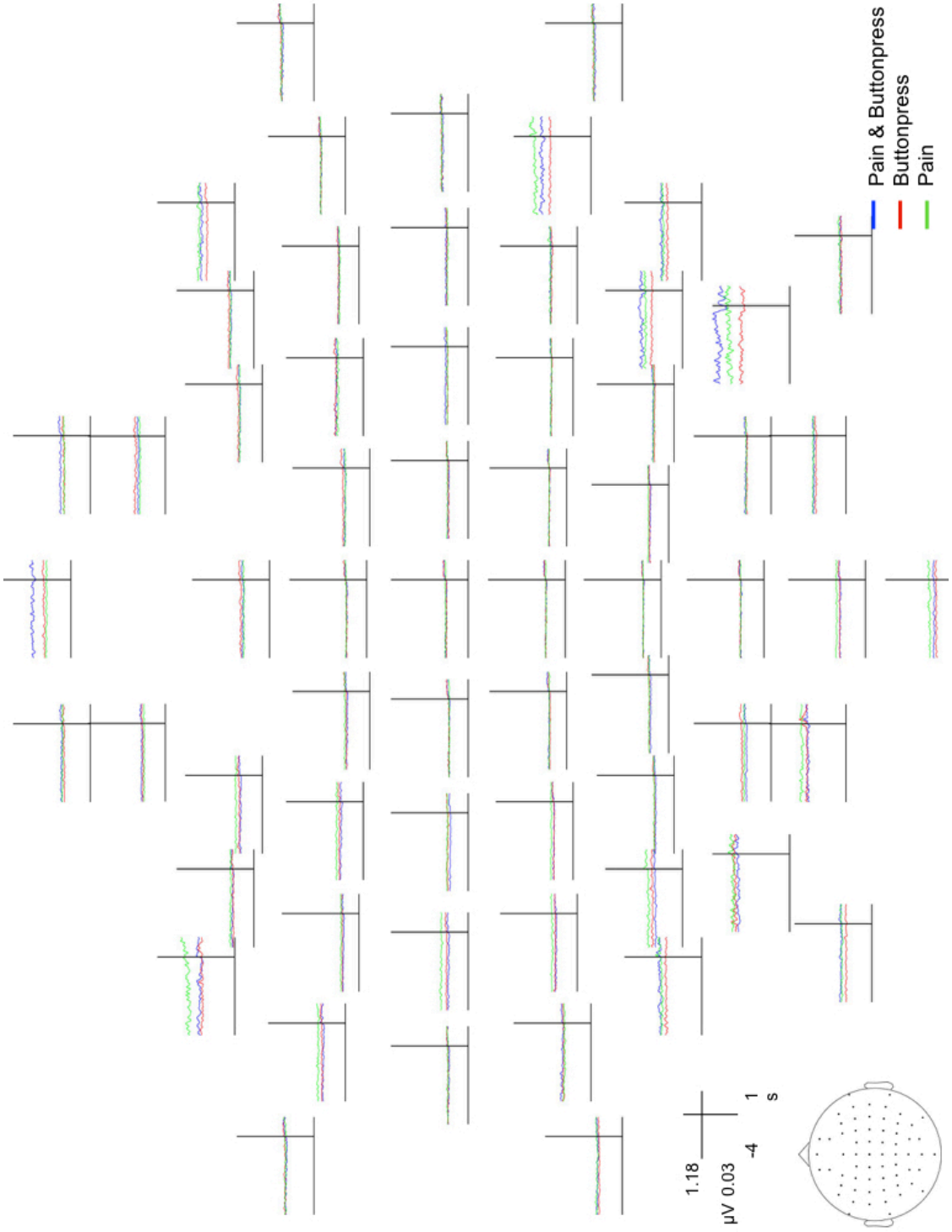

Figure 15: Grand average of gamma power (40-100 Hz) time courses.

Power evolution over time at each electrode site and for each experimental condition. No particular changes of power over time are observable.

respectively. Overall, we observed a decrease of power in the *pain & buttonpress* and in the *buttonpress* conditions, which was particularly pronounced at the alpha and beta band. We next assessed whether this power decrease was statistically significant and how this effect was topographically distributed (see 5.1.3.1). Next, we tested whether power changes over time at each frequency band statistically differed for the *pain & buttonpress* and the *buttonpress* condition (see 5.1.3.2).

5.1.3.1 Evolution of power over time

Results of the cluster-based comparisons between power during the initial and the final phase of motor preparation/thermal stimulation for each experimental condition are shown in Figure 16. Each row depicts the topographical distribution of the difference between early and late phase for the *pain & buttonpress*, *buttonpress* and *pain* condition, at the theta, alpha, beta and gamma band, respectively. Warm colors display brain areas where power was higher in the late phase compared to the early phase, while cold colors indicate areas where power was lower. Electrodes where changes of power were significant over time ($p < 0.025$) were marked with a bold black dot. Power decreased significantly over time in the theta band in the *buttonpress* condition, over a group of electrodes covering the sensorimotor areas. We detected a significant decrease of power over time in the alpha band in the *pain & buttonpress* and in the *buttonpress* condition, and in the beta band in each experimental condition. The effect was more pronounced contralaterally to the movement side in the *buttonpress* condition, contralateral to the stimulated hand in the pain condition, and bilaterally spread when both movement was performed and thermal stimuli were applied. In the gamma band power decreased over time in a cluster over electrodes widely spread over central and bilateral frontal regions. Taken together, these results confirm that motor preparation and pain

processing successfully engage brain areas involved in sensorimotor processes, as reflected by power desynchronization in the alpha and beta band.

