Simultaneous Assessment of Spinal and Supraspinal Activity during Experienced Pain

An Alternative Approach using Information Theory

Arguissain, Federico

DOI (link to publication from Publisher):
10.5278/vbn.phd.med.00027

Publication date:
2015

Document Version
Publisher's PDF, also known as Version of record

SIMULTANEOUS ASSESSMENT OF SPINAL AND SUPRASPINAL ACTIVITY DURING EXPERIENCED PAIN

AN ALTERNATIVE APPROACH USING INFORMATION THEORY

BY
FEDERICO GABRIEL ARGUISSAIN

DISSERTATION SUBMITTED 2015

AALBORG UNIVERSITY
DENMARK

SIMULTANEOUS ASSESSMENT OF SPINAL AND SUPRASPINAL ACTIVITY DURING EXPERIENCED PAIN

AN ALTERNATIVE APPROACH USING INFORMATION THEORY

Ph.D. thesis by

Federico Gabriel Arguissain

AALBORG UNIVERSITY
DENMARK

Thesis submitted:	August 25, 2015
PhD supervisor:	Associate Prof. Carsten Dahl Mørch, Aalborg University
Ph.D. co-supervisor:	Prof. Ole Kæseler Andersen, Aalborg University
PhD committee:	Associate Professor Laura Petrini (chairman) Aalborg University
	Dr. André Mouraux Université catholique de Louvain
	Dr. Marcelo Montemurro University of Manchester
PhD Series:	Faculty of Medicine, Aalborg University

ISSN (online): 2246-1302
ISBN (online): 978-87-7112-352-4

Published by:
Aalborg University Press
Skjernvej 4A, 2nd floor
DK – 9220 Aalborg Ø
Phone: +45 99407140
aauf@forlag.aau.dk
forlag.aau.dk

Printed in Denmark by Rosendahls, 2015

CV

Federico Gabriel Arguissain was born in Concepción del Uruguay, Argentina in 1985. He obtained his degree as Bioengineer in 2010 at the Faculty of Engineering of the National University of Entre Ríos (Argentina). His main areas of research are biomedical signal processing with focus on electroencephalography and electromyography in the study of pain and rehabilitation.

PREFACE

This Ph.D. thesis is the result of work carried out at the SMI center, Aalborg University between February 2011 and August 2015. During the period between October 2012 and April 2013 the work was performed at the University College of London as part of collaboration between these institutions.

ENGLISH SUMMARY

Pain research in humans has systematically involved the application of different experimental painful stimuli and the assessment of the elicited responses in order to investigate mechanisms of pain processing and the efficacy of treatments. Particularly, applying electrical stimulation to the skin elicits two synchronous electrophysiological responses that reflect spinal and supraspinal sensory processing: the nociceptive withdrawal reflex (NWR) and the somatosensory evoked potentials (SEPs). These responses have been traditionally assessed using features that are measured from the averaged signals across several repetitions of the eliciting stimulus (i.e. across trials). The averaging procedure has been typically applied to reduce the inherent across-trial variability of these two responses with the purpose of improving their signal-to-noise ratios. However, an increasing body of work suggests that across-trial variability should be considered by researchers not as a source of noise, but as a functional property of the nervous system that could index modulatory effects, task performance and different clinical conditions. In this Ph.D. project, the Information Theory (IT) framework is proposed as a viable approach to integrate single-trial data and to characterize signal variability which may be useful to analyze simultaneous spinal and supraspinal responses and to provide more insight about the mechanisms involved in pain processing.

In line with this, the main objectives of the present dissertation were to investigate the feasibility of using single-trial values extracted from both NWR and SEPs and to introduce IT as an alternative approach to assess these simultaneous spinal and supraspinal signals.

Study I assessed the level of agreement between two automatic methods and two human observers in the detection and estimation of single-trial SEP features. Study II quantified the amount of information about graded electrical stimulation that is carried by NWR and SEP features. Furthermore, the information carried jointly by pairs of these features was also assessed. Study III assessed the modulation exerted by two cognitive tasks over SEPs and the NWR during repeated electrical stimulation. Results emphasized the importance of the selection process of single-trial detection/estimation methods within the particular experimental protocol. Furthermore, it was shown that the IT framework can be used to quantify the information carried by NWR and SEP features simultaneously. Finally, it was found that the cognitive modulatory tasks were accompanied by changes in the variability of the NWR and SEPs, and this was reflected by differences in the amount of information they carried over repeated presentations of the stimulus.

In conclusion, the IT framework is an appropriate and promising methodology to quantify the relation between spinal and supraspinal activity in pain research.

DANSK RESUME

Human smerteforskning har systematisk anvendt forskellige eksperimentelle smertefulde stimuli og vurdering af de fremkaldte smerteresponser til at undersøge de mekanismer, der ligger til grund for smerter, samt virkningen af behandlingerne. For eksempel er der anvendt elektrisk stimulation på huden, som udløser to synkrone elektrofysiologiske responser, der afspejler spinale og supraspinale sensoriske processer: den nociceptive afværgerefleks (NWR) og somatosensorisk evokerede potentialer (SEP). Traditionelt er disse blevet vurderet som gennemsnittet af signalerne over adskillige gentagelser af stimuli (f.eks. over flere forsøg). Denne procedure er typisk anvendt for at reducere den naturlige variabilitet i de to responser på tværs af forsøgene for at forbedre deres signal-støj forhold. Et stigende antal studier viser dog, at variabilitet på tværs af forsøg ikke skal anses som en kilde til støj men som en funktionel egenskab i nervesystemet, som kan indeksere modulatoriske effekter, opgaveudførelse samt forskellige kliniske tilstande. Denne ph.d.-afhandling foreslår en ramme af informationsteori (IT) som en realistisk tilgang til at integrere data fra enkeltforsøg og karakterisere signalvariabilitet, hvilket vil kunne anvendes til at analysere samtidige spinale og supraspinale responser og til at give mere indsigt i de mekanismer, der ligger til grund for smerter.

I henhold til ovenstående var hovedformålet med denne afhandling at undersøge muligheden for at bruge værdier fra enkelt-forsøg fra både NWR og SEP samt at introducere IT som en alternativ tilgang til at vurdere disse samtidige spinale og supraspinale reflekser.

Studie I vurderede niveauet af overensstemmelse mellem to automatiske metoder og to observatører ved påvisning og vurdering af SEP-karakteristika fra enkelt-forsøg. Studie II kvantificerede mængden af information om graderet elektrisk stimulation, som bæres af NWR- og SEP-træk. Endvidere blev de informationer, der bæres i fællesskab af par af disse funktioner, vurderet. Studie III vurderede den modulation, som påvirkes af to kognitive opgaver over SEP og NWR under gentagen elektrisk stimulation. Resultaterne understregede vigtigheden af udvælgelsesprocessen af vurderingsmetoder til detektion i enkelt-forsøg. Endvidere blev det påvist, at IT-rammen kan anvendes til at kvantificere de informationer, der inderholdes i NWR- og SEP-karakteristika samtidigt. Endelig blev det påvist, at kognitive modulatoriske opgaver ledsages af ændringer i variabiliteten af NWR og SEP, hvilket blev afspejlet i forskellene mellem den mængde information, de inderholdte over gentagne påføringer af stimulussen.

Det kan konkluderes, at IT-rammen er en passende og lovende metode til kvantificering af spinal og supraspinal aktivitet inden for smerteforskning.

ACKNOWLEDGEMENTS

I would like first to express my sincere gratitude and appreciation to my supervisors, whose guidance and support throughout this Ph.D. work were invaluable to me. I would thus like to thank Ole Kæseler Andersen for the shared knowledge, our fruitful discussions but mostly for his endless patience and understanding. My deepest appreciation goes also with Carsten Dahl Mørch for his positive advice, the constructive criticism and for always finding the time to help me even when his schedule was really busy. I feel very fortunate for having the chance of being guided by these outstanding professionals and amazing persons.

I am very grateful also to Giandomenico Iannetti and his research group from University College of London for giving me the opportunity to share their working environment and to make me feel like member of their group during my stay in the United Kingdom. It was an incredible chance to learn new ways of doing research while also sharing funny moments that I really appreciated.

My heartfelt gratitude goes to my colleagues from SMI who have supported me during this learning process at Aalborg University. Mostly I would like to thank my colleague and friend José Biurrun Manresa, who is a *sine equa non* of this thesis. He has been next to me since the first day I arrived to Denmark and is still supporting me. We have shared long working hours which were always full of joy, laughter and accompanied by thousands of liters of our Argentinean drink, yerba mate. I could not ask for more from a friend. I would also like to express my gratitude to all the colleagues from our research group for their professional advice, their valuable feedback and also the amusing moments we had during our group meetings. I would like to give a big thanks to the technical and administrative SMI staff for their immense support and willingness to help at any time. It is heartwarming to stumble upon this kind of persons in life and I feel blessed for that.

I wish to thank my family from the bottom of my heart; my mom Patricia, my dad Gustavo and my sister Constanza for their love and immense support despite the distance. Finally, I wish to thank my partner, my other half, the woman who left all behind to follow me, my beloved wife Victoria. This thesis is dedicated to her.

F.G.A., August 2015

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

EEG	electroencephalography
ERPs	event-related potentials
EMG	electromyography
fMRI	functional magnetic resonance imaging
IT	information theory
LoA	limits of agreement
MEG	magnetoencephalography
MI	mutual information
NWR	nociceptive withdrawal reflex
PAG	periacqueductal gray
PTh	pain threshold
RMS	root-mean square
RRF	reflex receptive field
RTh	reflex threshold
RVM	rostroventral medulla
SEPs	somatosensory-evoked potentials
SNR	signal-to-noise ratio
TA	tibialis anterior

CHAPTER 1. INTRODUCTION

Chronic pain is a serious burden for society that implies suffering for patients and high economical costs to the health care system. In Denmark, it is estimated that chronic pain has a prevalence of approximately 20% within the adult population (Sjøgren et al., 2009). Similar values of prevalence of chronic pain have been found across Europe with reported percentages 15-30% (Breivik et al., 2006; Raftery et al., 2011; Häuser et al., 2013). Chronic pain has also been associated with a negative impact on the quality of social and working activities. Altogether, the importance of the study of pain and the translation of new knowledge to improve diagnosis and treatment is clear.

As stated by the definition given by the International Association for the Study of Pain (IASP), pain is a multidimensional experience that involves various sensory and emotional aspects. These observations have led researchers to apply various experimental pain modalities in order to investigate the different aspects of pain and the efficacy of pain relieving drugs (Arendt-Nielsen, 2007). This multi-modal approach comprises the assessment of pain responses that are evoked by different stimuli (e.g. mechanical, electrical, thermal, and chemical). Among these modalities, electrical stimulation has certain characteristics that made it a widely used technique in pain research. Electrical stimulation applied to the skin elicits synchronous electrophysiological responses that can be recorded non-invasively along the neural axis. Particularly, two commonly assessed responses are the nociceptive withdrawal reflex (NWR) and the somatosensory evoked potentials (SEPs).

The NWR is a spinal polysynaptic reflex elicited by noxious stimuli that evokes an involuntary withdrawal of the limbs in order to avoid potential tissue damage (Andersen, 2007). The elicitation and recording of the NWR is a standard electrophysiological technique regularly used in the study of spinal nociceptive pathways in both pharmacological and non-pharmacological interventional studies of acute pain and in healthy volunteers (Sandrini et al., 2005). Recently, the NWR has been used as a tool in human research for the assessment of spinal nociceptive excitability in chronic pain patients (Neziri et al., 2010; Lim et al., 2011, 2012; Biurrun Manresa et al., 2013).

The SEPs are transient responses observed in the continuous electroencephalographic (EEG) recordings elicited by a stimulus applied to the skin (Babiloni et al., 2004; Garcia-Larrea, 2006; Perchet et al., 2008). When elicited by high-intensity electrical stimulation, SEPs reflect the concomitant activation of both nociceptive and non-nociceptive fibers (Garcia-Larrea, 2006). This lack of specificity led to a gradual decrease on the use of electrical stimulation for assessment of nociceptive pathways in clinical settings in the past years, being replaced by other more nociceptive-specific stimuli such as those generated by

lasers (Cruccu et al., 2008). Nevertheless, recent studies that focused on the cognitive modulation of SEPs in particular, and event-related potentials (ERPs) in general, suggest that ERPs reflect mostly processes of detection and reorientation of attention to sensory stimuli rather than the modality of the stimulation (Legrain et al., 2012). These findings have given a new perspective of the functional significance of ERPs, suggesting that they reflect cortical processes related to detection of potential threats to the body.

The quantitative assessment of the NWR and SEP responses has traditionally included several features extracted from these signals. For the NWR, it usually involves the onset latency, amplitude, area under curve and root-mean square amplitude (RMS) among others. With regards to ERPs, the detection of features such as peak amplitudes and latencies is the most common and straightforward procedure. Regardless of the type of feature being used, these measurements are usually performed in the averaged responses across trials. Across-trial averaging in the time domain (Dawson, 1954) is usually carried out in the analysis of ERPs to increase their signal-to-noise ratio (SNR). Although SNR is not a major concern in the NWR, across-trial averaging is still widely used to get rid of the trial-to-trial variability observed in the single-trial traces. However, this technique has its drawbacks (Mouraux and Iannetti, 2008). Across-trial averaging does not take into account across-trial variability of these relevant features, which could contain valuable information in relation to the stimulus (Iannetti et al., 2005) and/or related to other modulatory effects (Lazzaro et al., 1997; Edwards et al., 2001; Jarchi et al., 2011b). Therefore, the averaging process can lead to a loss of vital information that could help understanding the different processes underlying these physiological responses. To overcome these issues, new single-trial methods have been proposed (Parra et al., 2002; Hu et al., 2010; Pernet et al., 2011), which provide additional information about brain mechanisms that could not be observed by only analyzing the averaged data.

One approach that has been previously used for studying the way in which the brain encodes sensory information is Information Theory. The concept of mutual information (MI) developed in the mathematical theory of communication (Shannon, 1948) has been applied on the analysis of spikes trains in single neurons (Optican and Richmond, 1987; Panzeri et al., 1999). Its application to other electrophysiological signals has recently started to be investigated (Magri et al., 2009; Ostwald et al., 2010), and its capabilities to extract the most informative features of a signal makes it a potential tool for studying different aspects of the neural activity. The information theory framework can be adopted to quantify the relation of simultaneously acquired signals from different modalities by taking into account the experimentally observed stimulus–response signal probability distributions. Mutual information can reveal which signal features are most informative regarding external stimuli or modulatory tasks, which signals are most

correlated to each other and which signals carry either the same or different information about the stimuli (Panzeri et al., 2008; Ostwald et al., 2010).

Few studies have combined NWR and SEPs (Willer et al., 1987; Dowman, 1991), probably due to difficulties in the simultaneous acquisition and proper quantification of both signals. The present work was intended to introduce alternative methods for the analysis of simultaneously acquired signals in order to evaluate the possibility of obtaining additional insight about the mechanisms behind sensory processing of nociceptive stimulation. The proposed approach may potentially allow a better characterization of the descending control mechanisms from the brain over the spinal structures during pain processing.

1.1. AIMS OF THE PH.D. PROJECT

The aims of the present Ph.D. project were: 1) to evaluate the use of single-trial analysis in the assessment of spinal and supraspinal activity in response to graded electrical stimulation and 2) to introduce MI as a new way to evaluate simultaneous spinal and supraspinal responses.

