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PAIN MODULATION INDUCED BY HETEROTOPIC NOXIOUS COUNTER-STIMULATION (HNCS)
PSYCHOPHYSIOLOGICAL ASSESSMENT OF ADEQUATE STIMULATION PARADIGMS AND SEX-RELATED EFFECTS

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Abstract

This work comprises three studies whose main concern was to find a valid tonic pain model able to trigger a genuine diffuse noxious pain inhibition. All studies were performed in healthy, drug-free volunteers and whereas the first two are validation studies, the third is an application study of the previous two.

The aim of the first study was to characterize the cold pressor (CPT) and hot water immersion test (HIT) from a physiological and a psychophysical point of view. A second issue was to clarify the origin of potential autonomic responses during both tests; are they related to baroreflex activity or rather a consequence of the pain experience per se? The study was performed in 30 volunteers aged 19-57 (median 24) years, and consisted of a single session including one CPT (4 ± 0.2°C) and one HIT (47 ± 0.5°C) with a cut-off-point of 5 minutes. Participants were randomly assigned to sequence order (the sequence of both trials was alternated) and groups were paralleled with respect to gender. Cardiovascular, respiratory and electrodermal activities as well as subjective pain intensity were continuously monitored. Pain detection and tolerance thresholds as well as pain unpleasantness and nervous tension were assessed additionally. Both tests were found to be comparable with respect to intensity of subjective pain and time course, but a significantly higher blood pressure increase during CPT could be observed, compared to the HIT. In conclusion, the HIT appears to be less confounded with baroreflex activity and hence seems to be a more adequate tonic pain model.

The second study tested the internal validity of inter-digital web pinching (IWP) with regard to its potential as DNIC-trigger. 24 gender-matched participants, aged 21-54 (median 25) years, volunteered for the controlled study. The protocol included the assessment of thermal and mechanical perceptual wind-up (WU) before and after a HIT (47.5 °C) or an IWP (15 N) of 2 minutes duration each. WU pain was induced by 10 repetitive (1 Hz) contact heat (max. 49°C; 5 5 mm thermode) or 10 ballistic impact stimuli (0.5 g at 9m/s) on the phalanges of the non-dominant hand. Cardiovascular and corrugator muscle activity as well as pain experience were permanently monitored. Both heterotopic noxious counter-stimulation (HNCS) types produced a similar pain experience, but a more pronounced cardiovascular activity was observed for the HIT. Painful water immersion is though accompanied by a stronger baroreceptor activity. WU pain was significantly reduced for both pain modalities, although the inhibition was somewhat stronger for the HIT than the IWP. The IWP, being practically uncontaminated by baroreflex sensitivity (BRS), proved its validity as DNIC-trigger.

The third study investigated temporal characteristics of electrically elicited pain and nocifensive RIII-reflex activity in a gender-balanced sample of 28 volunteers aged 21-38 (median 27) years, using IWP as HNCS, a tonic pain model previously validated to be BRS-unrelated. Sex-related differences in the post HNCS time courses of pain perception were identified with women demonstrating a more rapid return to baseline compared to men. Interestingly, an opposite pattern was observed regarding nociceptive reflex activity with a steeper return rate of electromyographic responses in males, whereas those of women remained attenuated over the entire observation period. These findings may reflect a stronger defensive response to pain in women.

Declaration of originality

I, Anouk Streff, declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institute of tertiary education. I did not use, not even partly, work of foreigners and I solely used the cited references for the articles and the list at the end of the thesis.

Luxembourg, 09/08/2010 Anouk Streff

Abbreviations

AM	arithmetic mean
ANOVA	analysis of variance
ANS	autonomous nervous system
BL	baseline
BP	blood pressure
bpm	beats per minute
bpt	beats per test
BRS	baroreflex sensitivity
CPT	cold pressor test
DH	dorsal horn
DLF	dorsolateral funiculus
DNIC	diffuse noxious inhibitory controls
EA	endogenous analgesia
ECG	electrocardiogram
EDA	electrodermal activity
EMG	electromyogram
FM	fibromyalgia
HIT	hot water immersion test
HNCS	heterotopic noxious counter-stimulation
HPA	hypothalamus-pituitary adrenal (axis)
HPG	hypothalamus-pituitary-gonadal (axis)
HR	heart rate
HRV	heart rate varaibility
Hz	hertz
IASP	International Association for the Study of Pain
IWP	inter-digital web pinching
LF/HF	low frequency/high frequency
LLFR	lower limb flexion reflex
MAD	mean average deviation
Md	median

mA	milliampere
mmHg	millimeters of mercury
mmho	milliohm
ms	milliseconds
mS	millisiemens
µS	microsiemens
(µ)V	(micro)volt
N	Newton
NA	noradrenaline
NRM	nucleus raphe magnus
NRS	numerical rating scale
NT	nervous tension
PAG	periaqueductal grey
RR	respiration rate
RVM	rostroventromedial medulla
SC	spinal cord
SEM	standard error of mean
SES	Schmerzempfindungs-Skala
SETT	submaximal effort tourniquet test
SG	substantia gelatinosa
SIA	stress-induced analgesia
SRD	subnucleus reticularis dorsalis
T	transmission
TENS	transcutaneous electrical nervous stimulation
VAS	visual analogue scale
VMM	ventromedial medulla
WDR	wide dynamic range
WU	wind-up

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Table of contents

1 Theoretical background – endogenous pain modulation

"One fire burns out another's burning,
One pain is lessened by another's anguish"
(W. Shakespeare)

Descending pain modulating processes are well-documented networks capable of regulating the actual pain processing both in an inhibitory (anti-nociception) and in a facilitatory (pro-nociception) way. Gebhart (2004) sees the teleological use of descending inhibitory systems in the avoidance of unnecessary stress or anxiety or in the preparation of the organism for flight and/or fight reactions which would be compromised by a concomitant suffering of intense pain. The importance of a negative feedback loop as observed under DNIC conditions could in turn lie in a contrast sharpening filter enhancing the sensitivity of the stimulated area as compared to surrounding body regions (Le Bars et al., 1979a, 1979b).

Descending facilitatory influences are now known to contribute to the development and maintenance of hyperalgesia and hence pain chronification under pathophysiological conditions (Perrotta et al., 2009, in press). For evolutionary biologists, endogenous pain facilitation (primary hyperalgesia) processes may be more difficult to explain at the beginning, but intensification of pain may prevent the organism from further damage and the resulting indisposition could impose a resting period.

To introduce the essence of this thesis and the main content of the three successive studies, I would like to make a detour to indispensable developmental steps in the description of endogenous pain modulation processes extending from spinal segmental mechanisms over supraspinal to stress-induced analgesia (SIA), heterogeneous pain modulation and eventually diffuse noxious inhibitory controls (DNIC). With DNIC being the gist of this work, I will focus on a very old concept (cf. the Hippocrates aphorism: "Of two pains occurring together, not in the same part of the body, the stronger weakens

the other.") that in modern times has originally been studied and defined by Le Bars et al. in 1979. I will also try to expose how it has to be understood within the framework of this thesis and which stimulation paradigms are adequate for the triggering of the inherent pain modulation mechanism. Heterotopic noxious counter-stimulation (HNCS) employing different experimental pain stimuli (thermal, ischemic, chemical or mechanical) is normally used to elicit DNIC. Many of the used stimulation paradigms may imply a certain number of modulating or confounding variables, which can in turn influence descending pain modulation and hence should be kept in mind: cardiovascular parameters and baroreflex sensitivity (BRS), stress, psychological variables and sex-related effects as well as time courses or a differential post DNIC recuperation time.

1.1 Spinal segmental pain modulation

The dorsal horn (DH) of the spinal cord (SC) is the major receiving zone for primary afferent axons transmitting information from sensory receptors in the skin, viscera, joints and muscles to the central nervous system (CNS). Among others, simple observations like vigourously rubbing one's toe after hitting it against a table-leg e.g. (a natural and quite effective reaction) led Melzack and Wall to develop their gate control theory of pain. In fact, in 1965, they proposed that inhibitory interneurons located in the superficial part of the DH play a crucial role in controlling incoming sensory information before the latter is transmitted to the brain through ascending pathways (Todd and Koerber, 2006). The "gating" of sensory inputs at their first synaptic relay constituted the actual innovation of this theory. The authors tried to elucidate how three SC systems are involved in the processing of noxious sensory inputs: the dorsal-column fibers that project towards the brain, the substantia gelatinosa (SG) and the first central transmission (T) cells both in the DH. The SG was described as the actual "gate control system that modulates the synaptic transmission of nerve impulses from peripheral fibers to central cells" (Melzack and Wall, 1965). Furthermore, the afferent input is divided into "large" (tactile, i.e. responsible for touch, pressure and vibration) and "small" (nociceptive, i.e. responsible for pain) fibers projecting to the "gate control system" where the inhibitory effect of the SG on the T cells is enhanced by the large (Aβ-fibers) and reduced by the small (C-fibers) afferent fibers. Pain is only felt when the T-neuron is activated due to

prevailing excitatory influences resulting from excessive nociceptor input or because of missing inhibitory processes normally triggered by non-nociceptive fibers. In addition, the brain exerts control on the mentioned gating system (central control trigger). This aspect stresses the importance of the integration of physiological and psychological components of pain processing. More to the point, pain sensation is subjective and, in addition to peripheral input, can depend on emotions and cognitive and attentional processes.

The main merit of the gate control theory was to emphasize dynamic and plastic aspects of pain and to draw the scientific community's attention to the importance of pain modulation as opposed to considering pain as a simple hard-wired alarm system (Cervero, 2005). The theory gave scientists a rationale to develop and use treatment methods based on transcutaneous electrical stimulation (TENS; Kalra et al., 2001), opioids and other analgesics, thus constituting a direct source of inspiration for pathological pain models (Dickenson, 2002).

In 1968, Melzack and Casey expanded the gate control theory and the spinal segmental influences to cerebro-spinal interactions and put pain experience into a more complete, multidimensional context. A general, objective pain characterization turns out to be an exceedingly problematic activity, because both valence and intensity of pain depend on the noxious input, the personality of the experiencing subject and the context in which the pain occurs. While the gate control theory was mainly concerned with how the CNS deals with sensory inputs, pain is now described as having sensory-discriminative, affective-motivational, cognitive-evaluative and behavioral (vegetative and motor) components.

1.2 Supraspinal pain modulation

In the late 70s, Basbaum and Fields began to develop a more detailed neurophysiological model of inhibitory descending pathways integrating the brainstem and its projections to the spinal cord (Fields and Basbaum, 1978; Basbaum and Fields, 1984). The periaqueductal gray (PAG) in the mesencephalon was soon established as a principal site of descending pain modulation (Ossipov and Porreca, 2005 for review) because of some very important discoveries: electrical stimulation of the PAG produced analgesia (later on: stimulation provoked analgesia) in rats strong enough to permit surgery, a radical

demonstration of descending antinociception (Reynolds, 1969; Mayer and Liebeskind, 1974). Morphine microinjected into the ventrolateral PAG also attenuated nociception (Jacquet and Lajtha, 1973; cf. figure 1). Since direct projections between the PAG and the SC are rare, other brainstem structures had to be involved. The rostroventral medulla (RVM), including the serotonergic nucleus raphe magnus (NRM) and the nucleus gigantocellularis pars alpha, has been identified as a principal relay station between ascending nociceptive inputs and descending inputs from rostral sites able to modulate nociception. Direct and reciprocal communications between the RVM and the PAG are assumed to be firm, and when the RVM is stimulated electrically, or microinjected with morphine, behavioral anti-nociception as well as inhibition of dorsal horn units to noxious inputs are the result (Basbaum and Fields, 1978). At each spinal segment, the axons of serotonergic neurons in the NRM project directly to the DH of the SC (plus noradrenergic projections frome the locus coeruleus (LC)). The reticular formation in the medulla and PAG project to the RVM on their part. Thus, the PAG and NRM are implicated in a spinal-medullary-spinal negative feedback loop as suggested by Basbaum and Fields (1984) and support the notion of an endogenous analgesic system triggered by nociceptive stimuli.