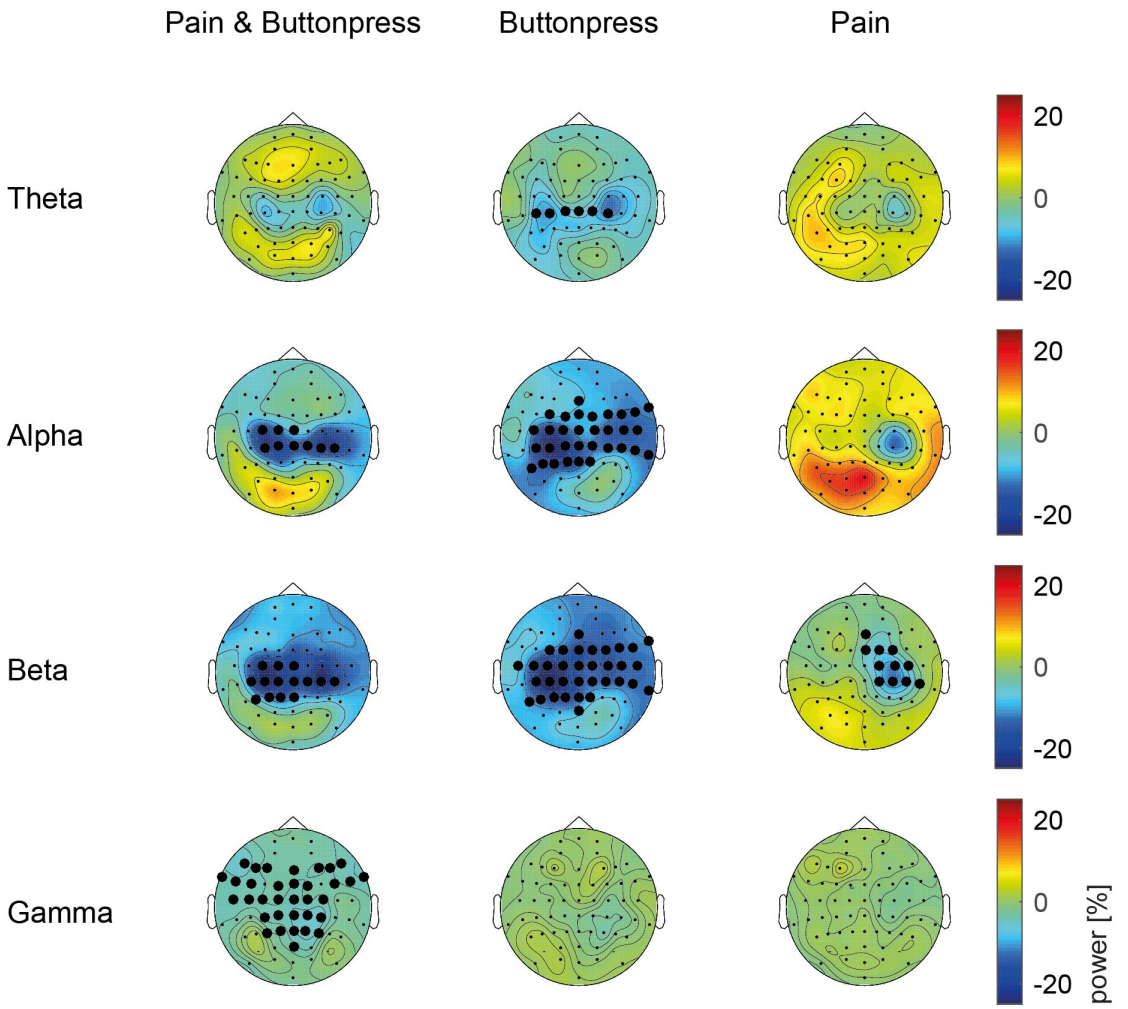

Figure 16: Topographical distribution of power changes over time.

The topographies show the results of the statistical comparisons between power activity during the initial and the final phase of motor preparation/thermal stimulation for each experimental condition at each frequency band. The most interesting results are detected in the alpha band in the *pain & buttonpress* and in the *buttonpress* condition, and in the beta band in each experimental condition. The decrease of power happens contralateral to the stimulation side or to the hand which performed the moving. Overall, the results suggest that motor preparation and pain processing recruit brain areas involved in sensorimotor processes.

5.1.3.2 Influence of pain on motor preparation

To specifically investigate the influence of a painful stimulus on motor preparation, we directly compared movement-related brain activity between the *pain & buttonpress* and the *buttonpress* conditions at theta (4-7 Hz), alpha (8-13 Hz), beta (14-30 Hz) and gamma (40-100 Hz) frequencies. We averaged frequency-specific power time courses across participants for each experimental

condition at a selection of electrodes covering the sensorimotor areas contralateral to the movement execution (Cz, CPz, C1, C3, CP1, CP3, see Figure 17A, that is where movement-related brain activity is expected to be more pronounced (Cheyne 2013; Pfurtscheller & Lopes da Silva 1999; van Wijk et al. 2012). As previously described, cortical activity desynchronized during the 2 s preceding the onset of the movement, especially at the alpha and beta band. Additional changes of brain activity after the execution of the movement were not further analyzed. However, movement preparatory brain activity did not significant differ between the two experimental conditions at any frequency band, neither when clustering across all the scalp electrodes nor when clustering across the selected electrodes (p>0.025) (see Figure 17B).