Therefore, the NWR was used as a measure of spinal activity and the SEPs as a measure of supraspinal activation, with the purpose of addressing the following specific research questions:

1. What are the expected differences when choosing automatic single-trial detection methods in comparison to human observers?
2. Is it possible to measure the mutual information carried by single-trial features extracted from both NWR and SEPs?
3. How is the interaction between single-trial features from NWR and SEPs in relation to the evoking stimulus?
4. Is it possible to derive a variability measure of NWR and SEP signals from MI?
5. How is the variability of NWR and SEP signals affected by cognitive modulatory tasks?

These questions were addressed in three main studies (Study I, II and III), published in three peer-reviewed scientific articles.

The three studies are:

Study I

Biurrun Manresa JA, **Arguissain FG**, Medina Redondo DE, Mørch CD, Andersen OK. On the Agreement between Manual and Automated Methods for Single-Trial Detection and Estimation of Features from Event-Related Potentials. *PLoS One* 10: e0134127, 2015.

Study II

Arguissain FG, Biurrun Manresa JA, Mørch CD, Andersen OK. On the use of information theory for the analysis of synchronous nociceptive withdrawal reflexes and somatosensory evoked potentials elicited by graded electrical stimulation. *J. Neurosci. Methods* 240: 1–12, 2015.

Study III

Arguissain FG, Biurrun Manresa JA, Mørch CD, Andersen OK, Iannetti GD. *Spinal and supraspinal responses show opposing modulatory effects during attentional tasks.* In preparation for *Journal of Neurophysiology.*

1.2. DISSERTATION OVERVIEW

The present thesis presents an evaluation of the use of single-trial measurements of simultaneous spinal and supraspinal responses with the objective of assessing the somatosensory pathways in humans and proposes mutual information as a methodology to objectively quantify their relationship.

The thesis is structured in four chapters, of which the first is the present introduction. Chapter 2 presents an overview of the different aspects related to the two electrophysiological signals that are subject of this thesis (NWR and SEPs), toghether with the current methodology to measure these two responses separately and simultaneously. Within this chapter, an evaluation of the agreement of different single-trial methods for the estimation of SEP responses is included (Study I). Chapter 3 first introduces IT as a framework to study concurrent spinal and supraspinal responses. Second, it demonstrates how IT can be used to quantify the information carried by NWR and SEP features about the evoking stimulus (Study II). Lastly, it proposes MI as a tool to quantify the variability of NWR and SEP signals and gives an example by examining the effect of attention on the variability of these two responses (Study III). Finally, a synthesis with future perspectives is presented in Chapter 4.

CHAPTER 2. CLASSICAL APPROACH FOR SOMATOSENSORY RESPONSE ANALYSIS

2.1. SPINAL RESPONSE: THE NOCICEPTIVE WITHDRAWAL REFLEX

2.1.1. Definition and functional significance

The NWR is a defensive polysynaptic reflex elicited by noxious stimuli that induces an involuntary fast movement, withdrawing the affected limb from the noxious source in order to protect the tissue from potential damage. This mechanism was first documented by Sherrington (Sherrington, 1910) after his observations in animal studies in the early 20th century. Sherrington described a stereotyped pattern, characterized by an ipsilateral limb flexion with a concurrent contralateral limb extension and defined the observed phenomenon as flexion reflex. Later studies performed in humans showed that the reflex can be elicited when the stimulus intensity is high enough to activate nociceptive fibers (Aδ) in the skin (Kugelberg, 1948). The size of the reflex is dependent on the stimulus intensity (Shahani and Young, 1971), and different reflex patterns (i.e. the group of activated muscles to produce the movement) can be evoked depending on the site of stimulation (Kugelberg et al., 1960). A modular organization of the NWR has been described (Sonnenborg et al., 2000; Andersen et al., 2001), where a reflex module consists of a reflex receptive field (RRF) located in the skin and a group of synergistic muscles that provide an optimal movement to withdraw the affected skin site from the noxious source (Andersen, 2007). Besides its protective function, the neural circuits included in the NWR are also part of a complex network involved in posture and locomotion (Andersen et al., 2001; Spaich et al., 2004).

In healthy subjects, there is typically a close relationship between the reflex threshold (RTh, the minimum stimulus intensity required to elicit a reflex) and the pain threshold (PTh, the minimum stimulus intensity reported as painful) (Willer, 1977). In line with this observation, several researchers have used the NWR to study the components of the spinal nociceptive pathways and the function of different neurotransmitters involved in pain processing in both healthy volunteers and patients suffering from chronic pain or altered pain perception (Sandrini et al., 2005).

2.1.2. Methodology for elicitation and recording

Electrical stimulation is the standard technique for eliciting the NWR (Andersen, 2007). Natural stimuli are not suitable for human studies since the stimulus intensity necessary to elicit the reflex can produce tissue damage. Similarly, reflexes evoked by radiant heat delivered by lasers require high intensity stimulation close to causing skin a burn (Mørch et al., 2007). Furthermore, the heat receptors habituate to the heat stimulation and it is therefore difficult to achieve consistent reflexes across time (Willer et al., 1979b; Mørch et al., 2007). On the other hand, electrical stimulation bypasses the receptor organs and provides a direct and synchronous activation of both Aβ and Aδ fibers, producing a strong afferent volley that evokes reliable responses. Generally, the stimulus consists of a constant current burst of 4-5 square-wave pulses, delivered by a computer-controlled electrical stimulator. Each pulse has a duration of 0.5-1 ms and the train of pulses is generally delivered at a stimulus frequency of 200-300 Hz which has been shown to be the most effective way to elicit NWR (Meinck et al., 1985). The electrical stimulations are applied with a random inter-stimulus interval ranging from 5 to 30 s to minimize habituation (von Dincklage et al., 2013).

The NWR has mainly been studied in the lower limbs, probably due to the lower number of degrees of freedom in the joints compared to the upper limbs. Muscle activity is measured by surface electromyography (EMG) recordings, where different muscles are assessed depending on the stimulation site. The most widely used setting is stimulation in the sural nerve below the malleolus and recording from the hamstrings (Willer, 1977; Dowman, 1991; Sandrini et al., 1993), although stimulation of tibial and plantar nerves and recordings from tibialis anterior muscle (TA) have also been used (Kugelberg et al., 1960; Shahani and Young, 1971; Meinck et al., 1981; Kolb et al., 2007). The latter have the advantage that the stimulation of the foot sole targets a receptive field which consists only of plantar skin. On the other hand, sural nerve stimulation targets both dorsal and plantar skin areas which might potentially evoke antagonistic reflexes (Meinck et al., 1985).

2.1.3. Supraspinal modulation of the NWR

There are multiple convergences of ascending and descending tracts in the reflex circuitry at the spinal cord. The interneuronal populations involved in the NWR integrate multisensorial input from different peripheral afferents and from several supraspinal centers (Schomburg, 1990; Dietz, 2010). This organization of the spinal circuits allows them to coordinate the course of movements by combining the information about the current state of centrally induced motor patterns, descending modulatory signals and the peripheral conditions (Andersen, 2007; Nakajima et al., 2014).

Evidence from animal studies suggest that several supraspinal structures such as the brainstem, cerebellum, basal ganglia and cortex modulate the NWR (Schomburg, 1990; Carlson et al., 2005). In particular, electrical stimulation of the periaqueductal gray (PAG) and the rostroventral medulla (RVM) induced an inhibition of reflex responses evoked by noxious skin heating in rats (Heinricher et al., 1987; Carstens and Campell, 1992; Fields et al., 1995). Injections of glutamate or morphine in the PAG also produced changes in reflex excitability (Carstens et al., 1990). The presence of projections from these supraspinal structures to laminae I-II and V of the dorsal horn were early reported and confirmed (Wall, 1967; Fields et al., 1995). Since these laminae contain most of the nociceptive populations that are involved in afferent nociceptive transmission, it is believed that the supraspinal structures can modulate ascending nociceptive information and nocifensive responses by targeting directly the nociceptive neurons located in the dorsal horn (Fields, 2004).

In humans, NWR elicited in spinal cord-injured patients usually result in a larger and longer-lasting response and expanded RRF compared to healthy subjects (Shahani and Young, 1971; Andersen et al., 2004; Biurrun Manresa et al., 2014), probably suggesting the presence of an inhibitory modulation exerted tonically by supraspinal structures in normal subjects. Patients with central nervous system disorders such as spasticity and Parkinson's disease present abnormal excitability of the NWR (Milanov, 1992; Gerdelat-Mas et al., 2007; Mylius et al., 2009).

The presence of descending control of the NWR pathways also arises from studies that investigated the impact of changes in the psychological/mental state on reflex excitability. Among the vast number of possible cognitive manipulations, one of the most investigated processes is attention. Many different strategies have been used to manipulate the focus and level of attention, either to incoming stimuli or away from them. Particularly, early studies employed mathematical tasks to divert attention away from stimulation (Bathien and Hugelin, 1969; Willer et al., 1979a). They reported a reduction of the NWR together with reduced pain ratings when participants performed the arithmetic task compared to when they focused on the electrical stimulation. Terkelsen et al. (2004) employed a paced auditory serial subtraction paradigm to distract also the participant from the nociceptive input. In this setup the volunteers experienced an altered autonomic activity and reduced pain ratings during the arithmetics but did not observe changes in the NWR. Edwards et al. (2006) replicated Terkelsen's group findings in terms of pain ratings and increased arousal during a serial addition task, although they observed an increased NWR when compared to a resting condition. This group also investigated the effect of mental arithmetic on the modulation of the NWR that is normally observed during the cardiac cycle (McIntyre et al., 2006). The NWR is inhibited during systole and this effect is believed to be mediated by arterial baroreceptor activation (also known as baroreflex) (Edwards et al., 2001). The baroreflex arc includes afferent fibers that project to the brainstem areas involved in descending inhibition

of spinal transmission and this inhibitory effect appears to be modulated with increased arousal (McIntyre et al., 2006).

Furthermore, recent studies used other types of demanding cognitive tasks such as computer game play (Edwards et al., 2007) and the Stroop test (Bjerre et al., 2011) to investigate its influence over the NWR. These authors reported a reduction of NWR thresholds and an expansion of the reflex receptive fields, respectively, when performing the tasks. Possibly the differences observed across the cited studies involving distraction paradigms could be related to the simultaneous changes in the subjects' emotional state or arousal elicited by the stimulation and/or the distraction tasks (Villemure and Bushnell, 2009). Rhudy et al. (Rhudy et al., 2005) demonstrated that emotional picture-viewing produces a consistent modulation of the NWR and subjective pain ratings. Particularly, they observed that NWR and pain magnitudes were lower during pleasant emotions and higher during unpleasant emotions. In a subsequent study it was shown that when stimulation were predictable due to a cue this modulatory effect of emotion was only reflected in the pain ratings but not in the NWR (Rhudy et al., 2006). All in all, evidence suggests that the supraspinal modulation of the NWR is highly complex and many different experimental factors can influence its measurement.

2.2. SUPRASPINAL RESPONSE: SOMATOSENSORY-EVOKED POTENTIALS

Among all techniques applied today to study neural activity in the human brain, EEG is the most suitable to study the temporal sequence in which different cortical regions are activated (Apkarian et al., 2005). EEG has a temporal resolution in the order of milliseconds, which is unsurpassed compared to other methods such as positron-emission tomography and functional magnetic resonance imaging (fMRI) (Apkarian et al., 2005; Kakigi et al., 2005). In contrast, EEG spatial resolution is relatively low, as signals recorded by each scalp electrode reflect a spatially blurred summation of neural activities. EEG offers similar performance to magnetoencephalography (MEG) in terms of spatiotemporal resolution. However, EEG detects primarily electric sources that are radial to the scalp while MEG is more sensitive to magnetic fields generated by electric sources in tangential directions. Moreover, the complexity of MEG instrumentation makes its costs several times higher than EEG instrumentation (Hämäläinen et al., 1993; Wendel et al., 2009).

Event related potentials (ERPs) are transient amplitude deflections in the continuous EEG recordings which are phased-locked to a sensory, motor or cognitive event. ERPs reflect the summed postsynaptic potentials generated by the synchronous firing of a large number of cortical pyramidal neurons (Peterson et al., 1995). Particularly, SEPs are transient changes in the ongoing EEG elicited by activation of the somatosensory pathways. ERPs have been widely used in experimental studies

to reveal different aspects of cerebral processing (Picton et al., 2000). Results support the idea that ERP components largely reflect the processes behind the detection and orientation of attention to the eliciting stimulus (Legrain et al., 2012).

2.2.1. Methodology for elicitation and recording of SEPs

SEPs can be elicited using non-invasive bipolar electrical stimulation of the skin and recorded using scalp electrodes following the 10–20 international system of EEG electrode placement. SEPs waveforms (and more generally ERPs) are composed by a set of waves or components which are typically characterized by their polarity (i.e. whether the voltage excursion is positive or negative), their amplitude, latency (measured as the time of peak deflection in relation to the time of stimulus onset) and scalp distribution (Picton et al., 2000).

SEPs elicited by high current stimuli usually display 5 components whose sources were suggested by dipole source localization studies (Dowman, 2004) and supported by intracranial recordings (Dowman et al., 2007): 1) a positive early peak at 45 ms (P45) usually related to innocuous stimulation and located in the primary somatosensory cortex leg area; 2) a central negativity at 70–110 ms located in the somatosensory association areas in the medial wall of the parietal cortex; 3) a negativity over the contralateral temporal scalp areas at 100– 180 ms situated in the parietal operculum and insula; 4) a fronto-central negativity at 130– 200 ms possibly originated in the medial prefrontal cortex and primary somatosensory cortex foot area; 5) a positive peak P2 at 280–320 ms post-stimulus apparently originated in the anterior cingulate cortex, inferior parietal cortex, and probably the somatosensory association areas in the medial wall of the parietal cortex. It might also be possible to observe a P3a ERP at 320–400 ms.

2.2.2. Use of SEPs in pain research

When SEPs are elicited by transcutaneous low-current stimuli, they display early components which are widely used in clinical settings to examine the state of large-diameter, low-threshold fast conducting afferents. Increasing the intensity of stimulation produces the activation of both low-threshold, non-nociceptive afferents and high-threshold, nociceptive fibers (Arendt-Nielsen, 1994). Although high-current stimulation evokes an aversive painful response, the use of SEPs in clinical settings is limited since they do not reflect specific nociceptive responses. Thus, other stimulation techniques such as lasers which selectively activate nociceptive afferents have been used more predominantly for the past three decades (Cruccu et al., 2004).

Using infrared laser stimulators, numerous studies have shown a strong correlation between the intensity of perceived pain and the magnitude of the laser-evoked potentials (LEPs) (Arendt-Nielsen, 1994). This consistent observation led some

researchers to consider LEPs as a direct index of pain intensity coding in the human brain (Tracey and Mantyh, 2007). On the other hand, a number of studies have shown that the manipulation of stimulus saliency (Iannetti et al., 2008; Mouraux and Iannetti, 2009; Ronga et al., 2013) (i.e. its ability to capture attention) produces dissociation between pain perception and LEP magnitudes. These observations brought a new perspective on the functional significance of LEPs, suggesting that these responses reflect neural activity that is not necessarily nociceptive-specific. Instead, LEPs (and more generally ERPs) reflect mechanisms of arousal or attentional reorientation regardless of the stimulus modality (Iannetti et al., 2008; Legrain et al., 2012).