Briefly, throughout the 1970s, a number of anatomical and physiological studies elucidated a major pathway from the PAG to the nucleus raphe magnus and the adjacent reticular formation of the ventromedial medulla (VMM). Furthermore, the ending zone of numerous VMM axons in the DH matches the region where nociceptors terminate, reinforcing the idea that the PAG and VMM specifically modulate nociception (Mason, 2005).

Since 1990, functional brain imaging studies of pain in humans have provided evidence for the role of several cortical and sub-cortical areas in pain perception. The limited anatomical and physiological evidence and insight available from primate studies on cortical pain processing could thus be complemented. Different brain imaging studies demonstrate a reduction in cortical responses to acute pain by analgesic drugs (Casey, 2000), the release of endogenous opioids (Zubieta et al. 2001, 2003; Petrovic et al., 2002) and psychological factors.

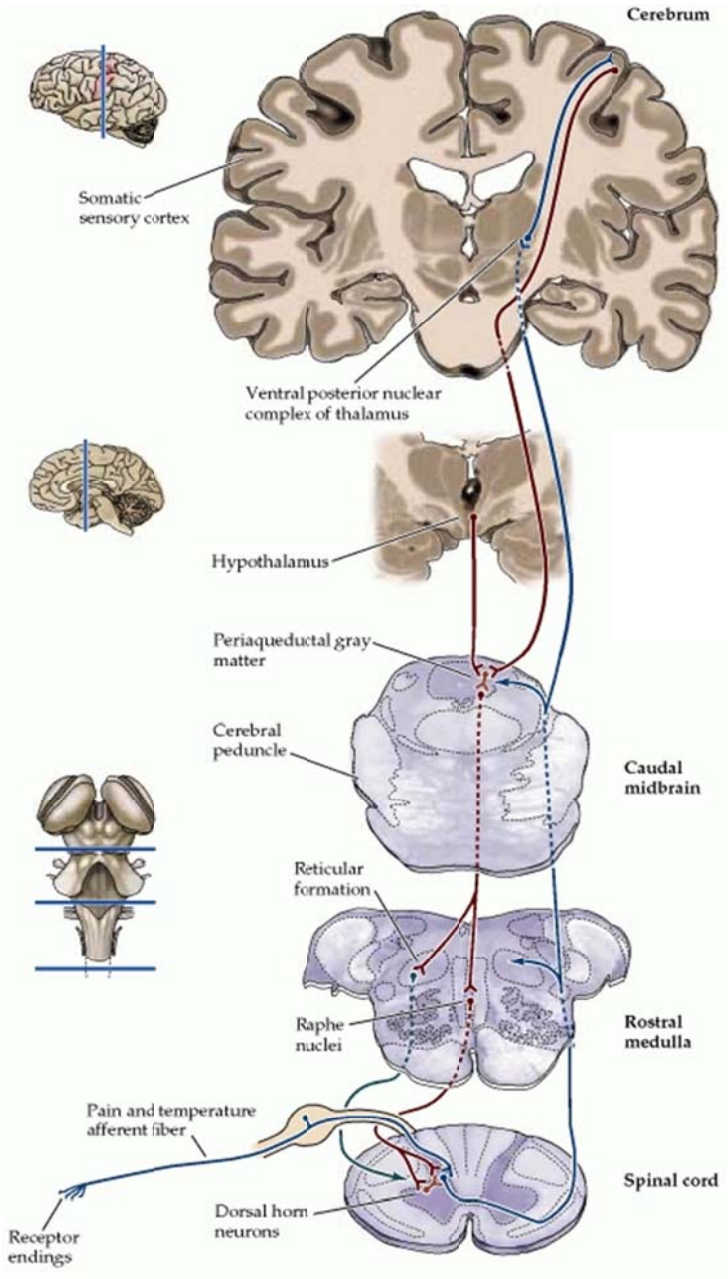

Fig. 1. The descending systems modulating the transmission of ascending pain signals.
These modulatory systems originate in the somatatosensory cortex, the hypothalamus, the periaqueductal gray (PAG) matter of the brain, and other nuclei of the rostral ventral medulla. Complex modulatory effects occur at each of these sites, as well as in the dorsal horn (DH; from Purves et al., 2001).

1.3 Opioids and non-opioids modulate nociception on a supraspinal level

The local synaptic inhibition is largely assumed to be conveyed through endogenous opioid relay stations. Shortly after the PAG and VMM were identified to be major relay stations of the descending pathways, opioid receptors and endogenous opioid peptides were discovered (Hughes et al, 1975). The well-known analgesic properties of opioids and the concentration of opioid signaling within the PAG and VMM consolidated the idea of an endogenous pain modulatory system (Mason, 2005).

Opioid and non-opioid analgesics are believed to activate neurons in the PAG and the RVM, in order to exert their actions (Fields, 2001). A subpopulation of RVM neurons projecting to the SC would inhibit nociception; this same system is responsible for electrically or chemically induced analgesia (cf. studies by Reynolds, 1969; Mayer and Liebeskind, 1974; Jacquet and Lajtha, 1973 cited above).

Opioids inhibit neurons rather than excite them. But strictly seen they "disinhibit" brainstem structures; they increase the activity of output neurons via inhibiting local GABAergic inhibitory inputs (Vaughan et al., 1997). Consequentially, the activation of the μ-opioid receptor in the PAG would hence for example disinhibit excitatory neurons projecting to the RVM. In the latter, the PAG could activate, or the local μ-opioid action could disinhibit spinally projecting neurons that mediate pain inhibition.

The mediators of the more recently observed descending facilitation of pain may be spinally projecting RVM neurons that express μ-opioid receptors. While the precise mechanisms are still to be determined, the facilitatory pathway was unveiled by selective lesions of RVM neurons expressing μ-opioid receptors, an intervention that resulted in the prevention of the development of hyperalgesia and allodynia.

The main non-opioid substance acting on pain modulation is noradrenaline (NA) and it is released through a host of physiological changes caused by a stressful event. The LC is activated and NA proves to have potent antinociceptive effects through spinal α2-adrenergic receptors (Kwiat and Basbaum, 1992). Since the LC is connected to the PAG and RVM, it seems to influence, through to these projections, at least partly, the analgesic action of the PAG (inter alia through an increase in blood pressure (BP) triggering the baroreceptor reflex, cf. point 1.5.1).

1.4 Stress-induced analgesia

Stress-induced analgesia (SIA) is a particular form of pain modulation leading to decreased nociceptive pain responses reminiscent of DNIC-induced hypoalgesia. It constitutes a defense mechanism protecting the organism from being constrained by an overwhelming pain experience in a life-threatening situation. Although the exact mechanisms of action are still not completely understood, endogenous opioids and cardiovascular reflexes (i.e. baroreflex sensitivity) have been shown to be involved in SIA (Koltzenburg, 2010).

During World War II, Beecher (1946) observed soldiers expressing less pain behavior than would be expected from their injuries. These observations provided first clues towards the strong dependence of pain sensation on context-related issues. After Beecher's observations, SIA and the inherent neuronal pathways have mostly been analyzed in rats. Jackson and Kitchen (1989) studied 20 and 25-day-old rats and tested their reactivity to forced swimming. They concluded that SIA could be observed in both rat groups. Short swims produced opioid-mediated pain inhibition whereas longer swims resulted in a non-opioid antinociception only in 25-day-old rats, suggesting that non-opioid pain modulatory systems develop more slowly than those dependent on endogenous opioids.

Bandler and Shipley (1994) also observed SIA in rats and stated that the PAG is a key element for controlling different reactions such as defensive behaviors, autonomic changes, and analgesia. In 1984 already, Basbaum and Fields had defined the PAG as a major module in the circuitry for inducing analgesia by stress (cf. chapter 1.3). Actually these descending inhibitory pathways are activated by the stimulation of opioid and non-opioid receptors in the PAG. In this region, there seems to be a dissociation of analgesia and immobility, supporting the model of two separate and competitive motivational systems which are defense and pain. While the brain areas mediating this inhibition are still unknown, it seems that the defense system inhibits the pain system through endogenous opioids. The amygdala could play a role in this inhibition (Wiedenmayer and Barr, 2000).

In humans it has been shown that the different endorphin receptors (μ, δ, κ) might be involved in different kinds of SIA. In this sense, the non-opioid form of SIA may simply

represent activation of naloxone resistant opioid receptors. Stress-induced antinociception at spinal levels is probably mediated by κ-receptors, whereas δ-receptors mediate the same phenomenon at a supraspinal level (Carlsson, 2002). It has been confirmed in humans that SIA has non-opioid components (Flor et al. 2002).

Ford and Finn (2008) highlighted the essential contribution of attentional and affective factors associated with the modality of the stressor, its context or the employed pain model. Additional brain imaging studies on SIA have to be made to improve our understanding of attentional and anticipatory factors in pain modulation. Increased knowledge of the neuroanatomy, –chemistry and –pharmacology of SIA will help us elucidate endogenous analgesia with a long-term aim of developing improved pharmacological and psychological approaches to pain treatment.

1.5 Spinal extrasegmental pain modulation - diffuse noxious inhibitory controls

Diffuse noxious inhibitory controls (DNIC) are the most convenient noninvasive way for quantifying descending pain modulation in humans, without stressing subjects.

DNIC describe the fact that responses to phasic pain stimuli presented at one body part may be dampened by an additional tonic pain stimulus applied to another body region, although this may seem paradoxical at first (Le Bars et al., 1992). Heterotopic (heterosegmental) noxious counter-irritation is a classical method to activate DNIC, a "pain inhibits pain" phenomenon (Villanueva and Le Bars, 1995; Le Bars, 2002;). In 1995, Le Bars and Villanueva noted that DNIC consist in a diffuse analgesia, covering the entire neuraxis that can be triggered by localized painful stimuli. Later on DNIC have been conceived as a differential contrast-sharpening filter, in the sense that noxious stimuli on a remote body site may activate a kind of surround inhibition of ongoing painful stimulation at adjacent or distal body sites (Villanueva, 2009). Newly arriving signals are better discriminated because background activity, related to stimuli that are currently less important (and less threatening) for the organism, may be considered as noise, and are literally filtered out. The focus on the strongest, most recent, and for the organism potentially most harmful pain stimulus, is hence considerably accentuated (Villanueva and Le Bars, 1995). DNIC represent an amplifier in the transmission system

which increases the potential alarm function of nociceptive signals by detecting the most intense pain and blocking all other pain input. It is short-acting, and does not seem to be somatotopically organized since it can be triggered by many types of noxious stimuli exerted on any part of the body outside the area of pain and outside their own excitatory receptive fields (Carlsson, 2002).

Pathological acute pain conditions would be associated with increased activation of DNIC; chronic pain states, however, are not always associated with a diffuse noxious inhibition of spinal nociceptive processes (Bouhassira et al, 2003).

Physiologically, DNIC represent pain control mechanisms, originating in the brainstem and modulating nociceptive activity in the spinal DH. This precise form of endogenous pain inhibition is operated through afferent Aδ- and C-fibers, who subsequently innervate wide dynamic range (WDR)–neurons of the DH. Ascending nociceptive pathways are activated by a counter-irritation stimulus and include projections to the dorsal reticular nucleus in the dorso-caudal medulla or the subnucleus reticularis dorsalis (SRD; inclusion of supraspinal processes). Widespread projections are sent back from these regions to the entire DH of the SC through the dorsolateral funiculus (DLF). In humans, thalamic structures and spino-thalamic pathways are not involved in DNIC, whereas a key role seems to be attributed to brainstem, probably reticular, structures (LeBars et al. 1992).

Regardless of the fact that DNIC are embedded in the neural pain control network and can thus be triggered by opioid transmission (e.g. PAG), even if those structures are not directly responsible for the triggering, they correspond to a basically stress-independent nociceptive system in the reticular formation of the brainstem (Le Bars, 1979a, 1979b).

As has been shown in electrophysiological and lesion studies in animals (Bouhassira et al., 1992) and humans (De Broucker et al., 1990), the above named neural substrates of DNIC are primordial for the production of the analgesic effect of counter-stimulation. In animals there is also evidence for the implication of other brainstem nuclei and higher-order brain structures in the descending pain modulation of nociceptive activity. As mentioned above, the RVM and the PAG modulate DNIC indirectly, but also the insular and medial cortices (including the anterior cingulated cortex; ACC) and the amygdala may also have a key role.