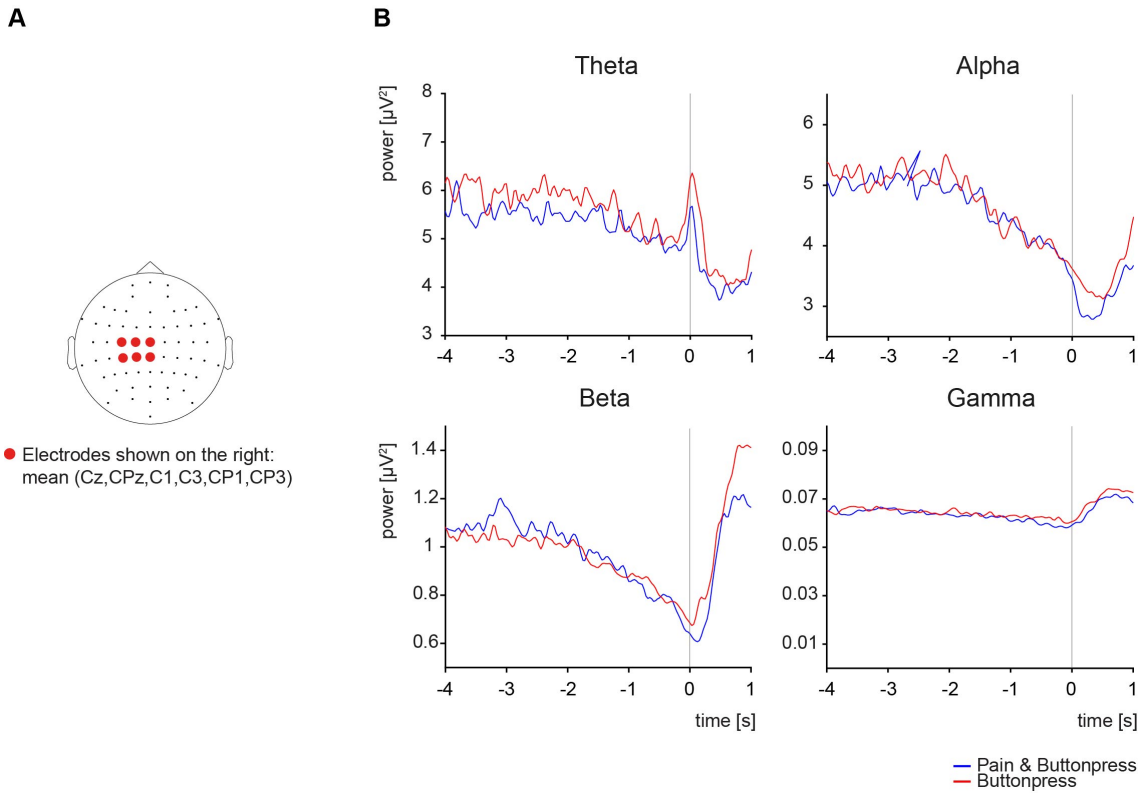

Figure 17: Statistical comparisons of power time courses of the Study 1.

A: Power time courses were averaged across a selection of electrodes (marked by red dots) covering the sensorimotor regions contralateral to movement preparation. B: We statistically compared time courses of frequency band specific brain activity during the last 2 s of motor preparation. We did not detect significant difference in movement preparatory brain activity between the *pain & buttonpress* and the *buttonpress* condition at any frequency band. Statistical analyses were performed using cluster-based permutation statistics (modified from Postorino et al., 2017).

5.2 Study 2

5.2.1 Behavioral results

We assessed whether depression, anxiety and individual locus of control were related to the individual warmth detection threshold. We therefore correlated questionnaires scores with the individual average temperature during the *warmth & buttonpress* condition. Again, no significant correlations were found ($p>0.05$). Likewise, no significant results ($p>0.05$), were detected when correlating the scores with the difference of the amplitude of the readiness potential in the *warmth & buttonpress* condition and in the *buttonpress* condition.

5.2.2 Modulation of the readiness potential is not pain-specific

To determine if the effect observed on the amplitude of the readiness potential were specifically attributable to pain, we analyzed the results from Study 2, in which participants had to perform a button press in response to a non-painful thermal stimulation. Brain activity was averaged time locked to the button press or to the temperature offset. We detected a pattern of movement-related potentials comparable to Study 1. Figure 18 shows the grand averages of the waveforms for each experimental condition of Study 2. Motor preparation is reflected by the readiness potential, which is followed by the motor potential time-locked to the onset of the movement and by the reafferent potential in the post-movement window. As before, since motor execution and temperature offset co-occurred at the same time, we analyzed exclusively brain activity preceding the onset of the movement. Moreover, we did not include the *warmth* condition in the following statistical comparisons, as no event-related potential were detected.