2.2.3. Single-trial assessment of SEPs

SEP components measured at scalp-surface are accompanied by ongoing neural and non-neural activity which is generally considered as background noise. Although the size of the SEPs elicited by high-current stimulation is considerably larger than the size of ERPs from other modalities (e.g. visual, auditory, laser), the magnitude of the signals of interest is still a fraction of the magnitude of the background EEG. Therefore, signal processing methods are required to enhance the signal-to-noise ratio (SNR). This is often performed by considering the mean response over several repetitions of the eliciting event, and therefore assuming that the resulting waveform is constant across trials (Dawson, 1954). However, the use of this procedure does not consider the across-trial variability of SEPs which could be attributed to ongoing activity (Arieli et al., 1996). Across-trial variability of SEPs could contain relevant information regarding the actual state of the cortical networks, possibly reflecting fluctuations in expectation, attention or other cognitive processes (Haig et al., 1995; Iannetti et al., 2005; Jarchi et al., 2011a). The averaging process may therefore distort the estimated ERP features and consequently eliminate vital information that could help to explain the different processes underlying the observed physiological responses (Mouraux and Iannetti, 2008).

This current interest in across-trial variability has led to the development of numerous single-trial methods for reliable automatic detection and estimation of ERP features (Mayhew et al., 2006; Hu et al., 2011b). The outcome of these automatic methods is usually validated against the expertise of a human observer (Hatem et al., 2012). Nevertheless, there are two main concerns related to the validity of automatic methods that have not been explored in depth: 1) the categorical agreement which evaluates the concordance between humans and algorithms on the presence (or absence) of an SEP component; 2) the quantitative agreement which quantifies the variation on the estimated features between methods. These aspects can be assessed using the following approaches:

- *Categorical agreement:* the presence/absence of a SEP component can be assessed using a group of indexes that describe different characteristics of the

level of agreement. The overall percent agreement (p_o) represents the sum of all trials in which the methods agree divided by the total number of trials. The positive percent agreement (p_{pos}) is derived from the number of positive trials (i.e. presence of an SEP component) in which both methods agree on divided by all of the positive trials for both methods. The negative agreement (p_{neg}) is calculated from the number of negative trials (i.e. a peak is absent) in which both methods agree on divided by all of the negative trials for both methods. These last two indices determine in which type of decision (i.e. presence or absence of a peak) there is a bigger disagreement between methods. Chance percent agreement p_e can be computed as the sum of the joint positive and negative responses, and represents the level of agreement that would still be present if the methods decided randomly on the presence/absence of a peak. Cohen's kappa (κ) is calculated as the ratio between the overall percent agreement corrected for chance ($p_o - p_e$), divided by the maximum possible percent agreement corrected for chance (100% – p_e). Normally, κ ranges from 0 (no agreement beyond chance) to 1 (perfect agreement), although it could be possible to obtain negative values of κ (if the agreement between methods is worse than what would be expected by chance).

- *Quantitative agreement*: the absolute variation of a particular SEP feature (e.g. peak amplitude and/or latency) between a pair of methods can be assessed using Bland-Altman's analysis. The method takes into account the single-trial differences between the quantities estimated by two methods. The mean difference is called bias, which gives a measure of *systematic error*. The standard deviation of these differences provides a reference of the absolute variation between methods, and gives a measure of *random error*. Normal distributions of the differences implies that 95% of the differences fall between ±1.96 standard deviations, and the limits of this span are considered the limits of agreement (LoA). The LoA therefore give a reference of the maximum differences that can be expected between methods when measuring the same quantity. Additionally, there are two other indexes that are frequently used to assess quantitave agreement. These are the coefficient of variation (CV), defined as the typical error divided by the average measurement, and the intra-class correlation (ICC), defined as the ratio between the variation observed within measures to the total observed variation between measures.

In Study I, the aim was to determine the categorical and quantitative agreement between manual and automatic methods for detection and estimation of SEP features. In this regard, a single experimental session with sixteen healthy volunteers was performed in which SEPs were elicited using electrical stimulation at six different stimulus intensities applied to the sole of the foot. Single-trial SEPs were defined by their characteristic peaks, named according to their latency and polarity: N1 (~70-110 ms), N2 (~100-180 ms) and P2 (~280-320 ms) (Treede et al., 1988). Two human observers performed the manual and blinded detection of single-trial

peaks. Additionally, two different automatic algorithms were also included, which consisted of an algorithm based on the derivative of the signal that classifies using fuzzy logic (DRIV) and an algorithm based on wavelet filtering and multiple linear regression (WVLT) (Hu et al., 2010). An example of the performance of the different detection methods can be observed in Figure 1.

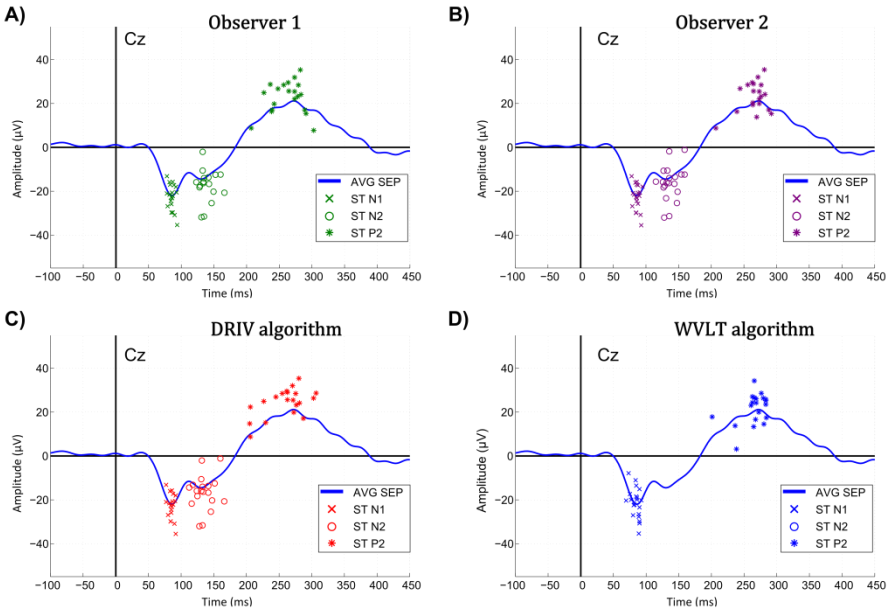

Figure 1. Single-trial somatosensory evoked potential (SEP) peaks detected by different methods. Panels display the average SEP signal (AVG SEP) elicited by electrical stimulation of a particular participant and the different single-trial SEP peaks detected by two different human observers (Observer 1, A) and (Observer 2, B) and two automatic algorithms (DRIV, C) and (WVLT, D). Crosses, circles and asterisks represent single-trial N1, N2 and P2 features, respectively, while the blue trace is the average of 20 trials elicited with the highest stimulation intensity.

The presence/absence of a SEP peak (categorical outcome) was assessed in each single-trial recording, together with the variation in the corresponding amplitude and latency (quantitative outcome) found by the four different strategies. Results of Study I showed that human observers generally displayed the highest categorical and quantitative agreement, and that there were significantly large differences between detection and estimation of quantitative features among methods.

Concerning the categorical agreement, results of Study I showed that there was an overall agreement ranging from good to excellent in all cases (the median p_o was higher than 80% for all possible pairings). The highest agreement was particularly observed in the decision of the presence/absence of the P2 peak (median p_o higher than 90%), since there was only one peak involved in the window of interest (unlike

the detection of N1 and N2). The main issue with the overall agreement given by p_o is that it is highly influenced by an imbalance in the presence-absence of the outcome. For example, if the number of trials in which a peak is present is large compared to the number of trials in which a peak is absent, then p_o will be ruled by the trials in which a peak is present. It is therefore necessary to also assess p_{pos} and p_{neg}, which quantify the relative agreement when a peak is present and absent, respectively. Indeed it was observed that although p_o was similar to the p_{pos}, (i.e., when a peak is present), there were large differences in the assessment of the absence of a peak, as reflected by the low p_{neg} values. Particularly, the categorical agreement regarding the presence/absence of all SEP peaks yielded significantly higher p_{neg} between human observers compared to any other pairing. Furthermore, all pairings between both human observers and the DRIV algorithm yielded significantly higher $p_{neg}$for N1 and P2 peaks compared to all pairings between both human observers and the WVLT algorithm.

When correcting for the agreement expected by chance, it was observed through the κ statistic that the agreement was very low, particularly in the cases involving the WVLT approach (Figure 2). This gives the idea that most of the agreement in those cases was probably due to chance. Moreover, the median level of agreement for the pairings involving the two human observers was significantly higher than the level of almost all other pairings, with a median κ ranging from 0.4 to 0.7. Overall, the observed differences could be mainly explained by the intrinsic differences of each detection approach, e.g., the WVLT algorithm is not designed to detect cases in which one or several peaks are not present. On the other hand, the DRIV algorithm purposely imitates the human decision-making process (Piater et al., 1995).

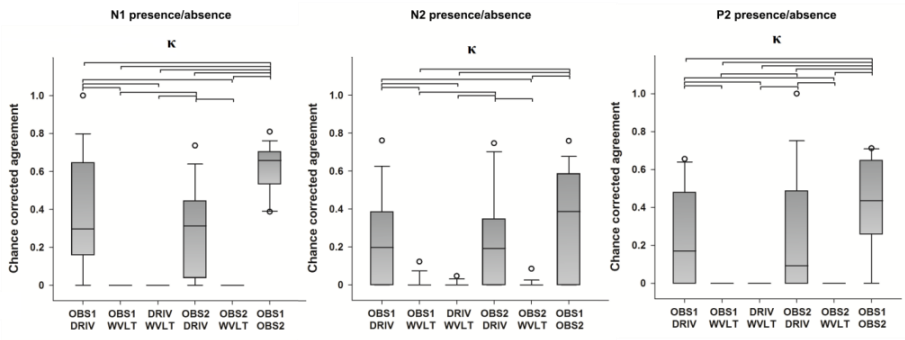

Figure 2. Cohen's kappa (κ) categorical agreement between different human observers (OBS1 and OBS2) and automatic algorithms (DRIV and WVLT). Horizontal lines on top of the bars represent statistically significant post hoc differences between pairings (Student-Newman-Keuls, $p < 0.05$).

Regarding the quantitative outcome, statistically significant differences in bias between pairings were found for all SEP component amplitudes and N2 and P2 latencies (Figure 3). In particular, it was found that the amplitude of the bias between human observers and the DRIV algorithm was irrelevant in practical terms (median bias < 1 μV in all cases). Pairings including the WVLT algorithm showed slightly larger amplitude bias with a median value around 5 μV. Similarly, the median latency bias was typically lower than 10 ms, although it was observed that that the maximum values could differ in up to 60 ms in cases in which the detected wave was N1 or N2 and the pairing included at least one of the algorithms. This was also observed in the quantification of P2, probably because the P2 wave is composed by a set of local maximae. On the other hand, the pairing between the two human observers showed a latency bias which was less than 20 ms with a final average latency difference close to zero (i.e. unbiased). In time, statistically significant differences in the LoA between pairings were found for SEP component amplitudes and for SEP component latencies, with the error being generally smaller for pairings between human observers and the DRIV algorithm (Figure 3).

Another relevant question in Study I was related to the effect of stimulation intensity on the categorical and quantitative agreement between methods for ERP feature detection and estimation. In order to address this question, two representative indexes for categorical and quantitative agreement (Cohen's κ and CV, respectively) were calculated from the best- and worst-performing pairings between manual and automated methods, and stimulation intensity was taken as a factor (with approx. 20 trials per intensity per subject).

Figure 4 represents the average SEPs across subjects for three levels of stimulus intensity. There were no differences in categorical agreement in relation to stimulation intensity, meaning that the number of peaks detected at each stimulus intensity did not change significantly between the human observers or between the algorithms. The quantitative agreement between the human observers was also not significantly different due to stimulation intensity for any of the peaks. However, there were some differences in quantitative agreement due to stimulation intensity when comparing the two algorithms. It was observed that the N1 peak amplitudes presented less variation at the highest stimulation intensity, whereas P2 peak latencies showed more variation at the lowest stimulation intensity. Nevertheless, it also has to be pointed out that the absolute differences were quantitatively small (7-18% in the case of N1 peak amplitudes and 2-3% for P2 peak latencies).

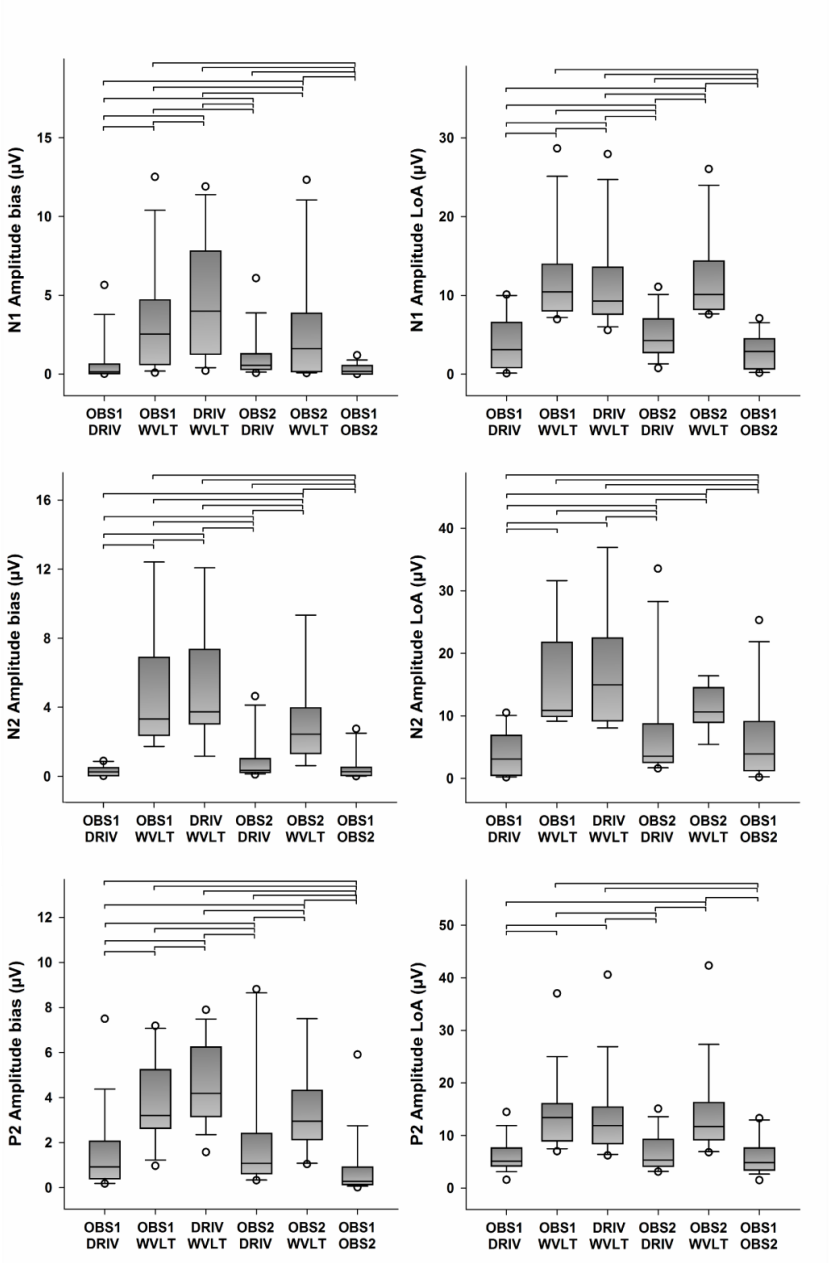

Figure 3. Bias and limits of agreement (LoA) for the quantitative agreement of N1, N2 and P2 peaks (n = 16 for each index). Horizontal lines on top of the bars represent statistically significant post hoc differences between pairings (Student-Newman-Keuls, p < 0.05).