Although initial studies stressed the inhibitory processes and analgesic effects of VMM neurons, this brainstem area is perfectly suited, anatomically and functionally, to modulate spinal nociceptive responses in *both* directions. Put this way, spinal nociceptive modulation depends on a balance between facilitation and inhibition provided by descending projections from the RVM. In fact, aiming to characterize the response properties of cells in the latter region, Fields and his colleagues (2006) have discovered that there are three different subpopulations of neurons, the ON- and OFF-cells and the neutral cells. Both ON-and OFF-cells are nonserotonergic (Gao and Mason, 2000) and in addition to them, the VMM contains nonserotonergic neutral cells as well as a heterogeneous population of serotonergic cells, non-reactive to both opioids and noxious stimulation.

The way to a better understanding of the role of the RVM in processing and "top down" modulation of pain was opened. Activation of ON-cells is associated with spinal facilitation, and not mere permission, of nociceptive responses, whereas the activation of OFF-cells is related to pain inhibition. The physiological characteristics of ON- and OFF-cells and their reciprocal responses to opioids and noxious stimulation have enabled the implication in the modulation of phasic nociceptive transmission. Therefore, descending modulation of the RVM activity, by higher-order structures, may increase or decrease spinal nociceptive processes, spino-thalamic activity and pain perception. Being part of the pain control network, the RVM does not only control sensory information, but it is an important region in homeostatic functions that themselves can be altered by pain (Suzuki et al, 2004).

DNIC seem to be functional without substantial opioid-dependent mechanisms underpinning them, but the definitive neurophysiology of DNIC in humans is not yet identified (Edwards et al, 2004). Coming back to the example of NA release mentioned earlier, experimental studies indicated that spinal (antinociception) and supraspinal (nociception) noradrenergic receptors produce differential influences on pain modulation depending on their localization and on the site of the stimulus input (Pertovaara and Almeida, 2006). Furthermore, Zhuo and coworkers (2002) presume that inhibitory mechanisms are principally mediated through the DLF, whereas the descending facilitation is actuated by the ventral and ventrolateral funiculi.

To use DNIC as an adequate paradigm for the characterization of descending pain modulation, the techniques for DNIC-induction should be unconfounded by other variables able to interfere with pain inhibition. On the following pages, more attention is paid to those potentially confounding variables which are, among others, cardiovascular activity and baroreflex sensitivity, stress, psychological variables, time and sex effects.

1.5.1 The autonomic nervous system and baroreflex sensitivity

The autonomic nervous system (ANS) significantly contributes to the maintenance of homeostasis because it functions with a conscious and autonomic control and regulates, among others, BP and thermoregulation. The ANS is composed of the sympathetic and the parasympathetic system. Both complementary systems are tonically active but anatomically and functionally distinct subdivisions. While the former predominates during emergency situations and prepares "fight-or-flight" reactions, the latter regulates "rest and digest" functions (McCorry, 2007).

The interaction between pain-regulatory systems and cardiovascular activity provides adaptive homeostatic mechanisms in the presence of pain (Edwards et al, 2004; see also Bruehl and Chung, 2004 for review). According to Möltner and colleagues (1990) autonomic changes are even an obligatory part of the complex, multidimensional pain response and are capable of providing objective addenda of affective-motivational pain processing. Dowling (1983) found an inverse relationship between HR activity and pain tolerance threshold and concluded that autonomic functions, such as skin conductance and HR level, related reliably to a behavioral measure of pain tolerance. Antinociception is associated with hypertension (cf. Bruehl and Chung, 2004, for review). This connection is not only present in subjects displaying a background of clinic hypertension in their family, but experimental studies showed that increased BP is accompanied by a reduced vegetative reactivity to nociceptive information (al'Absi et al., 1996; France et al., 2002b). Inverse relationships between BP and pain sensitivity have been observed in normotensives (Fillingim and Maixner, 1996; Fillingim et al. 1998).

Brain regions contributing to antinociception substantially overlap with those underlying control of the cardiovascular system (Randich and Maixner, 1984). In line with that, the pathways from VMM, a recognized pain control center, to sympathetic and

parasympathetic neurons target tissues like the heart and cutaneous blood vessels polysynaptically. The engagement of VMM neurons by situations and stimuli not associated with pain argue against a specific nociceptive modulatory system. It still remains unclear whether this is a common feature of all VMM neurons, or whether only a subset of the concerned neurons projects to multiple targets. Nevertheless, it can be taken for granted that pain is indirectly associated to homeostatic and behavioral adjustments. More to the point, intense pain accompanied by life-threatening injury achieves a primordial significance and outplays other homeostatic challenges. This functional activity is reflected in the interaction between pain sensitivity and the range of the arterial BP. The adaptative pain–BP–connection acts like a homeostatic feedback with the aim of regulating the negative arousal caused by the appearance of painful stimuli.

Arterial blood pressure is regulated by mechanoreceptors in the carotid sinus and aortic arch. The vagal cardioinhibitory and sympathoinhibitory vascular effects of the respective receptor activity have been studied since the 1930's. The effective reflexes activated by baroreceptors contribute to the dampening of relatively rapid changes in arterial pressure. Stimulation of those baroreceptors produces furthermore a general inhibition of central nervous processes (Dworkin et al., 1994). This effect includes a reduction of cortical excitability (Elbert et al., 1992), incitation of sleep (Koch, 1932), decrease of muscle tone (Dworkin et al., 1994) and reduction of pain sensitivity (Bruehl and Chung, 2004). In addition, descending pain inhibitory pathways are probably able to auto-regulate their activity through actions in autonomic centers of the SC modulating cardiovascular function through a negative feedback loop ("baroreceptor reflex"; Millan, 2002). Thus a significant role of baroreceptor activation in the relationship between resting BP and acute pain sensitivity is established in the functional model of this relationship: firstly, pain increases sympathetic arousal which results in elevated BP, secondly, elevated BP leads to enhanced baroreceptor stimulation, which thirdly triggers descending pain inhibitory activity allowing the arousal levels to return to a state of homeostasis (Bruehl and Chung, 2004).

1.5.2 Stress

Exposure to stress involves autonomic reactions and through this relationship already constitutes an additional important factor influencing pain modulation. In 1936, Selye introduced the notion of stress. The term "stresstrias" describes, a non-specific syndrome characterized by three typical symptoms: a diminution of the thymus gland, an enlargement of the adrenal cortex and bleedings in the stomach and duodenum. The "general adaptation syndrome" (GAS) is constituted of three phases: an alarm reaction, a resistance phase, i.e. adaptation, and a recovery period. In case of an intense and long-lasting stressor, the third phase can be characterized by exhaustion rather than recovery. Nowadays, stress has often been defined as a state in which the organism confronts a novel, threatening or challenging situation, or where the metabolic or physical status is compromised. Sooner or later, the described situations lead to a homeostatic imbalance, in which the organism recruits its arsenal of responses to fight the danger and return to its homeostasis (adaptation phase for Selye). This bodily answer usually constitutes a combination of complementary specific and non-specific responses to overcome all forms of physiological imbalance or injury (Zinder and Dar, 1999).

Behavioral stress plays a critical role in the etiology of hypertension and other cardiovascular disorders. Stress-induced changes in neural beta-adrenergic activity such as HR, pulse transit time and systolic BP changes have a potentially huge etiological importance in the psychosomatic theory of hypertension, not least because some sorts of stressful events, especially those involving opportunities for control, evoke cardiodynamic changes similar to the early stages of hypertension (Light and Obrist, 1980).

If one considers the definition of pain given by the IASP ("an unpleasant sensory and emotional experience, associated with actual or potential tissue damage, or described in terms of such damage"), pain induces stress by definition. Pain is indeed a potent stressor; its effects on BP are, on their own, indicative of a certain stress level, and Edwards and coworkers (2004) found changes in heat pain responses during noxious cold, maybe a function of stress in general rather than pain in particular. In the last few years, the study of SIA has contributed our knowledge of the relationship between pain and stress and showed that pain can be naturally inhibited. It is nevertheless very

important to take profit from laboratory settings to distinguish between pain and stress, leaving out the aversive component. In the natural environment stressful and painful stimuli are often inextricably entangled and in testing situations it has to be made sure that cardiovascular activity evoked during psychological stress is not due to concurrent pain stimuli (Caceres and Burns, 1997). This can only be achieved by using an unconfounded pain model.

Nonetheless, pain does also exist without stress. When considering disorders related to pain experience, it is important to exemplify pain asymbolia where pain is perceived without suffering.

In conclusion, pain is not always a defensive reaction, it can only be orientational and as seen above, whether all phases of Selye's description will be reached, or not, can eventually depend on evaluative factors (Lazarus, 1993). Pain is defined as a complex perceptual experience, where important emotional, cognitive and behavioral components are added to the sensory information about pain, such as intensity and spatio-temporal indicators.

1.5.3 Psychological factors modulating pain processing

The psychological factors above mentioned, like expectation, emotion, attention and cognition affect the neuronal activity of brain regions involved in descending pain inhibition, and expectation-dependent changes in pain are associated with genuine changes in the activation of spinal pain-modulating circuits.

The complete blocking of the normal analgesic response produced by DNIC supports the idea that anti-analgesic expectations can dramatically reduce the effect of active analgesic treatments, which implies that expectations can effectively block and potentially reverse the action of active drugs. Another idea is that of expectations activating a complex and highly modifiable pain-processing network. Interactions between prefrontal and cingulate cortical regions underlie subjective changes in the experience of pain. These changes are thought to reflect mechanisms recruited in an attempt to align the felt internal state of the body with an anticipated outcome (Rainville et al., 2002). Contrariwise, the coupling between the prefrontal cortex and the brainstem is thought to be directly related to decreases in subjective pain ratings and is assumed to reflect the engagement of

descending modulatory responses (Lorenz et al., 2003). It has been ascertained for the first time that expectation effects actually change physiological responses associated to both spinal inhibition and cortical activity. In a clinical perspective, a valid pain treatment may lose its efficacy if patients do not expect pain relief. The other way round, ineffective treatments will induce expectations of failure, which may interfere with the efficacy of future treatments (Charron et al., 2006). This situation may contribute to the poor efficacy of pain treatments in chronic pain conditions.

In addition to expectation, emotional factors related to pain, like anxiety or vigilance, may in turn affect autonomic activity and reactivity (Tousigant-Laflamme et al, 2005). Catastrophizing is another psychological variable with effect on the strength of endogenous pain inhibition. An fMRI study by Seminowicz and Davis (2006) pointed out that pain catastrophizing correlated negatively with the activity in brain structures involved in descending pain inhibition. Such results show that reduced descending pain inhibition processes play an important role in general pain processing and can be influenced by maladapted cognitive processes, like catastrophizing. This factor could be a possible explanation for sex-specific differences in endogenous pain modulation, because women show significantly higher catastrophizing scores than men (Dixon et al., 2004; Goodin et al., 2009).

1.5.4 Time and sex-related effects

When testing different pain models in humans, time effects are an important issue; different conduction velocities for fibers conveying different pain modalities, as well as post-counter stimulation recovery times will have to be considered (cf. study 2). Different time patterns for men and women are at the same time an essential factor when investigating sex-related differences in descending endogenous pain modulation. These differential temporal responses can be due to different characteristics of afferent fibers, i.e. the signal arriving in the cortex may already be different for men and women, or may be explained with respect to phenomenology, the experiencing subject and potential psychological, social, cultural and biological differences.

Starting from the idea that DNIC effects seem to depend on both local and descending pain-inhibitory mechanisms blocking nociceptive responses of DH spinal neurons (Le

Bars et al., 1992; Bouhassira et al., 1992), Staud and coworkers (2003) found a differential inhibition of wind-up (WU) pain by heterotopic heat conditioning stimuli for healthy men compared to healthy women or female FM patients. For both female subgroups, WU stimuli were not significantly reduced, pointing to considerable differences in pain modulation between men and women. However, the less effective central inhibitory mechanisms in women as compared to men may be a predisposing factor in the development of FM syndrome. Impaired DNIC has been found in chronic tension-type headache (Pielsticker et al., 2005), in FM patients (Staud et al., 2003) and in healthy females (Serrao et al., 2004). Granot and colleagues (2008) also came to the conclusion that EA is less effective in females. Besides differences in pain modulation, men and women differ in cardiodynamic reactions. Tousignant-Laflamme and coworkers (2005) found that the relationship between HR response and pain is sex-related suggesting a differential defensive versus orientational reaction for men and women.