We compare the amplitudes of the readiness potential in the *warmth & buttonpress* and in the *buttonpress* condition to assess the effect of a non-painful thermal stimulation on motor preparation. Figure 19 shows that a warm stimulus significantly modulates the preparation of the movement in a time window ranging from -0.26 s to -0.19 before the onset of the movement ($p < 0.025$), inducing a decrease of the amplitude of the readiness potential in the *warmth & buttonpress*

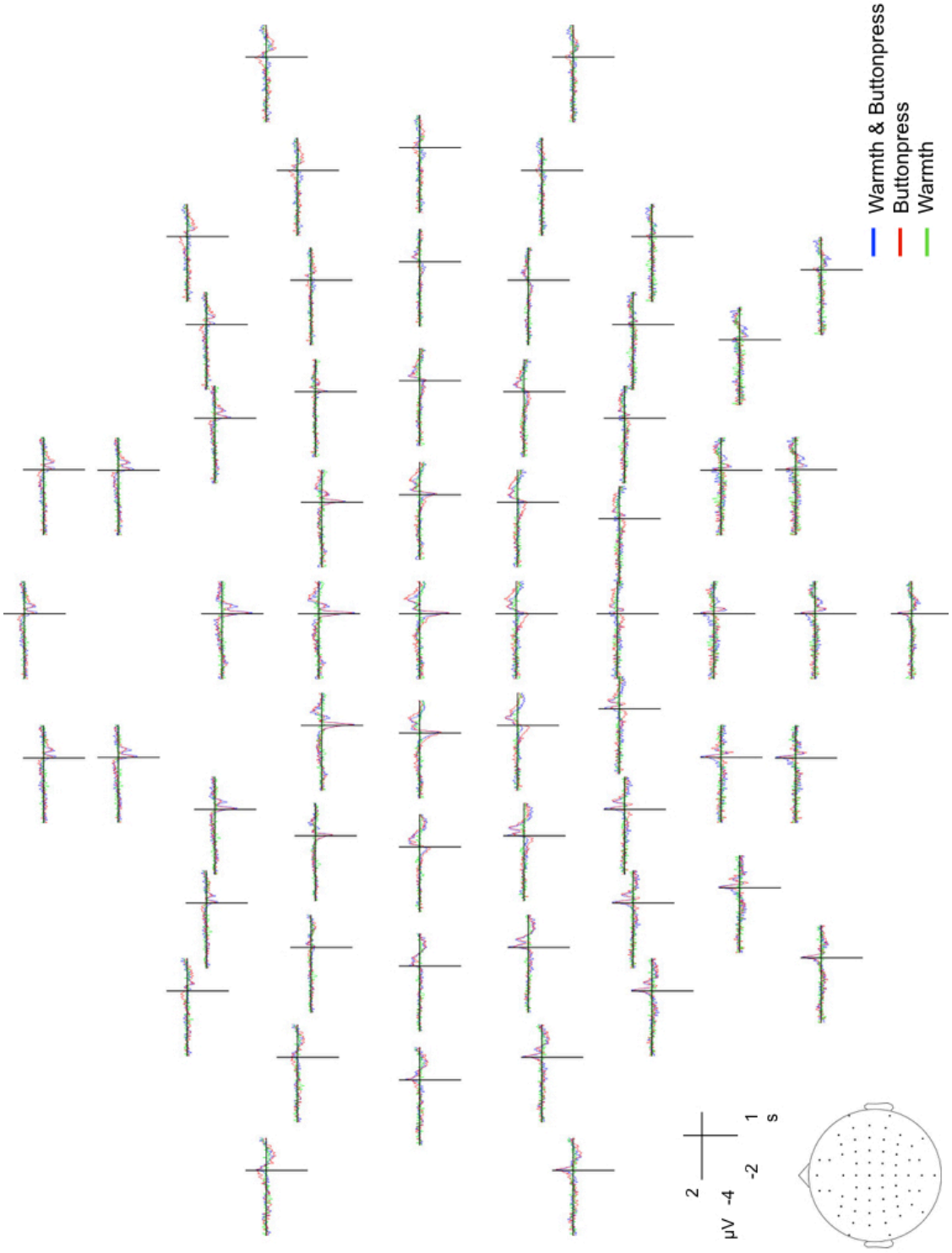

Figure 18: Grand average of the ERP waveforms of the Study 2.

Distribution of the movement-related potentials at each electrode site for each experimental condition. The onset of the movement is preceded by the readiness potential, a negative wave distributed principally at fronto-central electrodes. We did no detect movement-related potentials in the *warmth* condition, as no movement was performed.

condition. This effect was mainly localized over central and posterior electrodes (Figure 19A, p<0.025).