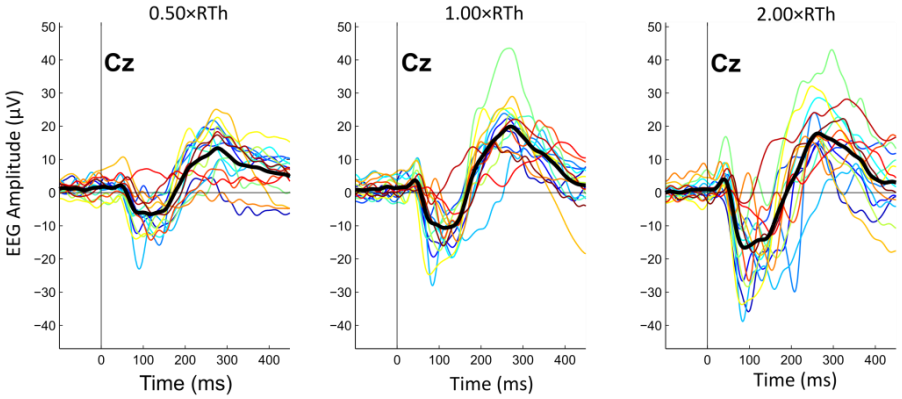

Figure 4. Effects of stimulation intensity somatosensory-evoked potentials (SEPs). Each panel shows the average SEP signal of all available trials from each subject (color-coded) for a single stimulation intensity. The overlapping thick black line represents the grand average of all subjects (n = 16). RTh: nociceptive withdrawal reflex threshold.

The results from Study I highlight the importance of having a criterion for detecting the presence/absence of a response. Particularly, studies interested in evoking responses near threshold might be in a situation where the physiological response might not be elicited. Therefore the single- trial estimation in the absence of that response could have a negative influence on the conclusions drawn.

Study I also emphasized the impact of selecting different pre-processing methods on the quantification process. Particularly, the WVLT algorithm uses wavelet filtering to reduce background noise and the outcome measures are further estimated using a multiple linear regression approach (Hu et al., 2010). On the other hand, the DRIV algorithm makes it selection and quantification over the signal with no further pre-processing.

It has to be noted that the aim of Study I was not to elucidate which method is better, since agreement between two methods is not enough to make such a statement. Instead, it was intended to provide reference values to the maximum differences that can be expected if a particular method is applied instead of another. In the light of these results, the DRIV method was selected for use in Study II as it showed the closest resemblance to human detection with lower computational cost than the WVLT method.

In summary, different detection/estimation methods may lead to substantial differences in the results; this implies that special care should be taken during the selection of the approach for feature extraction.

2.3. SYNCHRONOUS RECORDINGS OF SPINAL AND SUPRASPINAL ACTIVITY

The electrical stimulus required to elicit a NWR also evokes a SEP response that can be measured at scalp level. The simultaneous recordings of both NWR and SEPs appeared as a suitable approach for investigating spinal and supraspinal processes related to pain mechanisms occurring in the same subject at the same time (Dowman, 1991). Although this methodology seems very promising to study the nociceptive processing across the neuraxis, simultaneous recordings have not been widely used. Only a few studies have combined these two methods and were mainly focused on grand-average responses.

The modulatory factor that has been mostly used in experiments involving synchronous NWR and SEP recordings is the stimulation intensity. Early results showed that the reflex size was linearly correlated with stimulus intensity above the RTh, while the late SEP components amplitudes were maximal when the stimulus level reached the pain threshold and then remained plateaued (Debroucker and Willer, 1985). Dowman (1991) did pioneering research on concurrent NWR and SEPs using the current intensity that elicited the maximal sural nerve compound action potential (CAP) as normalization factor for selecting the different stimulus intensities (Dowman, 1991, 1994, 2001; Dowman and Darcey, 1994). Particularly for the relationship with stimulus intensity, Dowman (1991) reported a positive correlation between NWR and pain ratings when stimulus levels were above 1.5 times the current necessary for eliciting the maximal CAP. It was also observed that the different SEP components changed their amplitude across all stimulation levels, therefore concluding that SEPs possibly reflect the neural processing of both nociceptive and non-nociceptive afferent information. These findings were supported with intracranial recordings (Dowman et al., 2007).

Simultaneous recordings of NWR and SEPs have also been used in studies of the effect of modulatory tasks over the nociceptive system. Different cognitive approaches have been taken in the quest of understanding how and when supraspinal centers modulate spinal excitability. Most notably, Dowman (2001) investigated the effect of attention on NWR, SEPs and subjective magnitude ratings elicited by non-painful and painful sural nerve stimulation using an *attention-ignore* paradigm. A cue was given before each trial to inform the subjects that they had to focus to either a visual identification task or a somatosensory rating task. Twenty percent of the trials were invalidly cued and the participants had to rate the stimulus intensity regardless its validity. Whereas he observed changes in the perceived magnitude of the stimulus when the subjects directed their attention to it in the validly cued condition and away from it in the invalidly cued condition, he did not observe differences in the NWR magnitude between the two conditions. This differs with other studies that looked into the effects of attention on the NWR, where changes in reflex amplitudes were observed when the subjects were engaged in a distraction

task (see Section 2.1.3). Probably the difference between Dowman's study and the others cited in Section 2.1.3 is task relevance: whereas in Dowman's study the evoking stimulus is task relevant (i.e. rate its intensity) in both conditions (valid and invalid cue), in most of the other studies the evoking stimulus was only task relevant in the attend condition.

Another interesting study investigated the influence of expectations on descending modulatory mechanisms and spinal nociceptive processing (Goffaux et al., 2007). Using a counterirritation technique (application of a second painful stimulus) they observed that differences in expectations affected the endogenous mechanisms that typically lead to a reduction of the amplitude of pain ratings, NWR and SEPs. More interestingly, the expectations of analgesia also reduced the subjective pain ratings in fibromyalgia patients but did not affect their observed spinal hyperexcitability (Goffaux et al., 2009), showing that in certain cases spinal and supraspinal responses to painful stimuli can be dissociated. These are particular examples of the importance of counting on techniques for the simultaneous assessment of spinal and supraspinal activity during painful stimulation, which will be further analyzed in the next chapter.

CHAPTER 3. MUTUAL INFORMATION ANALYSIS

3.1. CONVENTIONAL ANALYSIS AND INTERPRETATION OF SPINAL AND SUPRASPINAL RESPONSES

The most used methodology to analyze the relationship between stimulus intensity and NWR and SEP amplitudes has been to measure signal covariance (e.g. correlation coefficient or general linear model), a model which assumes linearity and homogeneity of variance (Debroucker and Willer, 1985; Dowman, 1991). One of the main concerns regarding these methods is that the required assumptions to apply them are not always accomplished (Kisley and Gerstein, 1999; Osborne and Waters, 2002). Physiological variables such as the reflex size might have highly skewed and kurtotic distributions with large outliers (Figure 5A), resulting in non-normally distributed data. Although data transformation might solve these issues, the interpretation of the results might become difficult. Removal of outliers could improve the accuracy of the regression analysis, although it is not always appropriate to remove outliers unless the real origin/cause of the extreme outcome is known. In addition, it is common to observe non-linear relationships with the dependent variable (e.g. stimulation intensity). Another assumption which impacts in the result of statistical tests is the homogeneity of variance across different levels of the predictor variables, also known as homoscedasticity. When a random variable such as the reflex size shows a variable dispersion of its errors across different values of the predictor then it is said to be heteroscedastic (Figure 5B).

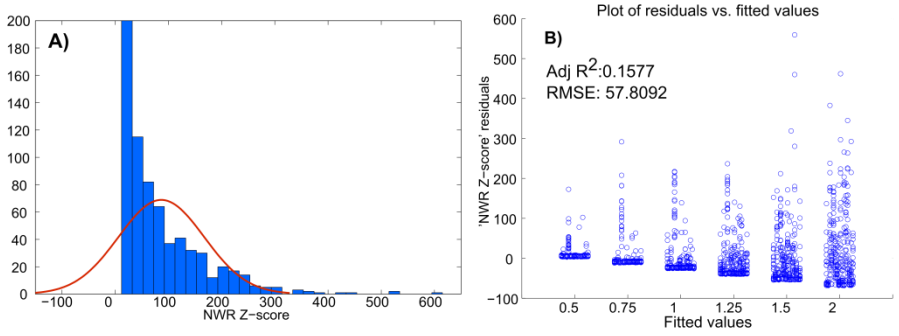

Figure 5. A) Histogram of the reflex amplitudes measured in terms of the interval peak z-score (Rhudy and France, 2007) *(n=16). Only trials with elicited reflexes are included. B) Residuals obtained from the difference between observed values of all reflex Z-scores and the estimated linear regression function with the normalized stimulation intensity as predictor (NWR-zscore ≈ C + intensity).*

Another aspect that might be interesting in studies involving several outcome measurements is the evaluation of interaction among these responses. Alternative methods such as multivariate outcome analysis can explain this relationship, although similar assumptions about the data hold for this approach. Nevertheless, several questions remain unanswered in relation to how modulatory effects influence each response independently, or if there is an interaction of any nature (linear or non-linear) between these responses that could provide more information than the analysis of each response individually. It is therefore of interest to apply a method which can be used to integrate simultaneously acquired data and that does not assume a specific signal distribution or linearity.

3.2. INFORMATION THEORY FRAMEWORK

The Information Theory (IT) framework was first introduced by Shannon in the middle of 20th century (Shannon, 1948). The concepts developed under this framework have been extensively applied in the study of the information transmission in neuronal populations (Quian Quiroga and Panzeri, 2009). The IT framework allows the quantification of stimulus-signal and signal-signal relationships based on the estimated stimulus-response signal probability distributions and without linear and Gaussian constraints, which makes it a promising approach to study biological processes (Ostwald et al., 2010). The use of the IT framework has been increasingly extended to other types of neurophysiological signals and fields (Ince et al., 2010).

The fundamental quantity provided by the IT framework to characterize the relation between two variables is defined as mutual information (MI). The main aspects that makes MI so attractive are (Schneidman et al., 2003): 1) it provides a general measure of correlation between stimulus and responses, taking into account correlations at any order; 2) it does not make prior assumptions on the relevance of the signal features in question, neither stimulus- nor response-related; 3) it meets various properties such as additivity of information for completely independent signals.

In order to interpret its concept, let's consider an experimental setup where the two variables are defined as the stimulus set $S = \{s_1, s_2, s_3 \ldots, s_n\}$ (controlled by the experimenter) and a consequent response R to be recorded. The response R would generally be a quantifiable, discrete signal that can take different values. MI therefore quantifies the level in which different response values discriminate between different stimuli. The information $I(S; R)$ between S and R can be expressed as the difference between the response entropy $H(R)$, a measure of the *overall* variability of the response, and the noise entropy $H(R|S)$, a measure of the variability exclusively attributable to trial-by-trial noise (Shannon, 1948; Cover and Thomas, 2006):

$$H(R) = -\sum_r P(r) log_2 P(r) \quad (1)$$

$$H(R|S) = -\sum_s P(s) \sum_r P(r|s) log_2 P(r|s) \quad (2)$$

$$I(S;R) = H(R) - H(R|S) = \sum_r P(s) \sum_r P(r|s) log_2 \frac{P(r|s)}{P(r)} \quad (3)$$

P(s) represents the probability of presenting a stimulus *s*, *P(r|s)* is the conditional probability of observing a response *r* when a stimulus *s* is presented. *P(r)* is the probability of observing a response *r* across all trials of all stimuli. Hence, *I(S; R)* quantifies the overall reduction of uncertainty that is achieved by observing a single-trial value of the response in relation to which stimulus was presented (Borst and Theunissen, 1999). *I(S; R)* is expressed in bits, where zero can be interpreted as a completely random stimulus response relationship and where the maximum information will be given by the entropy of the stimulus *H(S)* or the entropy of the response *H(R)*, depending on which one is smaller.

The MI expression can be generalized to calculate the information about the stimulus carried by the joint observation of two or more features. The response set can thus be considered as an array **R**. Particularly, the MI conveyed jointly by two responses R_x and R_y about the stimulus can be expressed as:

$$I(S; R_x, R_y) = I(S; \mathbf{R}) = \sum_s P(s) \sum_{r_x r_y} P(r_x, r_y|s) log_2 \frac{P(r_x, r_y|s)}{P(r_x, r_y)} \quad (4)$$

where $P(r_x,r_y|s)$ is the probability of observing the responses r_x and r_y in a single trial when stimulus *s* is presented; $P(r_x,r_y)$ is the probability of observing both r_x and r_y across all stimuli.

When two or more responses are considered, it is possible to describe *I(S; **R**)* as number of sub-terms that allow to quantify how correlations between signals contribute to information transmission (Pola et al., 2003):

$$I(S; \boldsymbol{R}) = I_{lin} + syn \quad (5)$$

The linear term I_{lin} represents the sum of the information by each of the response signals in **R** as if they were completely independent. The synergy term *syn* represents the information carried jointly by the two or more responses in **R**. Positive values of *syn* means that the joint observation of the responses carries more information about the stimulus than their individual contributions. Alternatively, negative values of *syn* imply that the combination of signals provides less information that their individual contributions, an effect also described as redundancy. If each response conveys independent information, the term *syn* is then zero and all the information would be a linear sum of the information conveyed by each individual signal, as expressed by the term I_{lin}. Although the synergy can be

further decomposed in a new subset of terms describing the possible contributions of correlations to information transmission (Pola et al., 2003), the present dissertation is only focused on the aforementioned terms.

3.2.1. Practical issues - Bias correction procedures

The computation of *I(S; R)* involves the usage of the probability distributions *P(r)*, *P(r|s)* and *P(s)*. Since these probabilities are not known in advance (except for *P(s)*, which would normally be controlled by the experimenter), it is necessary to estimate them from the data samples. This procedure can be performed by building a histogram of the experimental frequency of each response value across the available trials. Nevertheless, the estimation of the probabilities from a limited amount of data causes a systematic error (or bias) which is a key practical issue for the accurate application of the IT framework. Fortunately, several advanced methods have been developed to amend this problem and specific guidelines are available to help in the selection of an appropriate procedure for computing information and to produce accurate results (Panzeri et al., 2007).

The empirical estimation of the probabilities requires the discretization of the responses through a binning process. The number of bins is a free parameter which is determined in a tradeoff between the information that is lost due to discretization and the distortion caused by the limited size of data samples. The number of bins (i.e. the number of possible values that the response R can take, $|R|$) is constrained by the minimum number of stimulus $|S|$ and the maximum number of trials per stimulus N_s, where vertical bars denote cardinality of the sets (Golomb et al., 1997).