2 Experimental part

2.1 Two validation studies

2.1.1 Study 1: Differential physiological effects during tonic painful hand immersion tests using hot and ice water

The aim of study 1 was to compare and characterize the physiology and psychophysics of the well-documented cold pressor (CPT) and the less-used hot water immersion test (HIT), while challenging the internal validity of both pain models and verifying the exact origin of autonomic responses. In this context, the validity of CPT as a valid DNIC-trigger is studied and discussed: are the observed cardiovascular changes during CPT a consequence of the baroreflex mechanism or of pain per se? And furthermore, is the observed pain inhibition due to a genuine "pain inhibits pain" phenomenon or is it mainly related to baroreflex hypoalgesia?

An additional concern was to examine the relative usefulness of investigating CPT and HIT for studying specific DNIC-like effects not confounded by other variables. We combined psychophysiological and psychophysical methods to clearly characterize tonic pain models and to detect interfering autonomic reactions. The HIT turned out to be less sympathetically confounded and hence constitutes a valid and thus appropriate model to trigger distinct descending pain control models.

2.1.2 Study 2: Internal validity of inter-digital web pinching as a model for perceptual diffuse noxious inhibitory controls-induced hypoalgesia in healthy humans

Based on the findings of study 1, the more valid HIT was used in the second study, and compared to tonic inter-digital web pinching (IWP). BP rises during HIT were not significant compared to baseline (BL) values, but they were still present. With respect to tonic pain stimulation we hypothesized that the IWP might constitute an even less challenging paradigm on a cardiovascular level. If it were also capable of reducing phasic WU stimuli (heat versus ballistic impact stimuli) in a comparable way to HIT, it would constitute a preferable model for the study of specific DNIC-related effects. IWP proved to be a valid model and unrelated to BRS hypoalgesia. In a second approach, this model

will allow us to initiate clinical studies on the involvement of altered DNIC processing in chronic pain syndromes.

2.2 One application study: Sex-specific time course of diffuse noxious inhibitory controls-induced pain modulation and nocifensive reflex suppression in humans

2.2.1 RIII-reflex

Before introducing the third study, which is in a way a replication study of the findings from study 2, I would like to shortly expose the principal features of the nocifensive flexion reflex, considered to constitute an objective indicator of pain.

Since Sherrington's work (1906) we know that nociceptive reflexes are enhanced after a transaction of the spinal cord, indicating the importance of descending modulatory influences. More specifically, ON-cells are thought to be responsible for the facilitation of the nociceptive processing and thus encourage the occurrence of hyperalgesic pain states. The latter, contrary to the OFF-cells, are tonically inactive and show an increased firing rate immediately before the triggering of a nocifensive reflex (Fields and Basbaum, 1999).

In humans, nocifensive flexion reflexes are typically induced by applying electrical stimulations to the retromalleolar path of the sural nerve and measured by the magnitude of the EMG responses of the biceps femoris muscle (Willer, 1983). This nociceptive flexion reflex has a latency of about 90 ms, which is consistent with the conduction velocity of Aδ-fibers, and produces a brief motor response also lasting between 60 and 90 ms. Normally, the nocifensive flexion (RIII) reflex threshold corresponds to the human pain threshold and the amplitude of above-threshold responses are positively correlated with increases in subjective pain perception up to pain tolerance. It is therefore a very objective measure of pain sensation, stable in time and inter-individually. What is more, it is an internationally recognized technique that was recommended in 2004 by the European Federation of Neurological Societies, to assess normal and pathological pain-related spinal processes and pain modulation in humans.

In humans heterotopic noxious stimuli inhibit the spinal nociceptive flexion (RIII) reflex, which reflects the spinal transmission of nociceptive signals. In both animals and humans, these phenomena are sustained by a spino-bulbo-spinal loop with an ascending part located in the anterolateral quadrant of the spinal cord (De Broucker et al., 1990; Bouhassira et al., 1992).

Le Bars and Willer (2002) depicted a number of features that are shared by the RIII-reflex and associated painful sensations in humans and by DH WDR neurons in the rat SC. The authors concluded that these similarly shared characteristics are good evidence for the existence of DNIC in humans:

1) the RIII-reflex and the responses of WDR neurons to electrical stimulation of their cutaneous RF are similarly inhibited by various heterotopic nociceptive stimuli.
2) the extent of the inhibitions is directly related to the intensity of the conditioning stimulus.
3) the inhibitions are followed by after-effects, which can last for several minutes.
4) the inhibitions are mediated by a spino-bulbo-spinal loop, the ascending part of which is composed of the spinoreticular tract and synaptic relays in the brainstem.
5) the ascending pathways of the loop are mainly crossed while the descending pathways run ipsilaterally to the recording site.
6) there is at least one opioidergic link in this loop, both in the rat and in man.

2.2.2 Introducing study 3

There is a disproportionally high prevalence of chronic pain syndromes and multiple pain conditions among women. This may be due to psychosocial factors on the one hand (cf. pain catastrophizing and pain expressiveness), but may also be caused by sex-specific predispositions emerging from a differential CNS, implying the importance of different endocrine and nociceptive processing cascades (cf. chapter 1.5.4). Independent of the used endogenous pain modulation paradigm, whether DNIC (including different techniques to elicit them) or SIA, a valid pain measure should be able to elucidate those differences under experimental laboratory conditions.

The pain models used for the study of DNIC or DNIC-like effects are very heterogeneous and there are a lot of inconsistencies in the literature, the majority of which may be due to a differential implication of baroreceptor associated hypoalgesia. In studies using CPT or ischemic pain for instance, where contaminating cardiovascular parameters may play an important role, gender effects could not be identified. When employing inadequate pain paradigms, potential sex differences may therefore be missed. On the other hand, HNCS studies using physically or chemically induced muscle pain that is not or negligibly accompanied by cardiovascular challenge, have described obvious gender-related effects (Arendt-Nielsen et al., 2008, Weissmann-Fogel et al., 2008, Ge et al. 2009).

The aim of the third study was thus to investigate sex-related differences in defensive reactions and time patterns using IWP, which has been validated during the second study to be a non-confounded method for DNIC-induction.

3 Article 1

3.1 Abstract

The Cold Pressor Test (CPT) is an empirically validated test commonly used in research on stress, pain and cardiovascular reactivity. Surprisingly, the equivalent test with water heated to noxious temperatures (Hot Water Immersion Test, HIT) has not been thoroughly investigated. The aim of the present study was to characterize the physiological effects and psychophysics of both tests and to analyze whether the autonomic responses are mainly induced by baroreflexes or a consequence of the pain experience itself. The study consisted of a single session including one CPT (4 ± 0.2° C) and one HIT (47 ± 0.5° C; cutoff point 5 min) trial performed on 30 healthy drug free volunteers aged 19-57 (median 24) yrs. The sequence of both trials was alternated and participants were randomly assigned to sequence order and parallelized with respect to gender. Physiological parameters (cardiovascular, respiratory and electrodermal activity) and subjective pain intensity were continuously monitored. In addition, pain detection and tolerance thresholds as well as pain unpleasantness were assessed. Both tests were comparable with regard to the time course and intensity of subjective pain. However, a significantly higher increase of blood pressure could be observed during the CPT when compared to the HIT. The HIT appears less confounded with thermoregulatory baroreflex activity and therefore seems to be a more appropriate model for tonic pain.

Keywords: baroreflex hypoalgesia; cold pressor test; endogenous pain modulation; human pain models; psychophysiology; psychophysics.

Differential physiological effects during tonic painful hand immersion tests using hot and ice water

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Article Type: Original article

3.2 Introduction

The cold pressor test (CPT; Hines and Brown, 1936) was originally conceived as a clinical cardiovascular challenge test to identify blood pressure (BP) and heart rate (HR) reactivity after hand immersion into ice water. It also proved to be a reliable experimental model for tonic pain or pain tolerance assessment (Mitchell et al., 2004). It has been hypothesized that the relationship between cardiovascular excitability and pain induction is primarily due to the extensive rise in BP caused by the thermoregulatory vasoconstriction of blood vessels in deep tissue (Wolf and Hardy, 1941).

Hand immersion in painful cold or hot water has also been used for experimental characterization of endogenous pain modulation, especially as a trigger stimulus for diffuse noxious inhibitory controls (DNIC). The DNIC phenomenon relates to the inhibition of nociceptive dorsal horn activity and pain sensations induced by additional heterotopic noxious stimulation (Le Bars et al., 1979a,b, 1992). Animal studies have shown that it is mediated via an extra-segmental inhibitory process involving the medullary subnucleus reticularis dorsalis (Villanueva et al., 1996).

The validity of cold-water immersion as a heterotopic noxious counter-stimulus for DNIC induction may however be hampered by confounding interactions of cardiovascular and pain regulatory systems. Experimentally induced, as well as constitutional hypertension is associated with reduced pain sensitivity, a phenomenon commonly referred to as baroreflex hypoalgesia (for overview see Bruehl and Chung, 2004; Randich and Maixner, 1984; Ring et al., 2008). Observed cold-pressor related reductions in pain ratings may thus not selectively be attributable to DNIC, baroreflex mechanisms induced by thermoregulatory vasoconstriction may be involved as well.

Painful hot and cold water stimulations are comparable with regard to their inhibitory effects on subjective pain experience (Granot et al., 2008). The two stimulation paradigms are thus interchangeably used, although little is known about possible physiological specificities and underlying mechanisms.

In the present study we contrasted the hot and ice water immersion tests with regard to their psychophysical and physiological (cardiovascular, respiratory and electrodermal

activity [EDA]) characteristics. Our main goal was to validate the relative usefulness of the two paradigms for studies investigating DNIC effects.

3.3 Methods

3.3.1 Subjects

N = 35 healthy (18 female and 17 male; two left-handed) volunteers aged between 19 and 57 years (median [*Md*] age 24 yrs.) participated in the study. The subjects were recruited at the University of Luxembourg and received monetary compensation for their participation. All participants gave informed written consent, were drug free (no drug or alcohol intake > 24 h before the study, except oral contraceptives) and did not suffer from any medical, neurological, psychiatric or psychological disorder nor did they manifest any substance (incl. nicotine) abuse.

The study consisted of a single session (duration: 75 min.) including one hot water immersion trial (HIT) and one cold pressor trial (CPT). The sequence of both trials was alternated (AB-BA scheme) and participants were randomly assigned to sequence order and parallelized with respect to gender. The experimental protocol was in accordance with the ethical guidelines of IASP (Charlton, 1995) and met the criteria for an exemption from local ethical committee approval.

3.3.2 Algesimetry

Tonic thermal pain was induced by immersing the right hand up to the wrist in a 12 L tank with circulating hot (47-48° C) or cold (3-4° C) water. A cut-off point of 5 min. was predefined, which guaranteed a time interval sufficient for reliable psychophysiological recordings of cardiovascular parameters (Sollers JJ, personal communication, 03/09/2008). The temperature of the hot water bath was held constant with a commercially available submergible heater and a digital controller, whereas an external chiller was used for the coldwater bath (Aqua Medic GmbH, Germany). External aquarium pumps ensured water circulation in both water containers.

Subjective pain intensity was numerically rated on a verbally anchored scale (0 corresponding to *no pain* and 100 to the *maximal imaginable pain*) every 15 s during both pain tests. Pain unpleasantness was quantified using a 10-cm visual analogue scale (VAS;

verbal anchors: *not at all unpleasant* and *extremely unpleasant*) immediately after each test. Apprehension (nervous tension) associated with the pain test was determined using a 5-point Likert scale (1 = *minimal tension*; 5 = *maximal tension*). Furthermore, qualitative (i.e. affective and sensory) aspects of the pain experience during cold/hot water immersion were assessed with an adjective scale (*Schmerzempfindungs-Skala*, SES [Pain Sensation Scale]; Geissner, 1995).

In addition, detection thresholds for cold and warm sensation (method of limits) as well as cold and heat pain (staircase-method) were evaluated, employing a 30·30 mm contact thermode attached to the volar surface of the left forearm (TSA-II NeuroSensory Analyzer; Medoc Advanced Medical Systems Ltd., Israel).