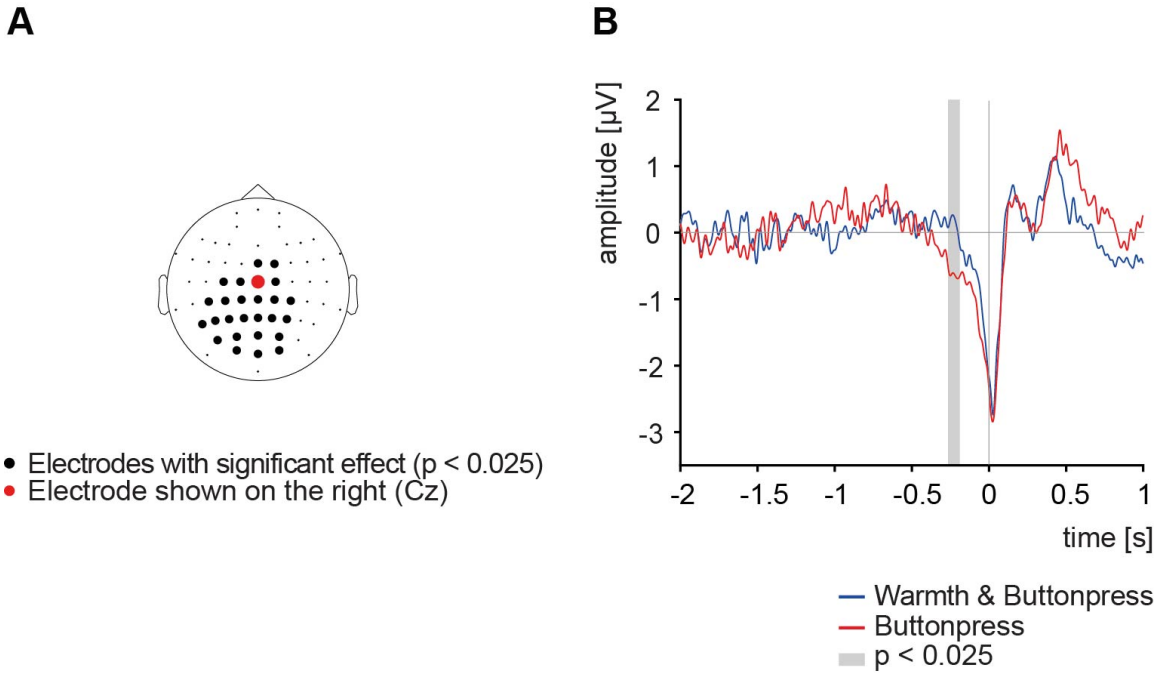

Figure 19: Statistical comparisons of movement-related potentials of the Study 2.

A: The topography shows electrodes where significant differences between the experimental conditions were detected. B: Movement-related potentials at electrode Cz. Using cluster-based permutation statistics in a time window covering the last 2 s of motor preparation, we observed a significant difference between the amplitude of the readiness potential in the *warmth & buttonpress* and the *buttonpress* conditions, as marked by the gray shaded area (modified from Postorino et al., 2017).

Finally, to evaluate whether pain and warmth differentially modulate motor-related brain activity, we contrasted the difference of the readiness potential amplitude between the *pain & buttonpress* and *buttonpress* conditions in the Study 1 with the difference of the readiness potential amplitude between the *warmth & buttonpress* and *buttonpress* conditions in the Study 2. Figure 20 shows the time course of the difference of the amplitude of the readiness potentials between experimental conditions. We did not detect any significant difference, that is both a painful and a non-painful stimulation induce in a similar way a decrease of the amplitude of the readiness potential compared to the *buttonpress* condition. Taken together, these findings indicate that pain and warmth impact movement preparatory brain activity in a comparable way, suggesting a non-pain-specific effect.

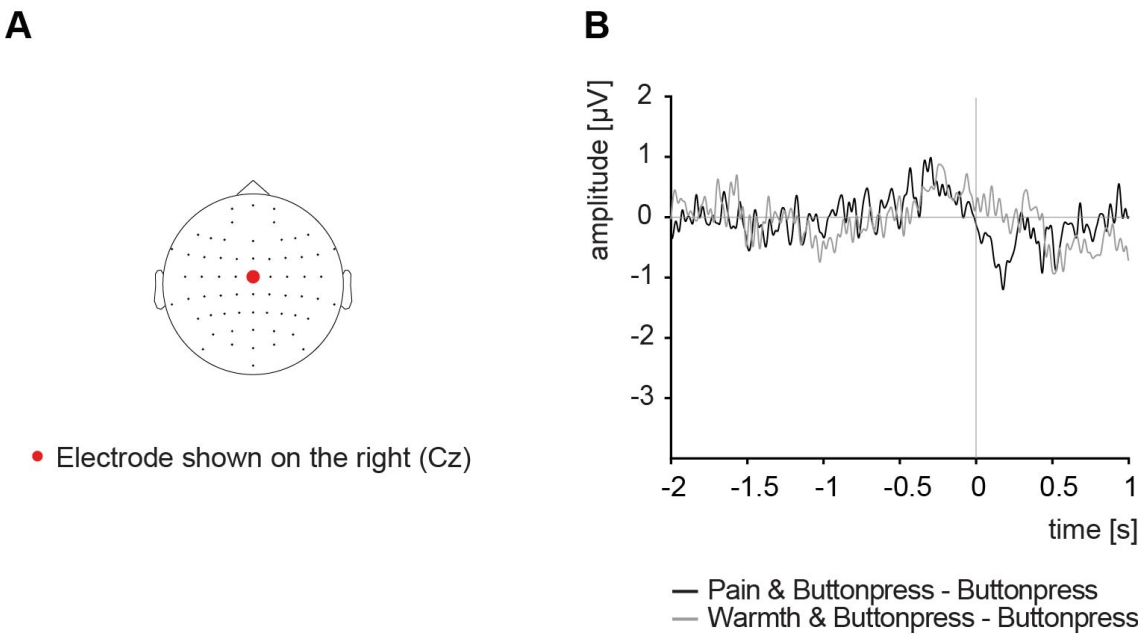

Figure 20: Comparison of the results of Study 1 and Study 2.