3.3. APPLICATION OF MUTUAL INFORMATION IN THE ASSESSMENT OF SIMULTANEOUS SPINAL AND SUPRASPINAL ACTIVITY

In Study II, the aim was to quantify the amount of information about electrical stimuli carried by simultaneous electrophysiological responses in humans using MI analysis. Simultaneous NWR and SEPs were assessed in sixteen subjects during repeated electrical graded stimulation and different features were extracted from the acquired signals to quantify their information transmission in relation to the eliciting stimuli. Four NWR features were extracted per trial: the root mean square (RMS) amplitude (NWR RMS), interval peak z-score (NWR Z-score), latency (NWR latency) and duration (NWR duration). Additionally, six single-trial features were extracted from the SEPs at the vertex using the DRIV algorithm from Study I: N1, N2 and P2 amplitudes and latencies, respectively. The six stimulation intensities applied were grouped into three subgroups that defined the stimuli set as $S = \{1, 2, 3\}$: 1; subthreshold intensities (0.5x, 0.75x RTh), 2; near threshold intensities (1.0x, 1.25x RTh) and 3; above threshold intensities (1.5x, 2.0x RTh). This grouping yielded a total of N_s=40 trials per stimulus group (120 trials in total per subject).

Each extracted feature was taken individually to calculate its univariate information about the stimulus and presented as $I(S;R_x)$. The maximal information value that was expected was the entropy or self-information of the stimulus, which was in this case 1.58 bits for a stimulus set S consisting of 3 stimulation intensity groups.

Moreover, a bivariate analysis was performed using pairs of features to calculate the information carried jointly and shown as $I(S; R_x, R_y)$. The bivariate analysis included the synergy and linear term quantities for each response feature pair. Only the two most informative features of each modality (NWR and SEP) were paired with all the remaining features. Additionally, the parameters used in the calculation of the MI such as the number of bins and the performance of the bias correction methods were also validated.

3.3.1. Univariate mutual information analysis

Results from Study II showed that the information carried by the reflex features that quantify the NWR amplitude was significantly higher than information contained in the SEP features (Figure 6).

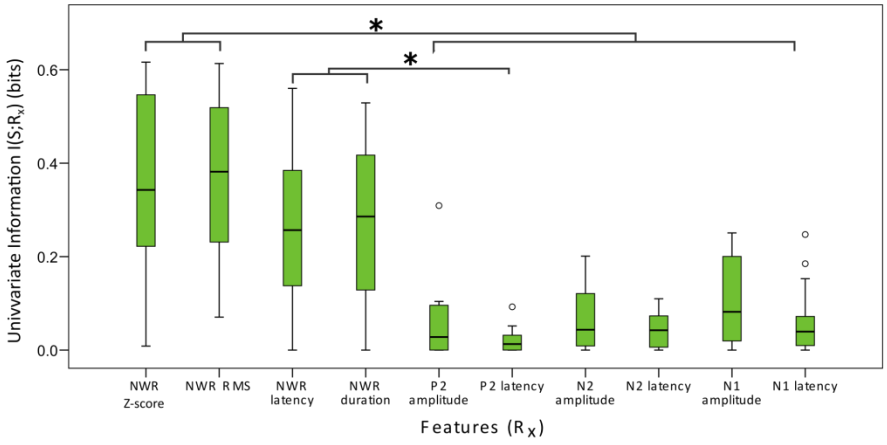

*Figure 6. Univariate information of spinal and supraspinal features about the stimulus. Median values of the information I(S;R) about the stimulus carried separately by single-trial features extracted from the somatosensory evoked potentials (SEPs) and the nociceptive withdrawal reflex (NWR) (n=16). *: p<0.05. Solid lines represent the median; box edges represent the 25 and 75 percentiles.*

These observations may result from the different concurrent processes occurring at spinal and supraspinal level which are reflected in the EMG and EEG signals, respectively. On one hand, the muscle was at rest prior to stimulation with minimal EMG activity compared to the high post-stimulus activation. On the other hand, the brain presents ongoing activity related to numerous brain states which is reflected at scalp level together with the evoked activity. It has been traditionally considered that

the ongoing activity is unrelated to the stimulus, which led to apply the aforementioned *signal+noise* model. However, it has been shown that these ongoing processes mostly explain the variability of subsequent evoked responses (Arieli et al., 1996; Pfurtscheller and Lopes da Silva, 1999; Engel et al., 2001; Busch et al., 2009). Either way, the fact that the amplitude of the ongoing EEG activity can be as large as the transient evoked responses represents a clear element that increases trial-to-trial variability and impoverishes the single-trial peak detection.

The differences found in the carried information may also arise from the different performance of the methods used for feature extraction. There are numerous methods for automatic single-trial detection of ERP components (Quian Quiroga and Garcia, 2003; D'Avanzo et al., 2011; Hu et al., 2011a; Jarchi et al., 2011b) which exert a strong influence in the quantification of the information contained in the signal of interest. In that matter, a potential strength of the MI metric is in fact that the MI analysis can be used as a measurement of performance for pre-processing and feature extraction methods (Ostwald et al., 2010).

The analysis of the impact of the discretization presented information quantities that did not change considerably when the number of response bins increased over 6-8 (Figure 7A). Likewise, the analysis of the performance of the bias correction methods showed that its application helped to obtain accurate information estimates with 40 trials per stimulus ($\log_2(40)\approx5.3$), as these median values did not change considerably with larger amount of trials (Figure 7B).

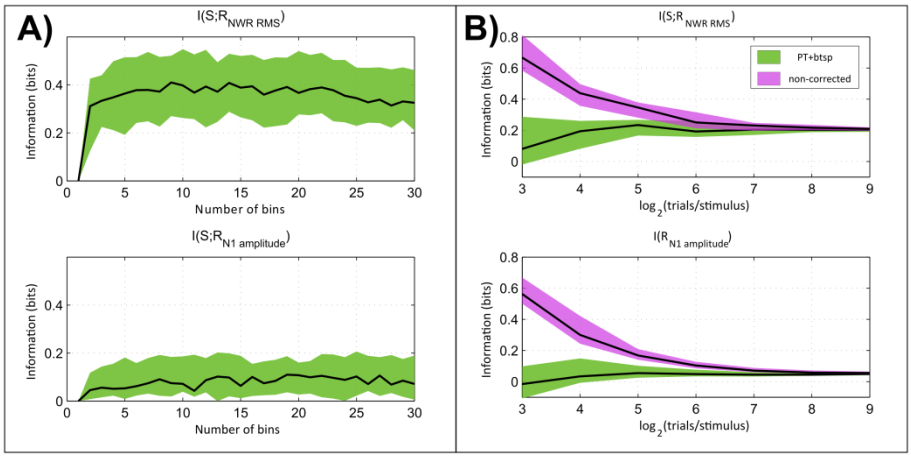

Figure 7. Validation of the parameters used in the calculation of mutual information for two response features. A) Univariate information I(S;R) of spinal (NWR RMS) and supraspinal (N1 amplitude) features about the stimulus as a function of the number of response bins. B) Monte-Carlo simulation of I(S;R) as a function of the number of trials. The information quantities are shown for corrected (PT+bstp) and non-corrected estimates. Solid line represents the median; shaded zones represent the 25 and 75 percentiles.

3.3.2. Bivariate mutual information analysis

Results from Study II indicated that the information carried jointly by pairs of features was generally more informative than their individual contributions. Particularly, combining the NWR RMS with the remaining reflex features resulted to be significantly more informative than the combination of NWR RMS with the P2 features (Figure 8A). Moreover, the combination of the N1 amplitude with the features that reflect the reflex amplitude (i.e. NWR RMS and NWR Z-score) provided more information about the stimuli than the combination of N1 amplitude with the other SEP features (Figure 8B).

Further evaluation was performed on the effect of correlations between different pairs of responses and whether these correlations lead to an increase (synergy) or decrease (redundancy) in the conveyed information compared to the amount they would carry if they were independent. First, it was observed that the combinations of NWR RMS with the other reflex features were redundant (synergy<0, Figure 8A). When comparing the different synergies produced by the combination of NWR RMS with the remaining features it was particularly found that the combination NWR RMS – Z-score was more redundant than the combinations between the NWR RMS and the SEP features (Figure 9A). Second, the synergies given by combining the N1 amplitude with the remaining SEP features were not significantly different from zero (Figure 8B). On the other hand, the combination of the N1 amplitude with the NWR features was redundant. Finally the comparison between pairs containing the N1 amplitude as one of the responses did not show significant differences.

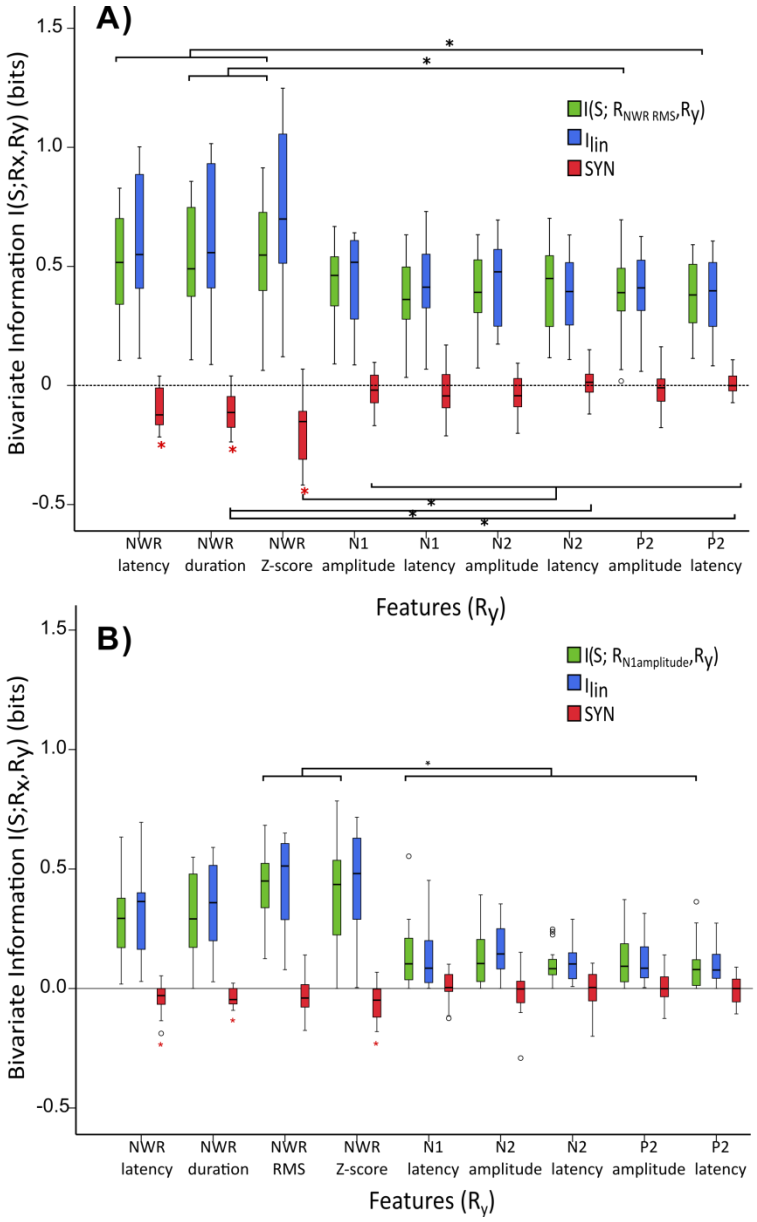

*Figure 8. Bivariate information carried jointly by spinal and supraspinal features about the stimulus. Median values of the information $I(S;R_x, R_y)$, synergy $SYN(S;R_x,R_y)$ and linear term $I_{lin}(S;R_x, R_y)$ about the stimulus that results from pairing single-trial values of NWR RMS (Panel A) and N1 amplitude (Panel B) with the remaining SEPs and NWR features,listed as R_y (n=16). Red * represent $p<0.05$ for Wilcoxon one sample signed rank test. Dark * represent $p<0.05$ for post-hoc Tukey test on the ranks.*

As with the univariate analysis, we tested the validity of the MI measurements (I, I_{lin} and SYN) as a function of the number of bins when all features were combined in pairs with NWR RMS and N1 amplitude. Figure 9A displays the behavior of MI quantities as a function of the number of bins for a particular feature combination. It was generally observed for all the different combinations that the MI quantities did not vary substantially when they were estimated with responses discretized with more than 4 bins per response (16 response bins in total). Furthermore, the behavior of MI quantities (*I* and *SYN*) as function of the number of trials was evaluated using Monte-Carlo simulation (Figure 9B). Again it was observed that the bias correction procedures provided accurate estimates of the MI quantities using 40 trials per stimulus ($\log_2(40)\approx5$).

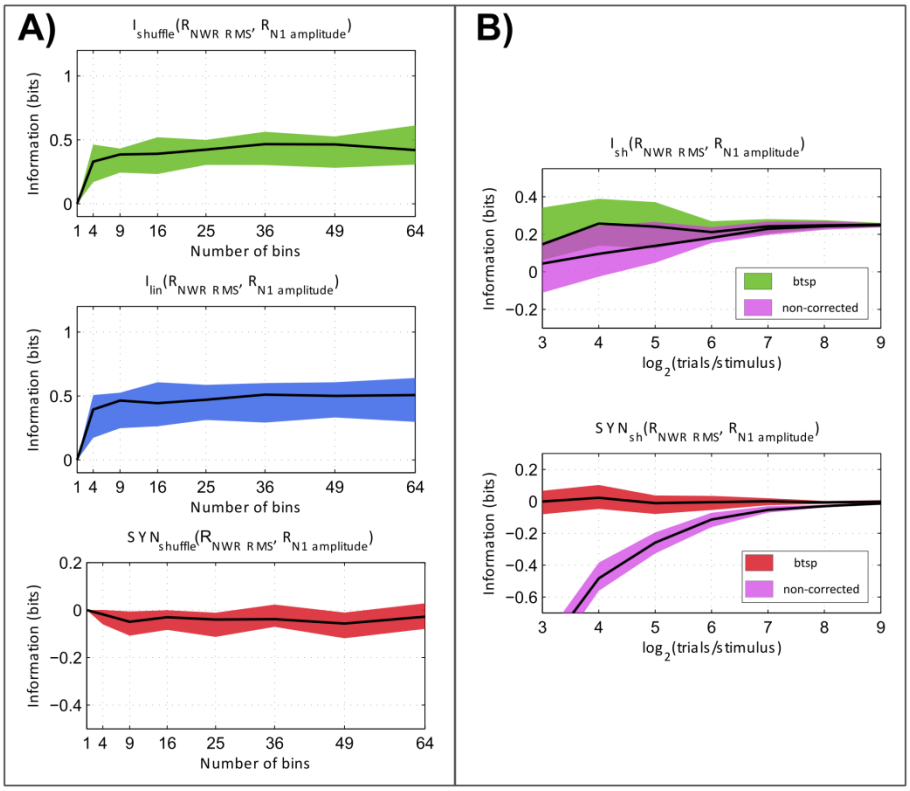

Figure 9. Validation of the parameters used in the calculation of mutual information quantities for a combination of response features. A) Median values of the information quantities I, I_{lin} and SYN provided by the combination of NWR RMS and N1 amplitude features (n=16) as a function of the number of bins. Solid line represents the median; shaded zones represent the 25 and 75 percentiles. B) Monte-Carlo simulation of bivariate information $I(S;R_{NWR\ RMS}, R_{N1\ amplitude})$ and $SYN(S;R_{NWR\ RMS}, R_{N1\ amplitude})$ about the stimulus as a function of the number of trials. Solid line represents the median; shaded zones represent the 25 and 75 percentiles for corrected (bstp) and non-corrected information.