3.3.3 Psychophysiological recording

BP was continuously monitored on the wrist of the left arm with a noninvasive BP amplifier (NIBP100A; BIOPAC Systems, Inc., USA). Cardiac activity was assessed with a pre-cordial lead II electrocardiograph (ECG100C; BIOPAC Systems, Inc., USA; with 0.5-Hz high pass and 35 Hz low pass filtering) employing disposable pre-gelled Ag-AgCl electrodes. Subjects were grounded through a surface electrode attached to the chest. Respiration rate (RR) was obtained (with 0.05-Hz high pass and 1-Hz low pass filtering) using strain gauge belts positioned on the thorax and the abdomen (TSD201; BIOPAC Systems, Inc., USA). EDA was recorded with two 6-mm diameter domed Ag-AgCl electrodes (SS3LA; BIOPAC Systems, Inc., USA) and processed through a constant voltage (0.5 V) coupler (GSR100C; BIOPAC Systems, Inc., USA; with 5 ⌈S/V signal gain and 1-Hz low pass filtering). Transducers were filled with isotonic electrode paste (formulated with 0.5% saline in a neutral base) and fixed on the mid-phalanx of the third and the fourth finger of the left hand. The skin temperature of both hands was measured on the palms by using a digital infrared thermometer (Sanowell Scaneo; Hofmann GmbH, Germany). The laboratory room was mechanically ventilated with ambient temperature maintained at 23.5 ± 0.5° C. The AcqKnowledge software package (BIOPAC Systems, Inc., USA) was used for the collection and analysis (online and offline transformations) of the psychophysiological data.

3.3.4 Psychometrics

To test whether inter-individual differences in behavioral inhibition or activation systems might influence reactivity in the CPT and HIT, subjects were asked to fill out the BIS/BAS-scales (Carver and White, 1994).

3.3.5 Procedure

Each session began with the installation of the subject in upright position onto the experimental chair (approx. 90° inclination) and electrode/transducer placement. This was followed by a 5-min adaptation period and the measurement of detection thresholds for thermal sensation and pain (see experimental protocol in figure 1).

Subsequently, the registration of physiological parameters was started with the recording of a 5-min resting baseline (BL1), succeeded by the first water test (CPT or HIT, depending on the individual sequence). The subjects were instructed to immerse their right hand up to the wrist in the corresponding water tank and to verbally indicate the time point of the first pain sensation (i.e. pain threshold). Further, they were instructed to rate their pain sensation every 15 s on a numerical rating scale (NRS). The subjects were asked to leave their hand in the water container until the pain tolerance level was reached. The alternate water immersion test (CPT or HIT, respectively) followed after a 10-min rest period serving for BL assessment (BL2). For adaptation of skin temperature, the test hand was immersed in a container with tepid water (32° C) during the first 3 min of this pause. Skin temperature on both hands was measured before and after each BL and test recording. Only the last two minutes of the corresponding BLs (BL1 and 2, respectively) were used for standardization of physiological data.

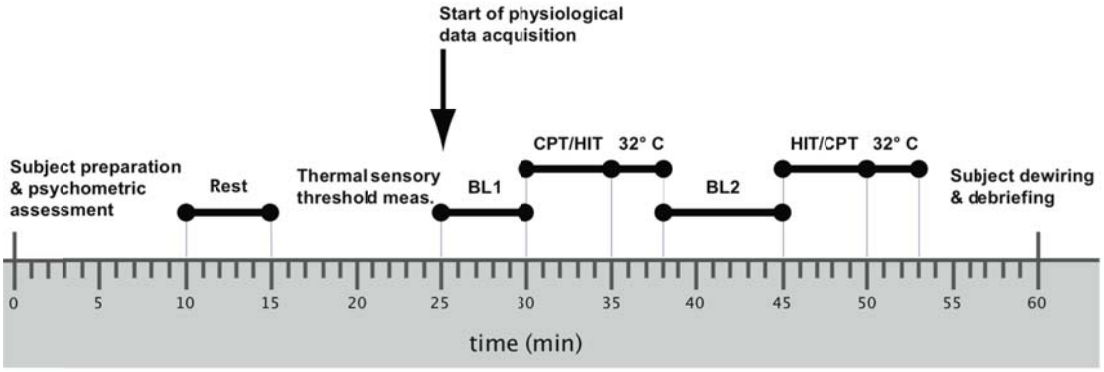

Fig. 1. Experimental protocol. CPT (cold pressor test), HIT (hot water immersion test).

3.3.6 Data reduction and analysis

Due to technical problems during psychophysiological recording, the data of three subjects were incomplete and thus not included in analysis. Furthermore, two participants felt no pain sensations during one or both water tests and had to be excluded as well, leaving a statistical population of $N = 30$.

The mean systolic BP and HR were calculated separately for both test periods and relativized to mean BL (1-min recording 2 minutes before the beginning of CPT and HIT, respectively) values. The mean RR was computed for thoracic and abdominal respiration separately (re-sampling rate = 50 Hz). The standard deviation of nonspecific EDA amplitudes for the first test minute was calculated offline and served as tonic EDA parameter (cf. Besthorn et al., 1989). The 1-min recording preceding test onset served as BL for RR and EDA. Overall pain experience during the immersion tests was computed as the geometrical grand mean of all subject's ratings different from zero.

All statistical analyses were conducted using the Statistical Package for Social Sciences (SPSS Inc., USA). Graphs were created with SigmaPlot (Systat Software Inc., USA) and Temporis (Bartas Technologies LLC, USA). Effect size computations were carried out with the G*Power program (Faul et al., 2007). Parametric (*t*-tests for paired samples; Pearson product-moment correlation coefficient [r]), non-parametric tests and correlation coefficients (Wilcoxon's signed rank test; Spearman's rho [r_s]) were computed as appropriate (e.g. non-parametric tests in the case of skewed data distributions). For normally distributed data, the arithmetic mean and standard error of the mean ($AM \pm$

SEM) were used as measures of central tendency and variability, whereas asymmetrically distributed data are represented as median plus mean absolute deviation (*MAD*) or range. As in the analysis of psychometric data we tested for the null hypothesis (that there is no difference between both tests), a more conservative two-tailed significance level of $\alpha = .20$ was chosen. For the analysis of psychophysiological data, a one-tailed *p*-value of less than .05 was considered significant.

3.4 Results

3.4.1 Psychophysical and psychometric data

Pain thresholds (i.e. latency to detection of first pain) correlated moderately between both tests ($r_s = .33$, $p < .05$) and were significantly higher for the CPT than for the HIT ($z_{29,1} = 2.9$, $p = .003$, effect size [d] = .52), although the absolute time difference of 3 s (CPT: *Md* = 13 s, range = 5-30 s; HIT: *Md* = 10 s, range = 1-28 s) may be considered negligible (see figure 1 and table 1). Pain tolerance levels (CPT: *Md* = 300 s, range = 63-300 s; HIT: *Md* = 150 s, range = 35-300 s) were also higher during cold-water immersion ($z_{29,1} = –1.91$, $p = .06$) and highly correlated between both tests ($r_s = .48$, $p < .01$). As expected, both immersion tests were comparable with regard to the time course of subjective pain experience (see figure 2a,c) and pain increase (see figure 2; 63 compared to 67 NRS-units for CPT and HIT, respectively; $t_{29,1} = –1.22$, $p = .22$ and $r_s = .41$, $p < .05$). However, when analyzing relative summation of pain as percent difference between the first and last pain rating, a significant difference could be shown between both tests (⊗% = 30 to 56 % for CPT and HIT, $z_{29,1} = –2.57$, $p = .01$; cf. figure 2e). No sequence effects were found with respect to subjective pain intensity (sequence CPT-HIT: *AM* ± *SEM* = 62 ± 4.7 and 67 ± 5.5 for CPT and HIT, respectively; $t_{29,1} = –.72$, $p = .49$; sequence HIT-CPT: *AM* ± *SEM* = 63 ± 6.1 and 66 ± 5.5 for HIT and CPT, respectively; $t_{29,1} = –.50$, $p = .63$). Nonetheless, pain thresholds were negatively correlated with the percent increases in pain for both tests ($r_s = –.40$, $p < .05$ for CPT and $r_s = –.54$, $p < .01$ for HIT). Interestingly, pain thresholds did not correlate with the pain tolerance levels, but with overall subjective pain intensity (see figure 2d), although this relationship became significant for the CPT only ($r_s = .63$, $p < .01$).

Both tests were perceived as highly unpleasant and were evaluated similarly with regard to the affective and sensory dimensions of the pain experience (cf. figure 2b). Unpleasantness correlated with overall subjective pain intensity in both tests ($r_s = .43$, $p < .05$ for the CPT and $r_s = .55$, $p < .01$ for the HIT) as well as with pain tolerance ($r_s = –.40$, $p < .05$), which again was only true for the CPT. On the other hand, significant correlations between unpleasantness ($r_s = .38$, $p < .05$), subjective pain intensity ($r_s = .43$, $p < .05$), pain tolerance level ($r_s = –.40$, $p < .05$) and the affective SES scale could only be observed during hot water immersion, but not for the CPT. These observations may constitute a first indication of a more discernable pain sensation induced by the HIT.

There were no consistent relations between the quantitative sensory parameters and inter-individual differences in behavioral inhibition or activation (i.e. on the BIS/BAS scales) with the exception of a positive correlation between unpleasantness and behavior inhibition during CPT (total BIS score; $r = .48$, $p < .01$). Thus a more intense pain experience may be associated with a stronger avoidance behavior, which is further supported by the fact that the total BIS score showed a negative correlation with pain tolerance ($r = –.40$, $p < .05$).

Table 1. Psychophysical data

	CPT		HIT				
	Measures of central tendency + dispersion	Range	Measures of central tendency + dispersion	Range	Correlation CPT/HIT	Test value (df=29)	*P*-value (2-tailed)
Pain threshold (s)	13±6 [a]	5-30	10±6	1-28	r_s = .33*	−2.93 [b]	.003**
Pain tolerance level (s)	300±93	63-300	150±124	35-300	r_s = .48**	–1.91	.06
Overall subjective pain intensity (aggregated over time)	63±4 [c]	30-93	67±4	22-96	r_s = .41*	−1.22 [d]	.22
Subjective pain increase (%Δ) relative to initial rating	30±5	1-88	56±10	0-250	r_s = .34	−2.57	.01**
Unpleasantness (VAS)	68±4	18-100	75±4	28-100	r = −.18	−1.19	.24
Affectivity (SES)	39	34-59	40	33-62	r_s =.34	−.32	.75

All data expressed as T-values w. norm values * *P*<.05; ***P*<.01;****P*<.001
[a] Md±*MAD (mean absolute deviation)* [b] *z*-value [e] T-value (mean=50, SD=10)
[c] AM±*SEM* [d] *t*-value