A: we calculated the difference of the readiness potentials between the experimental conditions for Study 1 and Study 2 and contrasted against each other both across all electrodes and at Cz only (marked by a red dot). **B:** Cluster-based permutation statistics during the last 2 s of motor preparation yielded no significant results, suggesting that pain and warmth affect motor preparation in a similar way (modified from Postorino et al., 2017).

6 Discussion

The current research project was designed to investigate the influence of pain on motor preparation in the human brain. We hypothesized that pain is fundamentally linked to motor preparation, and that it could be reflected by a modulation of movement-preparatory brain activity. The results of the first study showed that a movement directed to interrupt a painful stimulation is associated with a reduced amplitude of the readiness potential compared to a movement performed without concomitant pain. However, our second study revealed that a similar modulation of preparatory brain activity occurs, when a movement is directed to interrupt a non-painful thermal stimulation. Taken together, our findings suggest that the influence of pain on movement-related brain activity reflected by the readiness potential is not specific to pain, but might rather represent a modality-spanning phenomenon.

6.1 Pain modulates movement preparatory brain activity

Pain is a complex phenomenon, which comprises several dimensions, primarily the sensory, cognitive, affective and motivational ones. Yet pain crucially relies on behavioral responses to fulfill its fundamental protective function. Indeed, pain signals potential harm and threat to the organism; as a result, it motivates decisions and actions directed to prevent further injury, to avoid the source of danger, to minimize overall the experience of pain (Fields, 2006; Sullivan, 2008; Wiech & Tracey, 2013). Despite several lines of evidence emphasizing the tight connection between pain and motor processes (see 2.1), a comprehensive model of their interaction in the human brain is still lacking. Significant evidence of functional interaction of pain and motor processes in the human brain are provided by anatomical (Dum et al., 2009; Picard & Strick, 1996, Iwata et al., 2005) and neuroimaging (Misra & Coombes, 2014; Perini et al., 2013) studies, which identified the anterior midcingulate cortex (aMCC) as a core region where information about pain are redirected to motor centers to guide behavior (Shackman et al., 2011). In our research project, we used EEG to investigate

whether electrical brain activity related to movement preparation could be modulated by concomitant pain. Precisely, we expected to detect changes of specific measures of movement preparation, such as the readiness potential (Brunia et al., 2012; Colebatch, 2007; Shibasaki & Hallett, 2006) and the desynchronization of power in the alpha and beta range (Cheyne, 2013; Neuper & Pfurtscheller, 2001; Pfurtscheller & Lopes da Silva, 1999; van Wijk et al., 2012). We found that the amplitude of the readiness potential was reduced when a movement was performed to interrupt a painful stimulation, compared to a movement performed without pain (see 5.1.2). To the very best of our knowledge, no previous studies investigated the effect of a painful stimulation on the readiness potential. Piedimonte et al. (2017) investigated how the contingent negative variation (CNV), whose late component likely reflects motor preparation (Brunia et al., 2012), could be modulated by different expectations of pain. Similar to our study, they implemented a paradigm where a painful stimulation could be interrupted by a motor response. Interestingly, the motor performance and the late component of the CNV were not affected by different expectations of pain when the intensity of the stimulation was kept constant. In line with our hypothesis, it might suggest that, when confronted with potentially threatening stimuli, the motor system might be inherently prepared to rapidly react to efficiently avoid pain, regardless of expectations. However, that study cannot be directly compared to ours as it primarily assessed the influence of expectations on a different measures of motor preparation, that is the CNV, whereas we investigated the direct effect of pain on the readiness potential. Further studies demonstrated that the amplitude of the vertex potential elicited by salient stimuli (e.g. painful stimuli) predicts response times of defensive behavioral responses (Moayedi et al., 2015) and modulation of the applied force (Novembre et al., 2018). Although these studies do not directly assess the effect of pain on motor-related brain responses, they clearly indicate that cortical activity elicited by salient stimuli has an impact on the motor system to prepare appropriate protective responses, thus strengthening the hypothesis that sensory and motor processes are tightly linked in the human brain.

As regards brain oscillatory activity, we did not detect significant effects of pain on motor-related desynchronization in the alpha and beta band. In agreement with previous findings, we observed a contralateral decrease of alpha

and beta power in response to both a painful stimulation (Giehl et al., 2014; Nickel et al., 2017; Schulz et al., 2015) and to motor preparation (Cheyne, 2013; Crone et al., 1998; Pfurtscheller & Lopes da Silva, 1999; van Wijk et al., 2012) as an indicator of cortical activation (see 5.1.3.1). Interestingly, the decrease of power was spread bilaterally over the sensorimotor areas when pain perception on one hand and movement preparation on the other hand co-occurred. However, time courses of brain activity in the alpha and beta band did not significantly differ when a movement was performed in response to a painful stimulation, compared to a movement performed without pain (see 5.1.3.2). So far, few studies investigated changes of brain oscillatory activity in response to concomitant pain and motor processes. In a series of studies, Babiloni et al. (2006, 2008, 2010, see Babiloni et al., 2014 for a comprehnsive review) assessed changes of alpha desynchronization during the anticipation of a painful stimulus and a motor response. Their results revealed that expecting a sensorimotor interaction enhanced movement-related alpha desynchronization. However, as both the painful stimuli and the motor responses were following an initial warning stimulus, it was not possible to separate movement preparation from anticipation processes. Moreover, motor responses were performed simultaneously to the painful stimulation, and did not have an effect on it. Our research instead investigated specifically the effects of pain on the preparation of biologically meaningful motor responses intended to interrupt the concomitant aversive stimulation.