Study II presented a novel approach that allows the quantification of information content carried by electrophysiological signals at single-trial level. The use of mutual information in other types of neurophysiological signals has increased steadily in the last years (Ince et al., 2010). Besides being used in electrophysiological studies, MI has been applied in other fields as e.g. the study of speech perception and the role of low-frequency phase information contained in MEG signals (Cogan and Poeppel, 2011). Additionally, MI has been employed in the integration of concurrent EEG and fMRI (Panzeri et al., 2008; Ostwald et al., 2010).

As expressed before, one of the main advantages of mutual information is that it is not constrained by parametric assumptions about the relationship between the signals of interest (Magri et al., 2012). MI considers linear and non-linear correlations at any order, although the calculation of higher-order correlations are limited by practical constraints related to the increasing number of variables and the limited number of trials in real experiments (Panzeri et al., 2007). In practice, real data from studies in humans which include painful manipulations are limited due to ethical and experimental constraints (e.g. habituation or sensitization to electrical stimulation). It is therefore vital to select the adequate parameters for MI estimation in order to make them fit within the experimental constraints, and validate them accordingly.

3.4. MUTUAL INFORMATION AS AN INDEX OF VARIABILITY

As mentioned in section 2.2.3, electrophysiological responses that reflect neural activation often display evident variability across repeated presentations of the stimuli that evoke them. The presence of this across-trial variability might have numerous different sources, possibly reflecting how sensory processing is shaped by neural network dynamics whose activity is often related to varying physiological and cognitive states (Arieli et al., 1996; Engel et al., 2001). Hence, a metric that can quantify the variability of these physiological signals might help to elucidate the different mechanisms behind the influence of interroceptive and/or exteroceptive factors in the perception of sensory processing.

A possible way to identify how discriminative is a neurophysiological response to repeated stimulation and to assess the influence of ongoing activity is to measure the amount of information it carries over its time course. This approach has been taken before to investigate how local field potentials (Belitski et al., 2008, 2010; Montemurro et al., 2008) and EEG fluctuations (Magri et al., 2009) encode complex naturalistic visual and auditory stimuli. In Study III, the use of IT is proposed in order to study the effect of cognitive tasks over the NWR and the SEPs.

3.4.1. Using mutual information to study the modulatory effects of attention over NWR and SEPs

Understanding the neural basis of attentional and emotional modulation of pain perception has been a relevant topic of several studies (Apkarian et al., 2005). Pain naturally captures attention, generally promoting the interruption of ongoing tasks to prioritize appropriate actions in order to avoid a potential threat (Legrain et al., 2009). Still, this inherent capacity of pain to redirect attention might be detrimental in chronic pain conditions where patients can find it difficult not to focus on the pain at the expense of the rest of daily living (Crombez et al., 2005). Therefore, studying how nociceptive stimuli capture attention and how the processing of this input is influenced by top-down factors might help explaining the mechanisms involved in pain-related conditions.

Electrophysiological responses such as the SEPs and NWR often exhibit variability which is typically associated to various unrelated factors and thus minimized on behalf of improving the SNR. However as explained above, trial-to-trial variability might contain information regarding ongoing physiological states which might be lost when signals are averaged across-trials (Arieli et al., 1996). Hence, the ability to measure across-trial variability of SEPs and NWR would allow incorporating the potential information contained in the variability of these responses to investigate how different emotional and cognitive processes can modulate somatosensory processing of nociceptive input.

In Study III, an experimental setup was established to assess the modulation exerted by two cognitive tasks over SEPs and the NWR during repeated electrical stimulation. The experiment consisted of a single session of six stimulation blocks with two alternating experimental conditions. The different conditions consisted of two cognitive tasks that had to be performed while receiving electrical stimulation: 1) attention to the stimuli (“attention”), where the subjects had to count the number of stimuli received; 2) distraction from stimulation (“distraction”), where subjects performed a modified version of the Stroop test (Stroop, 1935). Each block consisted of 24 stimuli (72 in total). Averaged responses for the two conditions are shown in Figure 10.

In Study III, a different approach was taken to compute the information carried by the responses about the repeated presentation of electrical stimuli. Each time point of the correspondent window of interest was considered as a different “stimulus” and labeled by an index s (Belitski et al., 2008). Thus, the obtained responses (NWR and SEPs) were then quantified in each time point and each trial. Particularly, the NWR was quantified by taking the rectified signal amplitude; the SEPs were quantified using the instantaneous power and phase of the EEG by means of Hilbert transform.

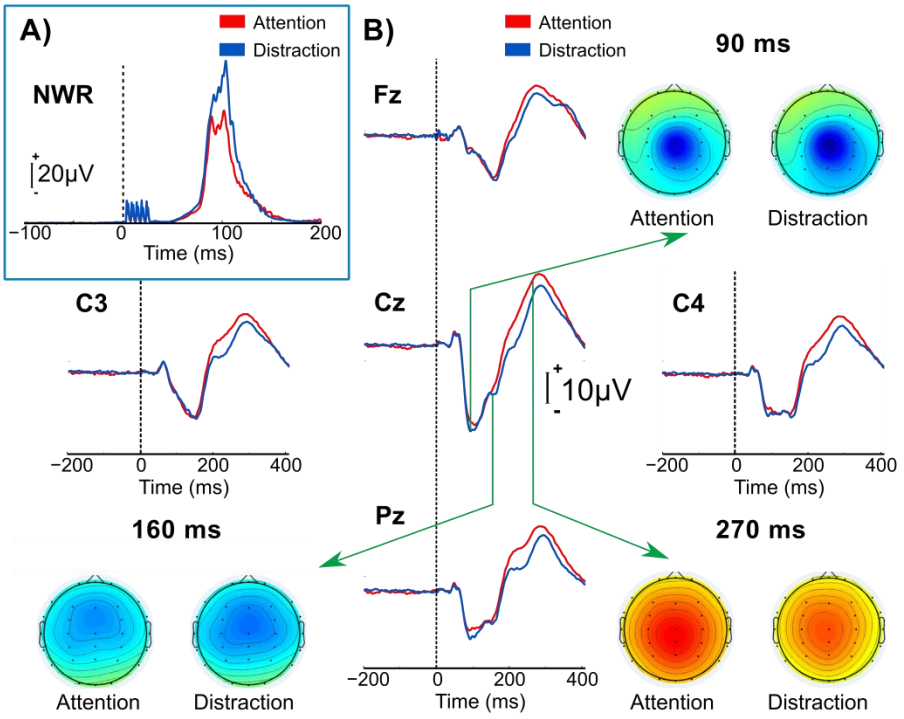

Figure 10. (A) Averaged NWR responses across participants for the two experimental conditions (n=13). (B)Averaged waveforms of the SEP responses and scalpmaps accross participants (n=13) for the two experimental conditions: attention to the stimulation and distraction from stimulation.

In order to estimate the probability distributions of NWR and SEPs, the response spaces where discretized by binning the NWR amplitude and the SEP instantaneous power into 12 equipopulated bins, and the SEP phase in 4 equipopulated bins. In total, one MI value was calculated for each subject, for each response (NWR amplitude and SEP power), for each channel and for each experimental condition (attention and distraction).

Results regarding the information carried by the NWR amplitude are shown in Figure 11. It was observed that the NWR was more informative (i.e. more discriminative) during distraction than the attention. Obtaining high information content is the result of good discrimination between the different stimuli (here referred as the different time points); this benefits from both distinct mean responses and high trial by trial reliability (Belitski et al., 2010). It is thus possible that the increased information observed in the NWR during distraction reflects a reliable lack of descending inhibition of spinal reflexes across trials under a demanding visual task. Instead, the attentional task of counting stimuli was probably not challenging enough to fully engage the subjects. This could lead to a variable focus

of attention on the stimulus and consequently varying level of descending control. As a result, smaller and less reliable spinal responses were observed in the attention condition and this was reflected in a reduction of their carried information.

The role of descending control from supraspinal centers over the NWR has been addressed in section 2.1.3. Descending control of spinal nociception is tonic, but there is a dynamical balance between inhibition and facilitation that depends on the behavioral, emotional and pathological state (Heinricher et al., 2009). It is possible that the lack of inhibition observed in the NWR during the distraction task in the present study is the result of descending modulatory control over the excitability of dorsal horn neurons. This mechanism might serve as an enhancement of nocifensive excitability advantageous for survival in situations where the attention is focused in another task.

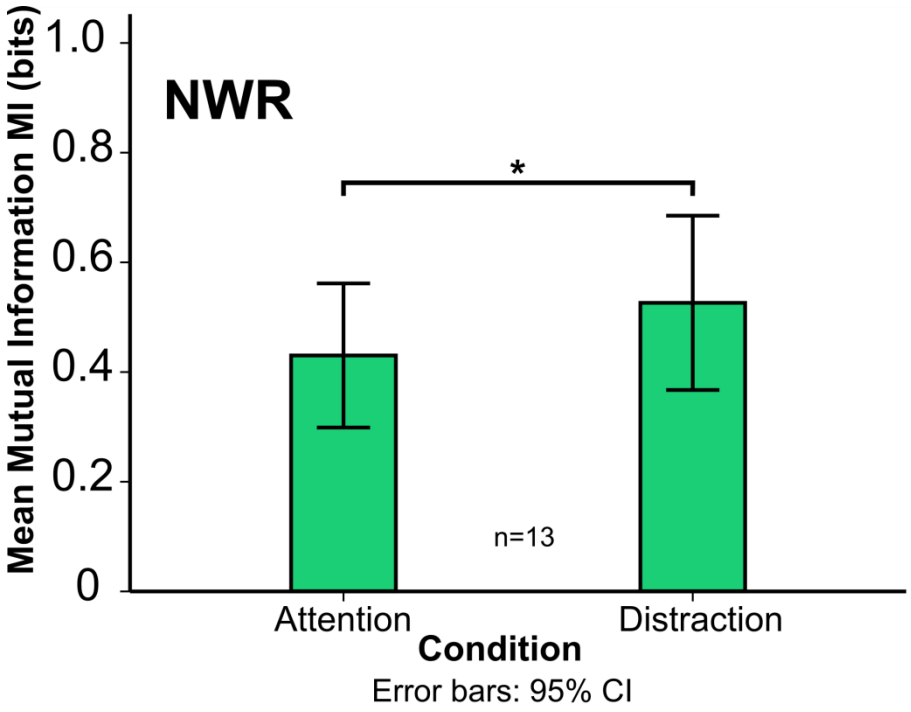

*Figure 11. Mutual information analysis of the NWR for the two experimental conditions. Mean values of the information I(S;R) about the stimulus carried by the amplitude of the nociceptive withdrawal reflex (NWR). The length of the window of interest where to perform this calculation was 50-160 ms. Error bars represent 95% confidence interval. *: $p<0.05$.*

Defining the stimulus set *S* by considering each time point as a different stimulus level *s* has the advantage that the information calculation considers the potential contributions of the effects of all time delays between the obtained response and the stimulus that evoked it. By using this definition, the observed response at eacth time point could be elicited by any sensory feature either occurring in that specific moment or in any previous time window (Belitski et al., 2010; Magri et al., 2012). Furthermore, it does not rely on any methods for extraction signal feature, e.g. peak detection as in Study II.

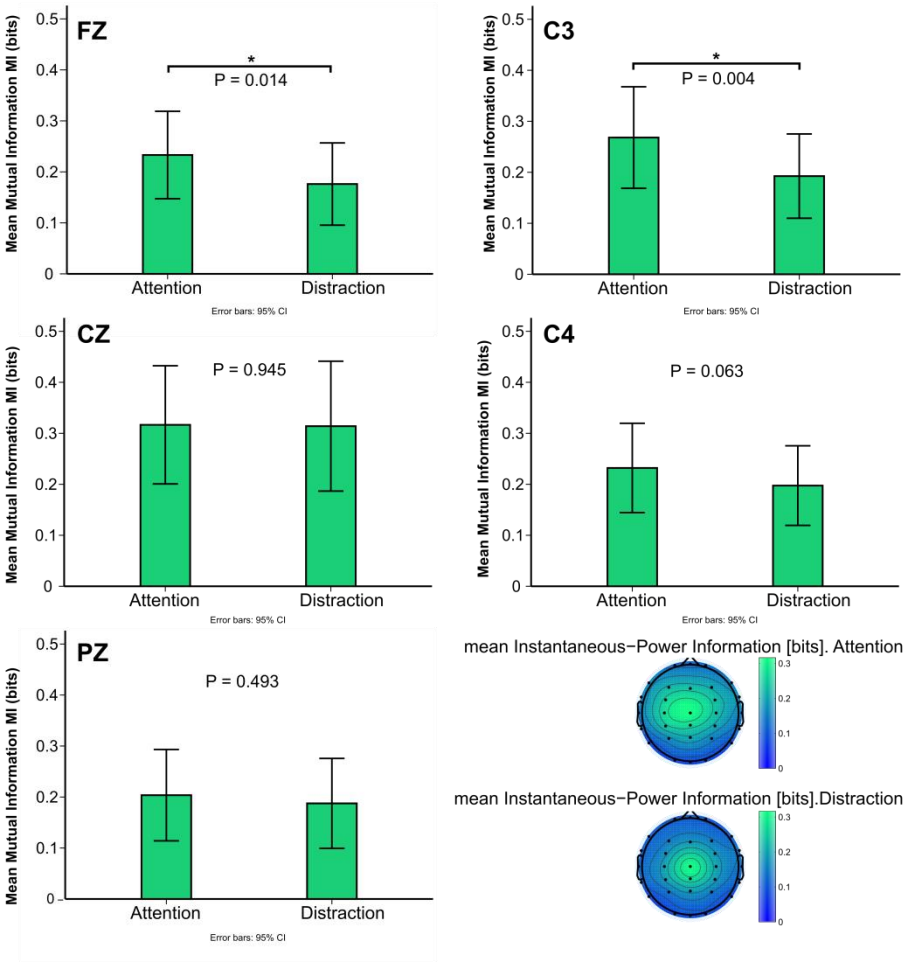

*Figure 12. Mutual information analysis of the SEPs for the two experimental conditions. Mean values of the information I(S;R) about the stimulus carried by the amplitude of the somatosensory evoked potentials at 5 different channels. The length of the window of interest where to perform this calculation was 40-400 ms. Error bars represent 95% confidence interval. *: p<0.05.*

On the contrary, information carried by SEP power was found to be less informative during the distraction task than the attention task (Figure 12) in Fz and contralateral to the site of stimulation (C3 channel). No significant differences were observed in the SEP phase between to the experimental conditions.

It is possible that the differences in information on the SEP power are reflecting the changes in the cognitive load between the two experimental conditions. The observed changes in contralateral and fronto-central scalp areas could be related to two SEP components which might index activity at the somatosensory association cortex and medial prefrontal cortex; these components are thought to be involved in changes in attentional control (Dowman, 2007).

CHAPTER 4. SYNTHESIS

The present dissertation described an evaluation of an alternative approach for the simultaneous assessment of single-trial spinal and supraspinal responses using Information theory. The present work introduced the first steps towards the use of mutual information for the integration of two electrophysiological methods (NWR and SEPs) that are widely used in the study of somatosensation.