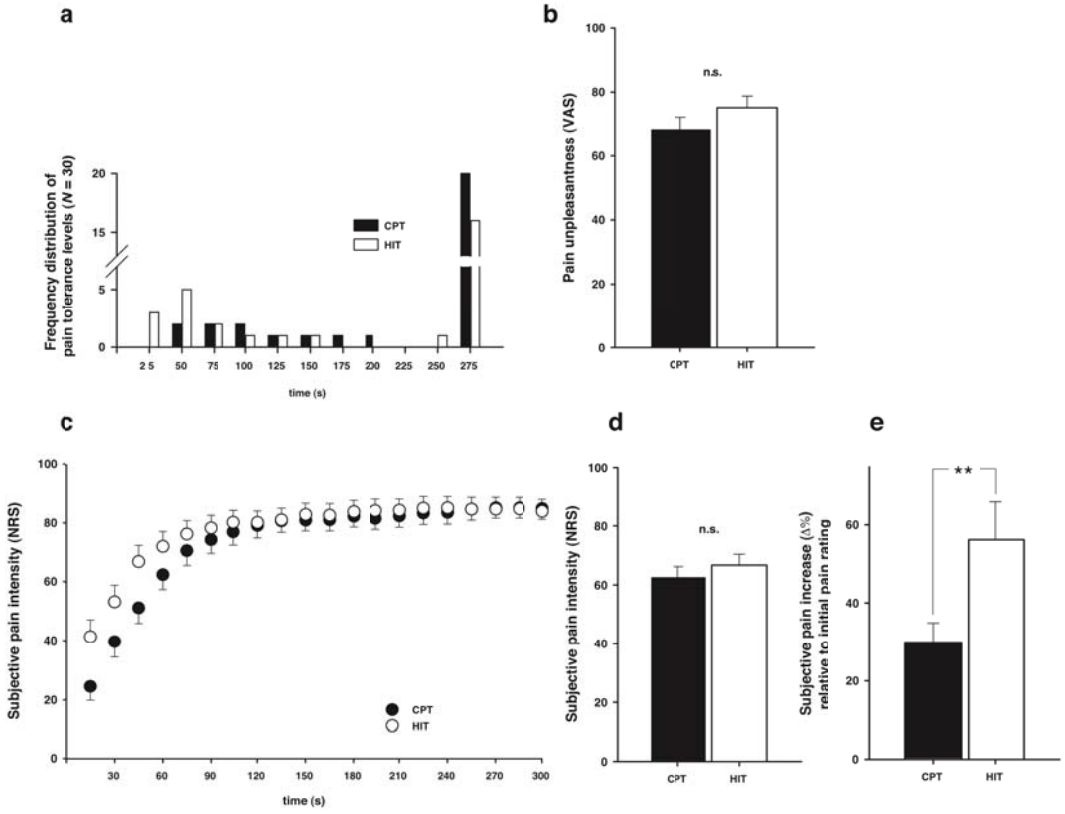

Fig. 2. Psychophysical data. (a) Frequency distribution of pain tolerance levels (N = 30) for both immersion tests. (b) Overall pain unpleasantness. (c) Time course of subjective pain intensity. (d) Overall subjective pain intensity (individual geometric means aggregated over test duration). (e) Temporal summation of subjective pain intensity (percent increase relative to initial pain rating). All data expressed as AM ± SEM. ** < .01.

3.4.2 Psychophysiological data

Significantly different overall (aggregated over test time) BP levels were observed during both tests (absolute values of 159 to 152 mmHg for CPT and HIT, respectively; $t_{29,1}$ = 2.81, p = .009). More to the point, the CPT produced a stronger rise in BP (Δ% = 16%) than the HIT (Δ% = 8%; $t_{29,1}$ = 0.85, p = .0002), calculated as percent differences relative to BL (see figure 3 and table 2).

Both tests also differed with respect to HR variability (ratio between low and high frequency components [LF/HF ratio] of the HR variability spectra relative to BL: 2.5 for CPT and 1.5 for HIT; $t_{29,1}$ = 2.49, p = .019) and with respect to the first test minute of EDA (or skin conductance level: 0.14 to 0.22 mS for CPT and HIT, $t_{29,1}$ = –1.81, p = .003).

HR on the other hand was highly correlated (r = .80, p < .01) during both tests and consequently did not differ significantly (80 to 81 BPM for CPT and HIT, $t_{29,1}$ = –.97, p =. 17). HRs recorded during BL were however significantly different from the ones recorded during test periods (76 to 80 BPM for BL and CPT, $t_{29,1}$ = –2.31, p = .01; and 76 to 81 BPM for BL and HIT, $t_{29,1}$ = –4.92, p = .00002). A significantly different HR between BL and test time was a result that could only be replicated for the HIT (76 to 81 BPM for BL and HIT, $t_{29,1}$ = 2.10, p = .04) when the initial 15-s phase was taken into consideration. The subjective pain intensity and the increase of the HR during this initial phase correlated (r_s = .46, p < .05).

The calculated percent difference in BP correlated with the EDA (r = .43, p < .05) and with the mean HR (r = .44, p < .05). This was again the case only for the HIT.

As to RR, no difference was found in thoracic (197 to 191 beats per test [BPT] for CPT and HIT, respectively; $t_{29,1}$ = .77, p = .22; r = .47, p < .05) nor in abdominal respiration (184 to 188 BPT for CPT and HIT, respectively; $t_{29,1}$ = –.57, p = .29, r = .45; p < .05) over the entire test duration. Additionally, no differences relative to BL (thoracic RR: 188 to 189 BPT for BL and CPT, $t_{29,1}$ = 0.04, p = .9; 191 to 183 BPT for BL and HIT, $t_{29,1}$ = –0.88, p = .4; abdominal RR: 183 to 177 BPT for BL and CPT, $t_{29,1}$ = –0.94, p = .4; 184 to 179 BPT for BL and HIT, $t_{29,1}$ = –0.52, p = .6) could be observed. A high correlation between thoracic and abdominal RR was only identified for the CPT (r = .59, p < .01). During the CPT, but not during the HIT, the respiration parameters correlated with the mean HR (thoracic RR · HR: r = .58, p < .01; abdominal RR · HR: r = .38, p < .05).

Table 2. Psychophysiological data

	CPT		HIT					
	Mean±*SEM*	Range	Mean±*SEM*	Range	Correlation CPT/HIT	*t*-value (df=29)	*P*-value (2-tailed)	Effect size (d)
Syst. blood pressure (mmHg)	159±4	118-211	152±4	113-196	r = .74***	2.81	.009**	.46
Increase of syst. blood pressure (%Δ)	16±2	4-48	8±1	7-21	r = .11	4.00	.0004***	.72
Heart rate variability (symp./parasymp.balance relative to BL)	2.5±0.2	0.2-4	1.5±0.4	−6-4	r = .25	2.49	.019*	.52
Heart rate (BPM)	80±2	65-104	81±2	64-114	r =.80***	−.97	.17	/
Thoracic respiration rate (BPT)	197±8	131-342	191±7	119-284	r =.47*	0.77	.22	/
Abdom. respiration rate (BPT)	184±7	109-298	188±8	101-333	r =.45*	−0.57	.29	/
EDA (mS)	0.14±0.02	0.004-0.45	0.22±0.03	0.003-0.6	r =.62***	−1.81	.003**	.53

All data expressed as T-values w. norm values * *P*<.05; **P*<.01;***P*<.001

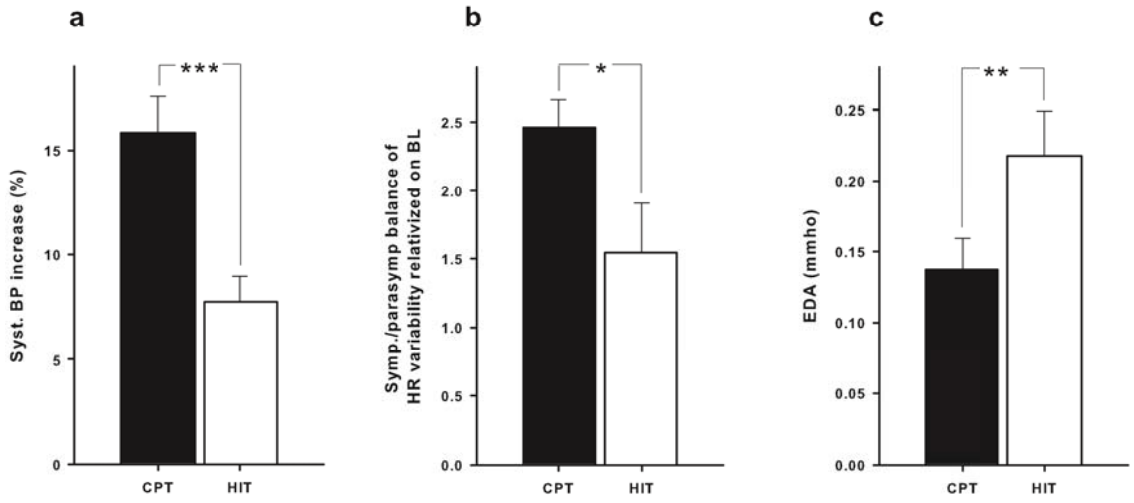

Fig. 3. Psychophysiological data. (a) Percent blood pressure increase relative to baseline (BL). (b) Sympathetic/parasympathetic balance rel. to BL. (c) Spontaneous electrodermal fluctuations rel. to BL. All data expressed as *AM* ± *SEM*. *** < .001, ** < .01, * < .05.

3.5 Discussion

The major goal of the present study was to investigate the *internal validity* of noxious water immersion as a tonic pain stimulus for DNIC induction. Since the cardiovascular regulations induced by local cooling of the extremities may themselves engender a reduced pain sensitivity in the sense of a baroreflex hypoalgesia (Duschek et al., 2007), using noxious cold as a DNIC trigger could result in *reactive testing* by producing pain reduction through the thermal and not the nociceptive qualities of the stimulus. Accordingly, it has already been postulated that pain processing and modulation may be highly intermingled with cardiovascular changes induced during the CPT (see Peckerman et al., 1991 for overview).

By contrasting cold to hot water immersion and analyzing the concurrent physiological arousal, especially cardiovascular reactivity, we wanted to investigate whether the HIT would be a less sympathetically confounded tonic pain model. We did not explicitly test for the capacity of both tests to induce pain inhibition, since both tests are analogous in this regard as Granot et al. (2008) documented.

We observed that both immersion tests were quite comparable with respect to temporal summation, unpleasantness and subjective intensity of pain. With the stimulation temperatures chosen in this study–on the order of those commonly used in DNIC investigations (cf. Granot et al., 2008; Lautenbacher et al., 2008)–, the HIT produced, however, a slightly higher subjective pain experience and was tolerated for a shorter period of time.

Both tests produced pronounced EDA fluctuations and tachycardia during the beginning of the immersion, an increase that returned to baseline levels within the second minute of the test. Spontaneous fluctuations of EDA were higher during the HIT, but contrary to Dowling (1983), who found a positive correlation between skin conductance level and pain tolerance, we could not identify any relationship between respiratory, electrodermal and algesimetric parameters. Correlations between mean thoracic and abdominal RR and HR were only found for the CPT (Steptoe et al., 1984; see also Weise et al., 1993), which could be the result of a potential respiratory sinus arrhythmia. This finding further supports a relatively higher baroreflex activity during cold-water immersion. The results

of the HR variability parameter substantiate this conclusion as well, since we observed a higher sympathetic activity during the CPT than during the HIT.

With regard to HR, we found enhanced values compared to BL in both tests, which is largely documented for the CPT and congruent with data from Kondo et al. (2001), who observed an overall increased HR during lower leg immersion even in innocuous 42° C water. Interestingly, the forehead CPT has even been shown to cause HR decreases (Peckerman et al., 1991), which could be explained by a reduced sympathetic innervation of the forehead.

Both immersion tests lead to increases in BP, which is also in line with data from former investigations (see Lovallo, 1975, 1985 for review on CPT and Tousignant-Laflamme et al., 2005 for HIT). The less pronounced cardiovascular effects during the HIT compared to the CPT are compatible with the observed inverse relationship between water temperature range (0-28°C) and size of HR rise (Kregel et al., 1992). Despite the observed increases in both tests and a more pronounced pain experience during the HIT, the cardiovascular changes were more prominent during the CPT with a higher increase of BP and a higher LF/HF ratio (i.e. sympathetic-parasympathetic balance).

The postulation that physiological changes induced by hot water are due to a genuine nocifensive rather than a thermoregulatory reaction was further corroborated by the positive correlation between pain tolerance and BP increase in the HIT trial, but the lack of such a correlation during the CPT. A positive, albeit gender-specific relationship between HR and pain experience was also found by Tousignant-Laflamme and colleagues (Tousignant-Laflamme et al., 2005) in an investigation using only the HIT. The absence of a correlative relationship between pain ratings during CPT and HR on the other hand, were in line with findings by other investigators (Peckerman et al., 1991). Interestingly, Dowling (1983) found a negative correlation between HR level and pain tolerance level during the resting and anticipation period before a CPT. This correlation became insignificant 40 s after the immersion, i.e. when pain had started to develop. This divergence between indicators of pain perception and cardiovascular reactivity observed in the two immersion tests is likely to be related to a lower sympathetic or thermoregulatory involvement during the HIT (Appenzeller, 2000).