In another recent study (Misra et al., 2017), movement-related brain activity was analyzed in response to voluntary movements performed during ongoing pain, ongoing warmth or without concurrent stimuli. Concomitant pain led to a reduction of reaction times along with an enhancement of movement-preparatory beta desynchronization in brain regions encompassing the premotor cortex and supplementary motor area, indicating a facilitating effect of pain on the motor system. In contrast to these findings, beta desynchronization was not modulated by concomitant pain in the present study. Some differences between the studies could account for the lack of an effect. Firstly, in our study participants performed biologically meaningful motor responses which had an effect on the ongoing stimulation, whereas in the previous studies there was no functional relationship between the movement and the painful stimulus. Moreover, in the

study from Misra et al. (2017), movements were externally cued by an auditory stimulus presented at a fixed interval after the onset of the heat stimuli, whereas participants in our study could freely execute button presses at any time. Due to these differences, the studies cannot be entirely compared to each other.

In summary, the results from our first study show that protective motor responses are associated with less preparatory brain activity than similar, but non-protective motor behaviors, as reflected by a reduction of the amplitude of the readiness potential. This reduction of voluntary motor preparation directly before a motor response might indicate that motor preparation occurs involuntarily and continuously during pain. Taken together, these results support the hypothesis that motor preparation represents an inherent part of pain processing in the human brain.

6.2 The link between pain and motor preparation is not pain-specific

Human behavior is triggered and regulated by a wide variety of stimuli present in the surrounding environments, e.g. visual, auditory and somatosensory ones. They signal particular circumstances in the environment and require the individuals to act upon them. Among those stimuli, painful stimuli have a preeminent relevance in signaling potential threats and driving appropriate protective responses. We therefore hypothesized that pain and motor processes are tightly wired in the human brain in a modality-specific manner and tested this hypothesis in a second experiment, in which we evaluated whether modulation of movement preparatory brain activity occurred also in association with non-painful thermal stimuli. Our results indicate that the amplitude of the readiness potential is similarly reduced when a movement is performed to interrupt a non-painful thermal stimulus, suggesting that the observed effect is not pain-specific, but it rather represents a modality-spanning phenomenon (see 5.2.2).

Some studies reported modulation of response time (Miller et al., 1999a), movement force (Miller et al., 1999b) and preparatory brain responses (Minelli et al., 2007) as a function of the intensity of visual and auditory stimuli. However, these findings are hardly comparable to our results as the studies were primarily designed to investigate the effect of physical properties of the stimuli on motor

preparation, regardless of any biologically meaningful impact on motor performance. Recent evidence highlights that both somatosensory and auditory stimuli modulate movement parameters and cortical responses in a comparable way, suggesting a supramodal mechanism regulating reactions to salient sensory stimuli (Novembre et al., 2018). Movement during both painful and non-painful stimulation has been found to engage similar cortical areas (Perini et al., 2013), confirming that pain activates a network of multimodal areas, which all eventually contribute to shape behavior. Thus, our results are in line with previous findings showing a non-specific interaction between pain and motor processes in the human brain.

Although specific interactions remain to be demonstrated, our results support the view that cortical pain processing comprises the generation and regulation of voluntary motor responses, as action constitutes undoubtedly an integral part of the pain experience.

6.2.1 Alternative explanations

According to our results, the amplitude of the preparatory readiness potential was reduced when a movement was performed in response to both a painful and a non-painful stimulation. It can be thus hypothesized that variables other than pain play a crucial role in modulating the amplitude of the readiness potential.

The presence of any sensory stimulus regardless of its salience might interact with attentional processes and re-direct the attention from the preparation of the movement to the stimulus itself. This altered attention could in turn influence the readiness potential (Birbaumer et al., 1990).

Alternatively, our observations could be explained by differences in the experimental tasks. In fact, although participants could freely determine when to stop the stimulation, this decision was influenced by the external stimuli in both the painful and the non-painful conditions. In contrast, in the corresponding *buttonpress* conditions the timing of the motor responses was internally generated, while no concomitant stimulation was present. Previous studies reported a difference in movement preparatory brain activity between internally generated and externally driven movements (Cunnington et al., 2002; Gerloff et

al., 1998; Jahanshahi et al., 1995; Jankelowitz & Colebatch, 2002). Interestingly, the amplitude of the readiness potential was significantly reduced for externally paced compared to for self-paced movements (Jahanshahi et al., 1995; Jankelowitz & Colebatch, 2002), suggesting that when the decision about the timing of the movement is externally cued and not internally generated, the readiness potential is attenuated as less voluntary control on the movement is involved. Our results are consistent with these previous findings, as they show that movement in response to an external stimulation, both painful and not, are associated with a reduced amplitude of the readiness potential. Thus, we cannot rule out that the differences highlighted by our study might instead reflect basic differences between stimulus-related and self-paced movements.