The Ph.D. project comprised two stages, the first of which was to establish a setup for the simultaneous recording of NWR and SEPs elicited by electrical stimulation at different intensities and to evaluate the possibility of using single trials to assess their relation with the stimulus. In regards to single-trial estimation, Study I investigated the impact of choosing different detection and estimation techniques in the assessment of SEP features. Results showed large differences in the agreement between the chosen methods which consisted of two automatic algorithms and two human observers. These results highlighted the importance of selecting an appropriate single-trial estimation method that suits the specific experimental conditions. Studies that include single-trial estimation should put special focus in the selection process of the detection/estimation method, since a particular choice may even sway the outcome (and consequently, the interpretation) of an experiment. Concerning the simultaneous elicitation of NWR and SEPs, stimulus intensity was the first obvious choice as an experimental parameter since it is a straightforward way to modulate the magnitude of these two responses. As shown before with averaged responses, it was observed in Study II that increasing stimulus intensity naturally modulated both single-trial NWR and SEPs to different degrees. Interestingly, stimulation intensity did not have a large effect on the level of agreement between manual and automatic methods, so the difference found could be attributed to other causes, most notably the different approaches taken to address the presence/absence of a response.

The second stage evaluated the possibility of quantifying the information about the stimulus carried by features extracted from both NWR and SEPs. The analysis of the individual amount of information carried by the signal features showed that NWR features that encode the amplitude of the NWR were generally the most informative. These observations most likely reflect the difference in the number of concurrent neural sources being active at these two levels of the neuraxis. It was then shown that the information carried by NWR and SEP features can be assessed simultaneously, and that the information carried jointly between features of the two modalities was mainly redundant. This synergy/redundancy obtained from the MI calculation could be further divided in subsequent sub-terms which disentangle the possible impact of correlations between the variables on information transmission. Although these terms were available, they were not analyzed mainly because most SEP features already carried relatively low information about stimulation intensity.

Since the calculation of these sub-terms is more prone to be biased due to the limited amount of experimental data, making inferences on those results could lead to imprecise interpretations. Finally, MI was considered a way to assess the effect of cognitive modulatory tasks on the variability of both NWR and SEP signals. MI was therefore employed in Study III to quantify the amount of information about the repeated presentation of electrical stimuli contained in the time course of these two signals under two different experimental conditions: attention and distraction from the stimulus. It was found that during the distraction condition the NWR was more informative than the attention condition, possibly reflecting changes in the tonic control of supraspinal centers over the excitability of spinal pathways. Furthermore, the top-down modulation exerted onto the SEPs by ongoing cognitive processes such as the distraction task was reflected in a reduction of the information carried by the SEPs in relation to the stimulus.

All in all, it is possible to conclude that the IT framework is an appropriate and promising methodology to quantify spinal and supraspinal responses and their relation in pain research.

4.1. FUTURE PERSPECTIVES

From a methodological perspective there are many challenges ahead towards the inclusion of information theory for the assessment of simultaneous NWR and SEPs. Particularly, the lack of specificity of electrical stimulation for activating nociceptive fibers logically reduced the use of SEPs in pain research in the past. However, new electrode configurations and stimulation patterns that aim to activate nociceptive fibers are continuously being developed, which might provide a new alternative in that matter. It is also of interest to use MI to analyze how the information carried by spinal and supraspinal responses is influenced by other stimulation techniques. Furthermore, MI could be also used to assess information transmission between different cortical areas (connectivity analysis) during somatosensory processing.

As discussed in Study I, pre-processing methods can have big differences between their outcomes. In this regard, MI could very well be used to quantify how different pre-processing strategies affect the estimation of certain SEP or NWR features in order to further maximize the information they carry about a particular process. Overall, given the non-linear nature of sensory processing, the IT framework should be considered as a viable approach for understanding the non-linear mechanisms behind somatosensation.

REFERENCES

Andersen OK, Finnerup NB, Spaich EG, Jensen TS, Arendt-Nielsen L. Expansion of nociceptive withdrawal reflex receptive fields in spinal cord injured humans. *Clin Neurophysiol* 115: 2798–2810, 2004.

Andersen OK, Sonnenborg FA, Arendt-Nielsen L. Reflex receptive fields for human withdrawal reflexes elicited by non-painful and painful electrical stimulation of the foot sole. *Clin Neurophysiol* 112: 641–9, 2001.

Andersen OK. Studies of the organization of the human nociceptive withdrawal reflex. Focus on sensory convergence and stimulation site dependency. *Acta Physiol (Oxf)* 189 Suppl: 1–35, 2007.

Apkarian AV, Bushnell MC, Treede R-D, Zubieta J-K. Human brain mechanisms of pain perception and regulation in health and disease. *Eur J Pain* 9: 463–484, 2005.

Arendt-Nielsen L. Characteristics, detection, and modulation of laser-evoked vertex potentials. *Acta Anaesthesiol Scand Suppl* 101: 7–44, 1994.

Arendt-Nielsen L. Translational human pain research. *Eur J Pain Suppl* 1: 38–40, 2007.

Arieli A, Sterkin A, Grinvald A, Aertsen A. Dynamics of ongoing activity: explanation of the large variability in evoked cortical responses. *Science* 273: 1868–71, 1996.

Babiloni C, Brancucci A, Arendt-Nielsen L, Babiloni F, Capotosto P, Carducci F, Cincotti F, Del Percio C, Petrini L, Rossini PM, Chen ACN. Attentional processes and cognitive performance during expectancy of painful galvanic stimulations: a high-resolution EEG study. *Behav Brain Res* 152: 137–47, 2004.

Bathien N, Hugelin A. Réflexes monosynaptiques et polysynaptiques de l'homme au cours de l'attention. *Electroencephalogr Clin Neurophysiol* 26: 604–612, 1969.

Belitski A, Gretton A, Magri C, Murayama Y, Montemurro MA, Logothetis NK, Panzeri S. Low-frequency local field potentials and spikes in primary visual cortex convey independent visual information. *J Neurosci* 28: 5696–709, 2008.

Belitski A, Panzeri S, Magri C, Logothetis NK, Kayser C. Sensory information in local field potentials and spikes from visual and auditory cortices: time scales and frequency bands. *J Comput Neurosci* 29: 533–45, 2010.

Biurrun Manresa JA, Finnerup NSB, Johannesen IL, Biering-Sørensen F, Jensen TS, Arendt-Nielsen L, Andersen OK. Central sensitization in spinal cord injured humans assessed by reflex receptive fields. *Clin Neurophysiol* 125: 352–62, 2014.

Biurrun Manresa JA, Neziri AY, Curatolo M, Arendt-Nielsen L, Andersen OK. Reflex receptive fields are enlarged in patients with musculoskeletal low back and neck pain. *Pain* 154: 1318–24, 2013.

Bjerre L, Andersen AT, Hagelskjær MT, Ge N, Mørch CD, Andersen OK. Dynamic tuning of human withdrawal reflex receptive fields during cognitive attention and distraction tasks. *Eur J Pain* 15: 816–21, 2011.

Borst A, Theunissen FE. Information theory and neural coding. *Nat Neurosci* 2: 947–57, 1999.

Breivik H, Collett B, Ventafridda V, Cohen R, Gallacher D. Survey of chronic pain in Europe: prevalence, impact on daily life, and treatment. *Eur J Pain* 10: 287–333, 2006.

Busch NA, Dubois J, VanRullen R. The phase of ongoing EEG oscillations predicts visual perception. *J Neurosci* 29: 7869–76, 2009.

Carlson JD, Selden NR, Heinricher MM. Nocifensive reflex-related on- and off-cells in the pedunculopontine tegmental nucleus, cuneiform nucleus, and lateral dorsal tegmental nucleus. *Brain Res* 1063: 187–194, 2005.

Carstens E, Campell IG. Responses of motor units during the hind limb flexion withdrawal reflex evoked by noxious skin heating: phasic and prolonged suppression by midbrain stimulation and comparison with simultaneously recorded dorsal horn units. *Pain* 48, 1992.

Carstens E, Hartung M, Stelzer B, Zimmermann M. Suppression of a hind limb flexion withdrawal reflex by microinjection of glutamate or morphine into the periaqueductal gray in the rat. *Pain* 43: 105–112, 1990.

Cogan GB, Poeppel D. A mutual information analysis of neural coding of speech by low-frequency MEG phase information. *J Neurophysiol* 106: 554–63, 2011.

Cover T, Thomas J. Elements of information theory. 2nd ed. New York: John Wiley & Sons, Inc., 2006.

Crombez G, Van Damme S, Eccleston C. Hypervigilance to pain: An experimental and clinical analysis. *Pain* 116: 4–7, 2005.

Cruccu G, Aminoff MJ, Curio G, Guerit JM, Kakigi R, Mauguiere F, Rossini PM, Treede R-D, Garcia-Larrea L. Recommendations for the clinical use of somatosensory-evoked potentials. *Clin Neurophysiol* 119: 1705–19, 2008.

Cruccu G, Anand P, Attal N, Garcia-Larrea L, Haanpää M, Jørum E, Serra J, Jensen TS. EFNS guidelines on neuropathic pain assessment. *Eur J Neurol* 11: 153–62, 2004.

D'Avanzo C, Schiff S, Amodio P, Sparacino G. A Bayesian method to estimate single-trial event-related potentials with application to the study of the P300 variability. *J Neurosci Methods* 198: 114–24, 2011.

Dawson GD. A summation technique for the detection of small evoked potentials. *Electroencephalogr Clin Neurophysiol* 6: 65–84, 1954.

Debroucker T, Willer JC. Etude comparative du reflexe nociceptif et des composantes tardives du potentiel evoque somesthesique lors de stimulations du nerf sural chez l'homme normal. *Rev d&'apos;Electroencéphalographie Neurophysiol Clin* 15: 149–153, 1985.

Dietz V. Behavior of spinal neurons deprived of supraspinal input. *Nat Rev Neurol* 6: 167–174, 2010.

Von Dincklage F, Olbrich H, Baars JH, Rehberg B. Habituation of the nociceptive flexion reflex is dependent on inter-stimulus interval and stimulus intensity. *J Clin Neurosci* 20: 848–50, 2013.

Dowman R, Darcey T, Barkan H, Thadani V, Roberts D. Human intracranially-recorded cortical responses evoked by painful electrical stimulation of the sural nerve. *Neuroimage* 34: 743–763, 2007.

Dowman R, Darcey TM. SEP topographies elicited by innocuous and noxious sural nerve stimulation. III. Dipole source localization analysis. *Electroencephalogr Clin Neurophysiol* 92: 373–91, 1994.

Dowman R. Spinal and supraspinal correlates of nociception in man. *Pain* 45: 269–281, 1991.

Dowman R. SEP topographies elicited by innocuous and noxious sural nerve stimulation. II. Effects of stimulus intensity on topographic pattern and amplitude. *Electroencephalogr Clin Neurophysiol* 92: 303–15, 1994.

Dowman R. Attentional set effects on spinal and supraspinal responses to pain. *Psychophysiology* 38: 451–64, 2001.

Dowman R. Topographic analysis of painful laser and sural nerve electrical evoked potentials. *Brain Topogr* 16: 169–79, 2004.

Dowman R. Neural mechanisms of detecting and orienting attention toward unattended threatening somatosensory targets. I. Intermodal effects. *Psychophysiology* 44: 407–19, 2007.

Edwards L, Ring C, France CR, al'Absi M, McIntyre D, Carroll D, Martin U. Nociceptive flexion reflex thresholds and pain during rest and computer game play in patients with hypertension and individuals at risk for hypertension. *Biol Psychol* 76: 72–82, 2007.

Edwards L, Ring C, McIntyre D, Carroll D, Clarke R, Webb O, Martin U. Increases in Arousal Are Associated with Reductions in the Human Nociceptive Flexion Reflex Threshold and Pain Ratings. *J Psychophysiol* 20: 259–266, 2006.

Edwards L, Ring C, McIntyre D, Carroll D. Modulation of the human nociceptive flexion reflex across the cardiac cycle. *Psychophysiology* 38: 712–8, 2001.

Engel AK, Fries P, Singer W. Dynamic predictions: oscillations and synchrony in top-down processing. *Nat Rev Neurosci* 2: 704–16, 2001.

Fields HL, Malick A, Burstein R. Dorsal horn projection targets of ON and OFF cells in the rostral ventromedial medulla. *J Neurophysiol* 74, 1995.

Fields HL. State-dependent opioid control of pain. *Nat Rev Neurosci* 5: 565–75, 2004.

Garcia-Larrea L. Chapter 30 Evoked potentials in the assessment of pain. In: *PAIN*, edited by Cervero F, Jensen TS. Elsevier, 2006, p. 439 – 462, X–XI.

Gerdelat-Mas a, Simonetta-Moreau M, Thalamas C, Ory-Magne F, Slaoui T, Rascol O, Brefel-Courbon C. Levodopa raises objective pain threshold in Parkinson's disease: a RIII reflex study. *J Neurol Neurosurg Psychiatry* 78: 1140–1142, 2007.

Goffaux P, Redmond WJ, Rainville P, Marchand S. Descending analgesia--when the spine echoes what the brain expects. *Pain* 130: 137–43, 2007.

Goffaux P, de Souza JB, Potvin S, Marchand S. Pain relief through expectation supersedes descending inhibitory deficits in fibromyalgia patients. *Pain* 145: 18–23, 2009.

Golomb D, Hertz J, Panzeri S, Treves A, Richmond B. How Well Can We Estimate the Information Carried in Neuronal Responses from Limited Samples? *Neural Comput* 9: 649–665, 1997.

Haig AR, Gordon E, Rogers G, Anderson J. Classification of single-trial ERP sub-types: application of globally optimal vector quantization using simulated annealing. *Electroencephalogr Clin Neurophysiol* 94: 288–97, 1995.

Hämäläinen M, Hari R, Ilmoniemi RJ, Knuutila J, Lounasmaa O V. Magnetoencephalography—theory, instrumentation, and applications to noninvasive studies of the working human brain. *Rev Mod Phys* 65: 413–497, 1993.

Hatem SM, Hu L, Ragé M, Gierasimowicz A, Plaghki, Bouhassira D, Attal N, Iannetti GD, Mouraux A. Automated single-trial assessment of laser-evoked potentials as an objective functional diagnostic tool for the nociceptive system. *Clin Neurophysiol* 123: 2437–45, 2012.

Häuser W, Schmutzer G, Hinz A, Hilbert A, Brähler E. [Prevalence of chronic pain in Germany. A representative survey of the general population]. *Schmerz* 27: 46–55, 2013.

Heinricher MM, Cheng Z, Fields HL. Evidence for two classes of nociceptive modulating neurons in the periaqueductal gray. *J Neurosci* 7: 271–278, 1987.

Heinricher MM, Tavares I, Leith JL, Lumb BM. Descending control of nociception: Specificity, recruitment and plasticity. *Brain Res Rev* 60: 214–25, 2009.

Hu L, Liang M, Mouraux A, Wise RG, Hu Y, Iannetti GD. Taking into account latency, amplitude, and morphology: improved estimation of single-trial ERPs by wavelet filtering and multiple linear regression. *J Neurophysiol* 106: 3216–29, 2011a.

Hu L, Mouraux A, Hu Y, Iannetti GD. A novel approach for enhancing the signal-to-noise ratio and detecting automatically event-related potentials (ERPs) in single trials. *Neuroimage* 50: 99–111, 2010.

Hu L, Zhang ZG, Hung YS, Luk KDK, Iannetti GD, Hu Y. Single-trial detection of somatosensory evoked potentials by probabilistic independent component analysis and wavelet filtering. *Clin. Neurophysiol.* (February 2011b). doi: 10.1016/j.clinph.2010.12.052.