Trying to differentiate between DNIC and baroreflex hypoalgesia using pharmacological manipulations has proven to be complicated. Although it has been demonstrated that opiates may reduce increases in subjective pain and BP induced by CPT, the causality and moderation of this effect remains elusive, due to the additional vasodilatory effectiveness of these substances (Posner et al., 1985; see also Edwards et al., 2004). The analgesic ibuprofen has, on the other hand, failed to reduce pain during CPT despite of its vasodilatory effects. The fact that pain was even increased in this study could speculatively be attributed to an inhibition of baroreflex hypoalgesia (Peckerman et al, 1991).

In summary, our data indicate that the HIT is less confounded with thermoregulatory baroreflex activity and therefore a more appropriate model to produce experimental tonic pain with less autonomic arousal. Nonetheless, the HIT might also provoke significant increases in BP, so that the induction of baroreflex hypoalgesia may not be excluded for this model. Due to the complex interactions between baroreflex, opioid and descending pain modulation mechanisms (see France, 1999 for review and discussion), it is difficult to experimentally characterize genuine DNIC effects as defined by LeBars et al. (1979a,b, 1992) in humans. Pain models employing water immersion as well as the ischemic tourniquet pain test (Smith et al., 1966) are massively confounded with cardiovascular regulations and consequently hypoalgesia (cf. Pertovaara et al., 1984). Thus, the pain modulation provoked by these models should strictly speaking be described as an unspecific form of descending inhibition rather than a perceptual correlate of DNIC. Further research with other tonic pain models, using psychophysics combined to psychophysiology, is needed to characterize tonic pain models that are less likely to induce interfering vegetative reactions, and therefore more appropriate for induction of distinct forms of descending pain control.

3.6 Acknowledgements

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4 Article 2

4.1 Abstract

Hot and ice-water immersions are commonly used for heterotopic noxious counter-stimulation (HNCS) in investigations on endogenous pain modulation. However, coincident sympathetic thermoregulatory activity does not allow to differentiate between perceptual hypoalgesia related to baroreflex sensitivity (BRS) or diffuse noxious inhibitory controls (DNIC). The present study analyzed the internal validity of another supposedly less confounded tonic pain model (inter-digital web pinching; IWP) regarding its potential as DNIC trigger.

We performed a randomized controlled study in 24 healthy gender-matched drug-free volunteers aged 21-54 (median 25) yrs. The study protocol comprised the assessment of mechanical and thermal perceptual wind-up before and after an IWP (15 N) or hot water immersion trial (HIT; 47.5° C) of 2 min duration. Wind-up was induced either by 10 repetitive (1 Hz) contact heat (max. 49° C; 5 × 5 mm thermode) or ballistic impact stimuli (0.5 g at 9 m/s) on the phalanges of the non-dominant hand. Cardiovascular activity, pain experience and corrugator muscle activity were continuously monitored.

Although both HNCS forms produced a similar pain experience (45% of scale), a more pronounced cardiovascular activity was observable for the HIT ($P < .01$). This indicates a higher baroreceptor activity and stronger contamination of painful water immersion by BRS-related hypoalgesia. Regardless of pain modality, wind-up was significantly reduced by HNCS, although this was stronger for painful water immersion than for noxious pinching ($P < .01$).

The HNCS types allow a differentiation between BRS-related and DNIC-like hypoalgesia. IWP proved its validity for DNIC induction, being practically non-confounded by BRS.

Keywords: baroreflex hypoalgesia; endogenous pain modulation; heterotopic noxious counter-stimulation; psychophysiology; psychophysics.

Internal validity of inter-digital web pinching as a model for perceptual diffuse noxious inhibitory controls-induced hypoalgesia in healthy humans

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Article Type: Original article

4.2 Introduction

Experimental pain models constitute important scientific tools for analyzing the intricate (patho-)physiological processes involved in nociception and pain. They serve as surrogates of (pre-)clinical pain processes that inter alia enable us to investigate the mechanisms of action of analgesics as well as to explore causal and modulating factors in chronic pain syndromes. The usefulness of these pain models, however, largely depends on their internal validity, namely the ability to mimic the pain phenomenon they purport to elicit. Clear interpretations and extrapolations can only be drawn when the observed effects on the dependent variables (e.g. subjective pain intensity) are non-confounded by extraneous factors or reactive measures (Campbell and Stanley, 1963).

In recent years, the focus in laboratory pain research has somewhat shifted from the analysis of basal nociceptive mechanisms to the study of modulating top-down processes referred to as endogenous pain modulation or descending inhibitory and facilitatory control (Ren and Dubner, 2002). Especially, the so-called diffuse noxious inhibitory controls (DNIC; Le Bars et al., 1979a, b) regained new research interest, not least because of their plausible explanation for the therapeutic efficacy of pain management techniques like transcutaneous electrical nerve stimulation (TENS; Carlson, 2002). These endogenous pain-modulating systems are activated by heterotopic noxious counter-stimulation (HNCS) and have been postulated to function as a differential contrast-sharpening filter, in the sense that noxious stimuli on one body site may activate a kind of surround inhibition of ongoing painful stimulation at adjacent or distal body sites (Le Bars et al., 1992).

Animal studies have shown that this extra-segmental inhibitory process relies on the suppression of activity in spinal wide dynamic range neurons via efferent projections from the medullary subnucleus reticularis dorsalis. Although a reticular involvement may also be assumed in humans, the exact neural structures have yet to be identified (Villanueva et al., 1996). Some authors have therefore proposed to use the term DNIC-like effects for psychophysical investigations on these counter-irritation phenomena (Pud et al., 2009).

DNIC-like effects are typically triggered through HNCS induced by tourniquet ischemia (Kosek and Hansson, 1997) or painful water immersion of the contra-lateral extremities

(Lautenbacher et al., 2008; Tousignant-Laflamme et al., 2005). These induction techniques do however represent a major cardiovascular challenge and the observed perceptual hypoalgesia might be an epiphenomenon related at least partly to baroreflex sensitivity (BRS) and not necessarily the expression of a genuine nociceptive filter mechanism subserved by DNIC (Streff et al., 2010). It is a well-documented phenomenon that experimental baroreceptor stimulation, as well as constitutional hypertension are accompanied by a reduced pain sensitivity (for review see Bruehl and Chung, 2004).

The aim of the present study was to analyze the internal validity of a supposedly less BRS confounded mechanical HNCS (i.e. inter-digital web pinching, IWP; Growcott et al., 2000), with regard to its potential as a DNIC trigger, by comparing it to the more largely studied water immersion procedure. A further question was to identify whether DNIC-like effects were modality-specific (thermal vs. mechanical).

4.3 Methods

4.3.1 Subjects

The study included $N = 24$ healthy drug-free (> 24 h) volunteers with a median [Md] age of 25 years (range 21-54 yrs.; gender ratio 1:1). We opted for a mixed-gender sample to control for potential gender differences with regard to DNIC (cf. Pud et al., 2009). Subjects were recruited via advertisements posted at the university campuses and received monetary compensation for their participation. Exclusion criteria were the presence of an acute medical condition or an anamnestic history of a neurological, psychiatric or cardiovascular disorder (checked via questionnaire and auscultatory blood pressure assessment). Participants were free of dermatological disorders and skin lesions on the stimulation sites. All of the subjects, with the exception of one, were right-handed. The stimulation protocol is in accordance with the ethical guidelines of IASP (Charlton, 1995) and was endorsed by the national research ethics committee (ref. 200703/01). Each participant gave informed written consent.

4.3.2 Algesimetry

Perceptual DNIC-like pain inhibition was tested by assessing the reduction of perceptual wind-up of phasic pain induced on the left hand by a preceding hetero-topic noxious counter-stimulation (HNCS) applied to the contra-lateral (i.e. right) hand. Tonic HNCS

had a duration of 2 min and was either induced (a) by immersion of the hand up to the wrist into hot water (temperature 47±0.5° C) or (b) by application of pinch pressure (force 15 N) to the corresponding inter-digital webs between the 2nd, the 3rd and the 4th digit of the right hand (Adriaensen et al., 1983). A 12-L tank with water circulated by an external magnetically driven pump and held at a constant temperature by a digitally controlled thermocouple heater was used for water immersion testing (HIT). For inter-digital web pinching (IWP), we employed a pair of pneumatically controlled plastic forceps with rounded tips (diameter 5 mm; modified version of the device used by Forster et al., 1992).

The latency until first appearance of a painful sensation during HNCS (≈ pain threshold) as indicated by the subjects was measured with a mechanical stopwatch (A. Hanhart GmbH & Co.KG, Germany). Subjective pain intensity was quantified on a verbally anchored numeric rating scale (NRS; 0 corresponding to *no pain* and 100 to the *maximal imaginable pain*) every 15 seconds during both pain tests. Pain unpleasantness was evaluated at the end of each test using a 10-cm visual analogue scale (VAS). Nervous tension perceived throughout the tonic stimulation was rated on a 5-point Likert scale (1 = *minimal* and 5 = *maximal tension*).

Perceptual wind-up served as a test stimulus for perceptual DNIC-like pain inhibition and was either evoked by ten controlled (a) contact heat or (b) ballistic mechanical impact stimuli of 1-s duration and repeated at a frequency of 1 Hz. Noxious thermal stimuli (adaptation temperature 32° C; target temperature 49° C; rise/return rate 1° C/s) were applied through a 5 × 5 mm thermode (TSA-II NeuroSensory Analyzer; Medoc Advanced Medical Systems Ltd., Israel) on the palmar side of the proximal phalange of the middle finger of the left hand. Phasic mechanical pain stimuli consisted of blunt plastic projectiles (mass 0.5 g; diameter 5 mm) that were accelerated through a guiding plexiglass tube and applied to the dorsal side of the distal phalange of the middle fingers with a velocity of 9 m/s via a pneumatically driven device (Beise et al., 1999; Kohllöffel et al., 1991). The subjective intensity of each phasic pain stimulus within a wind-up series was numerically rated (see NRS description above). Although mechanical temporal summation of pain is typically provoked by punctate stimuli like pinprick or von Frey hairs that elicit stinging pain (Magerl et al., 1998; Weissman-Fogel et al., 2009), we

chose a stimulus type that evokes a pain experience and intensity comparable to the ones commonly employed for thermal wind-up induction. In this sense, the projectiles used for mechanical impact stimulation had approximately the same surface area as the thermode and produced a similarly dull pain sensation than phasic contact heat (as assessed in pre-tests).

Pictures of the custom-built devices used for noxious mechanical stimulation are shown in Figure 1. The plastic forceps and projectiles had no sharp edges and were vertically applied (at an angle of 90°) to the skin, thus precluding skin penetration and risk of infection at the stimulation forces used. The stimulation procedures were well tolerated by all subjects.