Lastly, individual differences in psychological traits as the locus of perceived control can influence the way pain is perceived and the ability to cope with the painful experience (Arntz & Schmidt, 1989; Pellino & Ward, 1998), as well as modulate the neural responses to pain (Salomons et al., 2004; Wiech et al., 2006). That can in turn have a considerable impact on the motor performance (Feldner & Hekmat, 2002); in patients suffering from chronic pain, dysfunctional behavior is often maintained as a consequence of the lack of sense of control over pain (Jensen et al., 1991; Scharff et al., 1995). Thus, the way that pain modulates movement preparatory brain activity could differ between individuals with internal or external locus of control. Specifically, individuals who experience a low control on the ongoing painful stimulation, might likewise decrease their voluntary control on the motor response, and the preparatory readiness potential will be attenuated as a consequence. Therefore, we assessed individual locus of control by means of the Internality, Powerful Others, and Chance Scales (Levenson, 1981) and found no correlation between the individual scores and the difference of the amplitude of the readiness potential in the *pain and buttonpress* and in the *buttonpress* conditions (see 5.1.1). Thus, our findings do not support the view that the individually perceived locus of control has a relevant influence on the modulatory effect of pain on movement preparatory brain activity.

6.3 Limitations

We are aware that our studies have several limitations, which could have influenced the obtained results.

First, the experimental conditions were always performed following a fixed order. That was done to ensure that the length of trials in the *buttonpress* conditions and the ramping of the stimulation in the *pain/warmth* condition were always comparable with the *pain & buttonpress* condition. However, the fixed order entails the risk of an order effect, i.e. the effect of an experimental condition could carry over influencing the performance in the following condition, or the performance could improve over time due to practice (Shaughnessy et al., 2014). According to the effects of repetition in sensory (Grill-Spector et al., 2018) and motor (Hamilton & Grafton, 2009) systems, repeated movements are expected to be associated with an attenuation of related brain activity. Similarly, an effect of practice is reflected by a decreased amplitude of the readiness potential (Lang, 2003). However, in our study the repeated motor performance was associated with an increase of movement-preparatory brain activity over time. It is therefore unlikely that our findings can be explained by an order effect.

Second, movement kinematics were not assessed in our study. Previous studies reported that several factors including force, speed or precision of the movement could have an impact on motor-related brain activity (Lang, 2003; Shibasaki & Hallett, 2006). Thus, we cannot rule out that differences in movement characteristics could account for the observed difference between movement-preparatory brain activity with and without concomitant stimulation. Moreover, we did not collect subjective ratings of the perceived intensity of the thermal stimulation. In this way, it was not possible to determine whether the effect of pain on preparatory brain activity could vary as a function of different levels of pain intensity.

Lastly, based on the present data, we cannot precisely localize where in the brain pain modulates the preparation of movements. We were most interested in differences of brain activity across conditions rather than in the location of brain activity in certain conditions. Considering the inherent limitations of source modelling of EEG data we have deliberately decided not to perform source localizations on our data. The precise location of the observed effects remains

therefore to be determined by other imaging techniques. Moreover, despite the lack of pain-specific effects on motor preparation and the lack of effect on alpha and beta power in the present study, such effects could still exist and be more adequately identified by means of other approaches such as connectivity between brain regions implicated in complex sensorimotor interactions. Such effects could also occurs at other location such as subcortical regions which are difficult to localize by means of EEG.

6.4 Conclusions and future perspectives

In our research project, we used EEG to investigate whether and how pain influences the preparation of a biologically meaningful motor responses in the human brain. In a first study, our participants performed button presses in response to a painful heat stimulation as well as without concurrent stimulation. The results from this study show that protective motor responses are associated with attenuated preparatory brain activity, likely indicating that motor preparation occurs involuntarily and continuously during pain. However, the results from a second study in which participants performed comparable motor responses to a non-painful thermal stimulation revealed a similar modulation of the preparatory readiness potential, suggesting that the modulation of movement preparatory brain activity is a modality-spanning phenomenon.

Future work needs to be performed to demonstrate pain-specific interactions between pain and motor preparation. An integrated research approach should conceptualize pain as a multifactorial experience which includes perceptual, emotional and motor components. The motor dimension should be acknowledged as central to the pain experience as pain alters movement and movement influences the way pain is experienced. In this view, a better understanding of how pain and motor brain networks are engaged and interact with each other is crucial for developing novel rehabilitative strategies for pain management.

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List of figures

List of abbreviations

ACC	Anterior cingulate cortex
aMCC	Anterior midcingulate cortex
CNV	Contingent negative variation
DC	Direct current
EEG	Electroencephalography
ERP	Event-related potential
fMRI	Functional magnetic resonance imaging
LEP	Laser evoked potential
MEG	Magnetoencephalography
MRP	Movement-related potential
PAG	Periaqueductal grey
PET	Positron emission tomography
RVM	Rostral ventromedial medulla
SD	Standard deviation
SMA	Supplementary motor area
STT	Spinothalamic tract

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