Iannetti GD, Hughes NP, Lee MC, Mouraux A. Determinants of laser-evoked EEG responses: pain perception or stimulus saliency? *J Neurophysiol* 100: 815–28, 2008.

Iannetti GD, Zambreanu L, Cruccu G, Tracey I. Operculoinsular cortex encodes pain intensity at the earliest stages of cortical processing as indicated by amplitude of laser-evoked potentials in humans. *Neuroscience* 131: 199–208, 2005.

Ince RAA, Mazzoni A, Petersen RS, Panzeri S. Open source tools for the information theoretic analysis of neural data. *Front Neurosci* 4: 62–70, 2010.

Jarchi D, Sanei S, Mohseni HR, Lorist MM. Coupled particle filtering: A new approach for P300-based analysis of mental fatigue☆. *Biomed Signal Process Control* 6: 175–185, 2011a.

Jarchi D, Sanei S, Principe JC, Makkiabadi B. A new spatiotemporal filtering method for single-trial estimation of correlated ERP subcomponents. *IEEE Trans Biomed Eng* 58: 132–43, 2011b.

Kakigi R, Inui K, Tamura Y. Electrophysiological studies on human pain perception. *Clin Neurophysiol* 116: 743–63, 2005.

Kisley MA, Gerstein GL. Trial-to-trial variability and state-dependent modulation of auditory-evoked responses in cortex. *J Neurosci* 19: 10451–60, 1999.

Kolb TFB, Lachauer S, Schoch B, Gerwig M, Timmann D, Kolb FP. Comparison of the electrically evoked leg withdrawal reflex in cerebellar patients and healthy controls. *Exp. Brain Res.* 177: 493–508, 2007.

Kugelberg E, Eklund K, Grimby L. An electromyographic study of the nociceptive reflexes of the lower limb. Mechanism of the plantar responses. *Brain* 83: 394–410, 1960.

Kugelberg E. Demonstration of A and C fibre components in the Babinski plantar response and the pathological flexion reflex. *Brain* 71: 304–319, 1948.

Lazzaro I, Anderson J, Gordon E, Clarke S, Leong J, Meares R. Single trial variability within the P300 (250-500 ms) processing window in adolescents with attention deficit hyperactivity disorder. *Psychiatry Res* 73: 91–101, 1997.

Legrain V, Van Damme S, Eccleston C, Davis KD, Seminowicz DA, Crombez G. A neurocognitive model of attention to pain: behavioral and neuroimaging evidence. *Pain* 144: 230–2, 2009.

Legrain V, Mancini F, Sambo CF, Torta DM, Ronga I, Valentini E. Cognitive aspects of nociception and pain: bridging neurophysiology with cognitive psychology. *Neurophysiol Clin* 42: 325–36, 2012.

Lim ECW, Sterling M, Pedler A, Coombes BK, Vicenzino B. Evidence of spinal cord hyperexcitability as measured with nociceptive flexion reflex (NFR) threshold in chronic lateral epicondylalgia with or without a positive neurodynamic test. *J Pain* 13: 676–84, 2012.

Lim ECW, Sterling M, Stone A, Vicenzino B. Central hyperexcitability as measured with nociceptive flexor reflex threshold in chronic musculoskeletal pain: a systematic review. *Pain* 152: 1811–20, 2011.

Magri C, Mazzoni A, Logothetis NK, Panzeri S. Optimal band separation of extracellular field potentials. *J Neurosci Methods* 210: 66–78, 2012.

Magri C, Whittingstall K, Singh V, Logothetis NK, Panzeri S. A toolbox for the fast information analysis of multiple-site LFP, EEG and spike train recordings. *BMC Neurosci* 10: 81, 2009.

Mayhew SD, Iannetti GD, Woolrich MW, Wise RG. Automated single-trial measurement of amplitude and latency of laser-evoked potentials (LEPs) using multiple linear regression. *Clin Neurophysiol* 117: 1331–44, 2006.

McIntyre D, Edwards L, Ring C, Parvin B, Carroll D. Systolic inhibition of nociceptive responding is moderated by arousal. *Psychophysiology* 43: 314–9, 2006.

Meinck HM, Küster S, Benecke R, Conrad B. The flexor reflex—influence of stimulus parameters on the reflex response. *Electroencephalogr Clin Neurophysiol* 61: 287–298, 1985.

Meinck HM, Piesiur-Strehlow B, Koehler W. Some principles of flexor reflex generation in human leg muscles. *Electroencephalogr Clin Neurophysiol* 52: 140–50, 1981.

Milanov IG. Flexor reflex for assessment of common interneurone activity in spasticity. *Electromyogr Clin Neurophysiol* 32: 621—629, 1992.

Montemurro MA, Rasch MJ, Murayama Y, Logothetis NK, Panzeri S. Phase-of-Firing Coding of Natural Visual Stimuli in Primary Visual Cortex. *Curr Biol* 18: 375–380, 2008.

Mørch CD, Andersen OK, Graven-Nielsen T, Arendt-Nielsen L. Nociceptive withdrawal reflexes evoked by uniform-temperature laser heat stimulation of large skin areas in humans. *J Neurosci Methods* 160: 85–92, 2007.

Mouraux A, Iannetti GD. Across-trial averaging of event-related EEG responses and beyond. *Magn Reson Imaging* 26: 1041–54, 2008.

Mouraux A, Iannetti GD. Nociceptive Laser-Evoked Brain Potentials Do Not Reflect Nociceptive-Specific Neural Activity. *J Neurophysiol* 101: 3258–3269, 2009.

Mylius V, Engau I, Teepker M, Stiasny-Kolster K, Schepelmann K, Oertel WH, Lautenbacher S, Möller JC. Pain sensitivity and descending inhibition of pain in Parkinson's disease. *J Neurol Neurosurg Psychiatry* 80: 24–28, 2009.

Nakajima T, Mezzarane RA, Hundza SR, Komiyama T, Zehr EP. Convergence in Reflex Pathways from Multiple Cutaneous Nerves Innervating the Foot Depends upon the Number of Rhythmically Active Limbs during Locomotion. *PLoS One* 9: e104910, 2014.

Neziri AY, Haesler S, Petersen-Felix S, Müller M, Arendt-Nielsen L, Biurrun Manresa JA, Andersen OK, Curatolo M. Generalized expansion of nociceptive reflex receptive fields in chronic pain patients. *Pain* 151: 798–805, 2010.

Optican LM, Richmond BJ. Temporal encoding of two-dimensional patterns by single units in primate inferior temporal cortex. III. Information theoretic analysis. *J Neurophysiol* 57: 162–78, 1987.

Osborne JW, Waters E. Four assumptions of multiple regression that researchers should always test. *Pract assessment, Res Eval* 8: 1–9, 2002.

Ostwald D, Porcaro C, Bagshaw AP. An information theoretic approach to EEG-fMRI integration of visually evoked responses. *Neuroimage* 49: 498–516, 2010.

Panzeri S, Magri C, Logothetis NK. On the use of information theory for the analysis of the relationship between neural and imaging signals. *Magn Reson Imaging* 26: 1015–25, 2008.

Panzeri S, Schultz SR, Treves A, Rolls ET. Correlations and the encoding of information in the nervous system. *Proc Biol Sci* 266: 1001–12, 1999.

Panzeri S, Senatore R, Montemurro MA, Petersen RS. Correcting for the sampling bias problem in spike train information measures. *J Neurophysiol* 98: 1064–72, 2007.

Parra L, Alvino C, Tang A, Pearlmutter B, Yeung N, Osman A, Sajda P. Linear Spatial Integration for Single-Trial Detection in Encephalography. *Neuroimage* 17: 223–230, 2002.

Perchet C, Godinho F, Mazza S, Frot M, Legrain V, Magnin M, Garcia-Larrea L. Evoked potentials to nociceptive stimuli delivered by CO2 or Nd:YAP lasers. *Clin Neurophysiol* 119: 2615–22, 2008.

Pernet CR, Sajda P, Rousselet GA. Single-trial analyses: why bother? *Front Psychol* 2: 322, 2011.

Peterson NN, Schroeder CE, Arezzo JC. Neural generators of early cortical somatosensory evoked potentials in the awake monkey. *Electroencephalogr Clin Neurophysiol* 96: 248–60, 1995.

Pfurtscheller G, Lopes da Silva FH. Event-related EEG/MEG synchronization and desynchronization: basic principles. *Clin Neurophysiol* 110: 1842–57, 1999.

Piater J, Stuchlik F, von Specht H, Mühler R. Fuzzy sets for feature identification in biomedical signals with self-assessment of reliability: an adaptable algorithm modeling human procedure in BAEP analysis. *Comput Biomed Res* 28: 335–53, 1995.

Picton TW, Bentin S, Berg P, Donchin E, Hillyard SA, Johnson R, Miller GA, Ritter W, Ruchkin DS, Rugg MD, Taylor MJ. Guidelines for using human event-related potentials to study cognition: recording standards and publication criteria. *Psychophysiology* 37: 127–52, 2000.

Pola G, Thiele A, Hoffmann KP, Panzeri S. An exact method to quantify the information transmitted by different mechanisms of correlational coding. *Network* 14: 35–60, 2003.

Quian Quiroga R, Garcia H. Single-trial event-related potentials with wavelet denoising. *Clin Neurophysiol* 114: 376–390, 2003.

Quian Quiroga R, Panzeri S. Extracting information from neuronal populations: information theory and decoding approaches. *Nat Rev Neurosci* 10: 173–85, 2009.

Raftery MN, Sarma K, Murphy AW, De la Harpe D, Normand C, McGuire BE. Chronic pain in the Republic of Ireland--community prevalence, psychosocial profile and predictors of pain-related disability: results from the Prevalence, Impact and Cost of Chronic Pain (PRIME) study, part 1. *Pain* 152: 1096–103, 2011.

Rhudy JL, France CR. Defining the nociceptive flexion reflex (NFR) threshold in human participants: a comparison of different scoring criteria. *Pain* 128: 244–53, 2007.

Rhudy JL, Williams AE, McCabe KM, Nguyen MAT V, Rambo P. Affective modulation of nociception at spinal and supraspinal levels. *Psychophysiology* 42: 579–87, 2005.

Rhudy JL, Williams AE, McCabe KM, Rambo PL, Russell JL. Emotional modulation of spinal nociception and pain: the impact of predictable noxious stimulation. *Pain* 126: 221–33, 2006.

Ronga I, Valentini E, Mouraux A, Iannetti GD. Novelty is not enough: laser-evoked potentials are determined by stimulus saliency, not absolute novelty. *J Neurophysiol* 109: 692–701, 2013.

Sandrini G, Arrigo A, Bono G, Nappi G. The Nociceptive Flexion Reflex as a Tool for Exploring Pain Control Systems in Headache and Other Pain Syndromes. *Cephalalgia* 13: 21–27, 1993.

Sandrini G, Serrao M, Rossi P, Romaniello A, Cruccu G, Willer JC. The lower limb flexion reflex in humans. *Prog Neurobiol* 77: 353–95, 2005.

Schneidman E, Bialek W, Berry MJ. Synergy, redundancy, and independence in population codes. *J Neurosci* 23: 11539–53, 2003.

Schomburg ED. Spinal sensorimotor systems and their supraspinal control. *Neurosci Res* 7: 265–340, 1990.

Shahani BT, Young RR. Human flexor reflexes. *J Neurol Neurosurg Psychiatry* 34: 616–627, 1971.

Shannon CE. A Mathematical Theory of Communication. *Bell Syst Tech J* 27: 379–423, 1948.

Sherrington CS. Flexion-reflex of the limb, crossed extension-reflex, and reflex stepping and standing. *J Physiol* 40: 28–121, 1910.

Sjøgren P, Ekholm O, Peuckmann V, Grønbaek M. Epidemiology of chronic pain in Denmark: an update. *Eur J Pain* 13: 287–92, 2009.

Sonnenborg FA, Andersen OK, Arendt-Nielsen L. Modular organization of excitatory and inhibitory reflex receptive fields elicited by electrical stimulation of the foot sole in man. *Clin Neurophysiol* 111: 2160–9, 2000.

Spaich EG, Arendt-Nielsen L, Andersen OK. Modulation of lower limb withdrawal reflexes during gait: a topographical study. *J Neurophysiol* 91: 258–66, 2004.

Stroop JR. Studies of interference in serial verbal reactions. *J Exp Psychol* 18: 643–662, 1935.

Terkelsen AJ, Andersen OK, Mølgaard H, Hansen J, Jensen TS. Mental stress inhibits pain perception and heart rate variability but not a nociceptive withdrawal reflex. *Acta Physiol Scand* 180: 405–14, 2004.

Tracey I, Mantyh PW. The cerebral signature for pain perception and its modulation. *Neuron* 55: 377–91, 2007.

Treede RD, Kief S, Hölzer T, Bromm B. Late somatosensory evoked cerebral potentials in response to cutaneous heat stimuli. *Electroencephalogr Clin Neurophysiol* 70: 429–41, 1988.

Villemure C, Bushnell MC. Mood influences supraspinal pain processing separately from attention. *J Neurosci* 29: 705–715, 2009.

Wall PD. The laminar organization of dorsal horn and effects of descending impulses. *J Physiol* 188: 403–423, 1967.

Wendel K, Väisänen O, Malmivuo J, Gencer NG, Vanrumste B, Durka PJ, Magjarević R, Supek S, Pascu ML, Fontenelle H, Grave de Peralta Menendez R. EEG/MEG Source Imaging: Methods, Challenges, and Open Issues. *Comput Intell Neurosci* 2009: 656092, 2009.

Willer JC, Boureau F, Albe-Fessard D. Supraspinal influences on nociceptive flexion reflex and pain sensation in man. *Brain Res* 179: 61–68, 1979a.

Willer JC, Boureau F, Berny J. Nociceptive flexion reflexes elicited by noxious laser radiant heat in man. *Pain* 7: 15–20, 1979b.

Willer JC, De Broucker T, Barranquero A, Kahn MF, Debroucker T. Brain evoked potentials to noxious sural nerve stimulation in sciatalgic patients. *Pain* 30: 47–58, 1987.

Willer JC. Comparative study of perceived pain and nociceptive flexion reflex in man. *Pain* 3: 69–80, 1977.

SUMMARY

The nociceptive withdrawal reflex (NWR) and the somatosensory evoked potentials (SEPs) are two physiological responses that reflect spinal and supraspinal sensory processing, respectively. Although they can be elicited synchronously, its concurrent use in pain research is limited. Still, its assessment has mainly focused on the averaged signals across trials. Yet, an increasing body of work suggests that across-trial variability should be considered as a functional property of the nervous system that could index modulatory and clinical conditions.

In this Ph.D. project the aims were to study the viability of using single-trial (ST) features from both NWR and SEPs and to introduce Information Theory (IT) as a viable approach to integrate ST data and to characterize signal variability of these two signals to provide more insight about pain processing mechanisms.

Results emphasized the impact of selecting different ST detection methods. Moreover, it was shown that the IT framework can be used to quantify the information carried jointly by NWR and SEPs. Finally, it was found that cognitive modulatory tasks were accompanied by changes in the variability of the NWR and SEPs, and this was reflected in the information content across conditions.

In conclusion, the IT framework is a suitable and promising methodology to quantify the relation between spinal and supraspinal activity in pain research.

ISSN (online): 2246-1302
ISBN (online): 978-87-7112-352-4

AALBORG **UNIVERSITY PRESS**