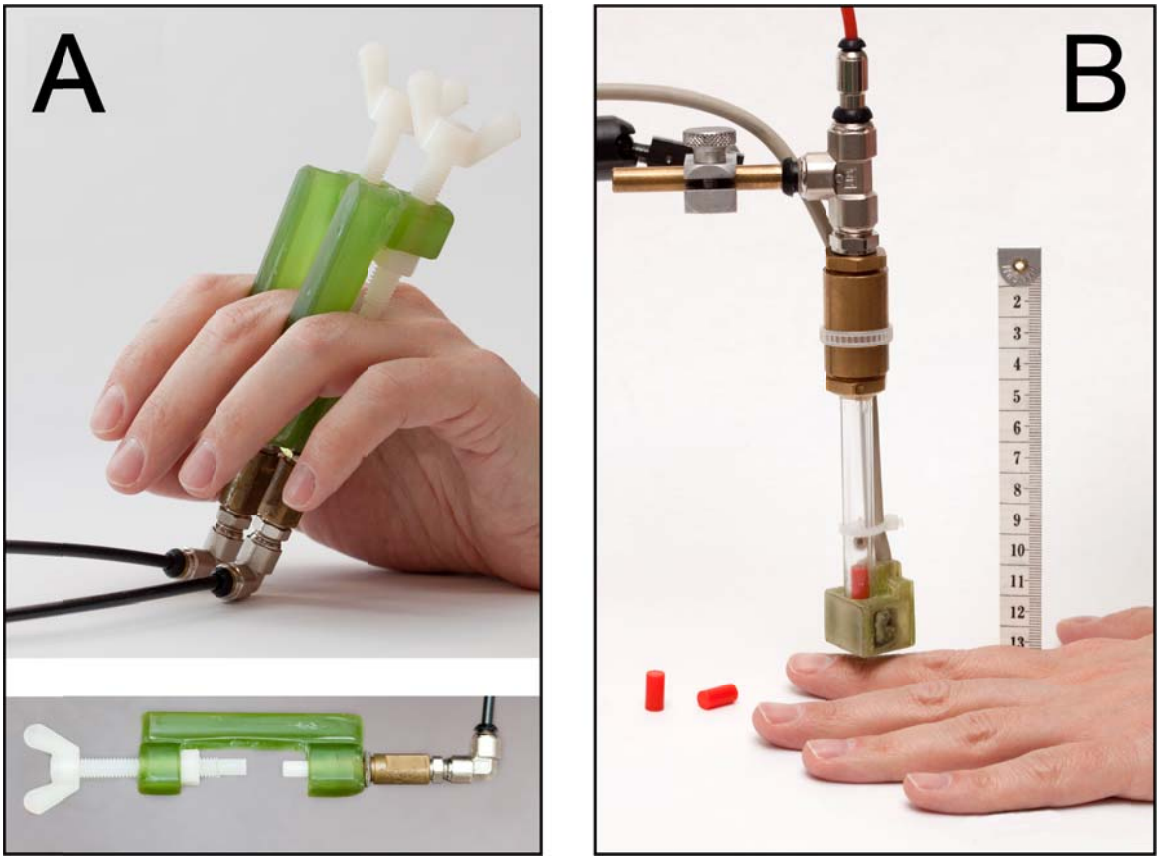

Fig. 1. Stimulation devices. (A) Inter-digital web pinching (IWP): A constant pressure force of 15 N was applied to the inter-digital skin folds between the index, middle and ring fingers using a pair of pneumatically controlled plastic forceps with blunt tips and a diameter of 5 mm (adapted from Forster et al., 1992). (B) Controlled mechanical ballistic impact stimulation: Rounded cylindrical plastic projectiles with a mass of 0.5 g and a diameter of 5 mm were accelerated through a guiding plexiglass tube by a pneumatically driven device and applied to the dorsal side of the distal phalange of the middle (2^{nd}-4^{th}) fingers with a velocity of 9 m/s (adapted from Kohllöffel et al., 1991).

4.3.3 Psychophysiological assessment

Pain-related physiological reactions during HNCS were continuously recorded (sampling rate 1000 Hz) on a MP150 Data Acquisition System with the corresponding amplifiers and transducers (BIOPAC Systems Inc., USA). Blood pressure (BP) was manometrically monitored on the wrist of the left arm. Cardiac activity (heart rate [HR]) was assessed with a standard pre-cordial lead II electrocardiogram (ECG; 0.5-Hz high pass and 35 Hz low pass filtering) using disposable pre-gelled Ag-AgCl electrodes placed below the right clavicle and on the left lower ribcage, respectively. Subjects were grounded through a surface electrode attached to the right lower ribcage. To control for breathing artefacts on cardiovascular measures, thoracic and abdominal respiratory effort was recorded via strain gauge belts. Facial muscle activity was electromyographically (EMG) recorded with two 4-mm diameter reusable Ag-AgCl electrodes filled with non-irritating electrode gel and fixed over the left eyebrow in parallel to the corrugator supercilious muscle (separated by approx. 1.5 cm). Skin was cleaned with ethylic alcohol prior to electrode placement.

Subjects were seated in upright position (inclination 90°) in an upholstered chair. Room temperature was held constant at 21±0.5° C by a mechanical ventilation system. The AcqKnowledge Software package (BIOPAC Systems Inc., USA) was used for data collection and offline analysis (incl. HR variability [HRV] analysis automation routines).

4.3.4 Experimental protocol

The study consisted of a single session (duration 90 min.) involving the assessment of perceptual wind-up before and after HNCS. The study protocol was based on a combined group (two gender-matched comparison groups for the two HNCS types: HIT vs. IWP) and repeated measurements crossover (two test blocks for perceptual DNIC-like pain inhibition in counterbalanced order: thermal vs. mechanical wind-up) design. Subjects were randomly assigned to one of the two HNCS groups. We chose a group comparison design with regard to the HNCS, since the after-effects of the potentially underlying BRS- and DNIC-related hypoalgesia might have had different time courses, which could have been confounded when using a within-subjects design.

Thus, depending on group affiliation, each subject underwent two HNCS trials of the same kind (i.e. either two HIT or two IWP) in a single session. The potential inhibitory effect on perceptual wind-up was assessed before (baseline [BL]) and at two time-points after each HNCS stimulation, i.e. immediately (t_1) as well as 10 min after (t_2) the 2-min conditioning stimulus. The inter-stimulus interval between both HNCS was 20 min (see Figure 2). The second post-HNCS time-point was chosen to make sure that the observed inhibition was not due to adaptation or habituation. Since DNIC-like effects appear to subside in less than 10 min, the chosen time window should have sufficed to observe a recovery from HNCS-induced inhibition (Jinks et al., 2003). Every wind-up test comprised three single runs of the above-described series of 10 stimuli. Half of the subjects in each HNCS group were first tested for mechanical (test block I) and then thermal wind-up (test block II), whereas the other half was tested contrariwise (AB-BA scheme).

Physiological registrations and stimulation procedures started subsequent to an adaptation period of 2 min after electrode placement, beginning with the assessment of the thermal and mechanical pain detection thresholds and followed by the various tests for perceptual DNIC-like effects. The experimental protocol is schematically illustrated in Figure 2 (experimental timeline). The same experimenter conducted all examinations.

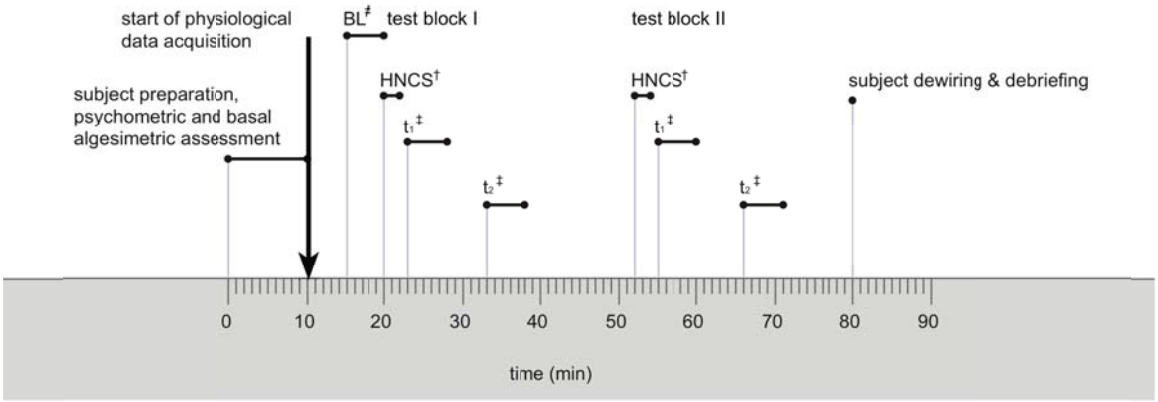

Fig. 2. Experimental timeline. BL = baseline, HNCS = heterotopic noxious counter-stimulation. # Mechanical and thermal wind-up testing (in counterbalanced order). † Half of the subjects received thermal, the other half mechanical HNCS during both test blocks. ‡ Sensory modalities (thermal/mechanical) for wind-up testing varied between the test blocks, but were fixed for both time-points t_1 and t_2.

4.3.5 Data analysis

Individual wind-up data were first standardized to the initial rating in a given stimulus series and then averaged over the three stimulation runs for the respective test point. In order to estimate potential changes in perceptual pain wind-up, we computed the slope ($\Delta y/\Delta x$) of the linear regression curve fitting the aggregated ratings for a specific test point to the corresponding stimulus repeats.

Mean HR, systolic and diastolic BP as well as the integrated EMG were calculated post-acquisition and separately for both test blocks. Integrated EMG was derived from raw EMG data with a smoothing factor of 100. All data were relativized to the corresponding BL values corresponding to the 1-min recording at 2 min before the beginning of HNCS. HRV was assessed by frequency-domain analysis and we report the ratio of low-to-high frequency spectra power (LF/HF) as a broad index of sympatho-parasympathetic balance.

Differences in physiological data between both tonic pain tests were analyzed with t-tests for paired samples, whereas non-parametric Wilcoxon signed rank tests were computed for threshold data, nervous tension and unpleasantness ratings due to skewed data distributions. All data are represented as arithmetic mean and standard error of the mean (*AM*±*SEM*), with the exception of the asymmetrically distributed psychophysical data, where *Md* and mean absolute deviation (*MAD*) or range were used as parameters of central tendency and distribution. Pearson product-moment correlation coefficients (r) and Spearman's Rho correlation coefficients (r_s) were used as appropriate.

Differences in the time course of subjective pain intensity between both tests were analyzed by a factorial analysis of variance (ANOVA) with HNCS type as a between-subjects factor and stimulus duration as within-subjects factor. Wind-up data were analyzed separately for HCNS and wind-up type with a repeated measures ANOVA with time-point (BL, t_1 and t_2) as the independent within-subjects variable. Huynh-Feldt corrected values were considered, in case the normal distribution assumption was not met, as verified by the Kolmogorov-Smirnov test ($P > .20$). For post-hoc analyses, t-tests with sequential Bonferroni-correction were performed.

A one-tailed *P*-value of less than .05 was considered significant in all tests, except when psychophysical differences between both HNCS types were analysed. Since in these

cases we tested for the null hypothesis (i.e. that there was no difference between both HNCS), a more conservative two-tailed significance level of $\alpha = .20$ was chosen.

All statistical analyses were performed using STATISTICA (StatSoft Inc., USA). Graphs were created with SigmaPlot (Systat Software Inc., USA) and Temporis (Bartas Technologies LLC, USA). Effect sizes were computed post hoc with G*Power (Faul et al., 2007).

4.4 Results

Descriptive analysis revealed no differences in psychophysical and psychophysiological data between both genders. For all subsequent analyses, data of male and female participants were thus combined.

Table 1. Psychophysical data

	IWP		HIT				
	Measures of central tendency and dispersion	Range	Measures of central tendency and dispersion	Range	Correlation IWP×HIT	Test value (df = 11)	*P*-value (1-tailed)
Pain latency (s)	5±12 [a]	2-32.5	10±8	5-40	r_s = .62	Z = 0.97 [b]	.33
Overall subjective pain intensity (aggregated over time)	44±8 [c]	2-78	43±6	16-67	r = 1**	t = –0.10 [d]	.46
Subjective pain increase (Δ%) relative to initial rating	177±114	–67-600	237±66	0-900	r = –.16	t = 0.52	.31
Unpleasantness (VAS)	62±18 [a]	8-86	57±15	38-86	r_s = –.02	Z = 0.01 [b]	.99
Nervous tension (Likert Scale)	2.2±0.9	0.5-3.5	2.2±0.9	0-3.5	r_s = .03	Z = 0.04	.97

[a] *Md*±*MAD* (mean absolute deviation); [b] *Z*-value; [c] *AM*±*SEM*; [d] *t*-value; ** $P < .01$

4.4.1 Comparability of HIT and IWP

There were no substantial differences in the ratings (cf. table 1) and extremely high retest reliabilities ($r = .99$, $P < .01$) between the two test blocks for both tonic tests, wherefore data from the first and second test blocks were aggregated for each test for further analyses. Both tonic tests had an analogous time course (Fig. 3A), albeit the mean initial ratings were slightly but not significantly higher for the IWP (31.5±7.5) than the HIT

(23.5±6). Pain onset was in average 5 s earlier for the IWP (5±12 s) when compared to the HIT (10±8 s), although this difference in latency was not statistically significant. Temporal summation of pain calculated as percent difference of the last to the first non-zero rating was again comparable under both tonic pain conditions. With a cut-off time of 2 min, pain experience remained always under the pain tolerance level for all subjects and all tests. Overall subjective pain intensity ratings (grand mean) did not differ between the IWP (44±8 NRS-units) and the HIT (43±6 NRS-units). Both tests were quite similar with regard to the amount of unpleasantness (IWP: 62±18, HIT: 57±15 VAS-units; see Fig. 3B) and even identical with regard to the amount of nervous tension (IWP and HIT: 2±1 on the Likert-scale) they induced.

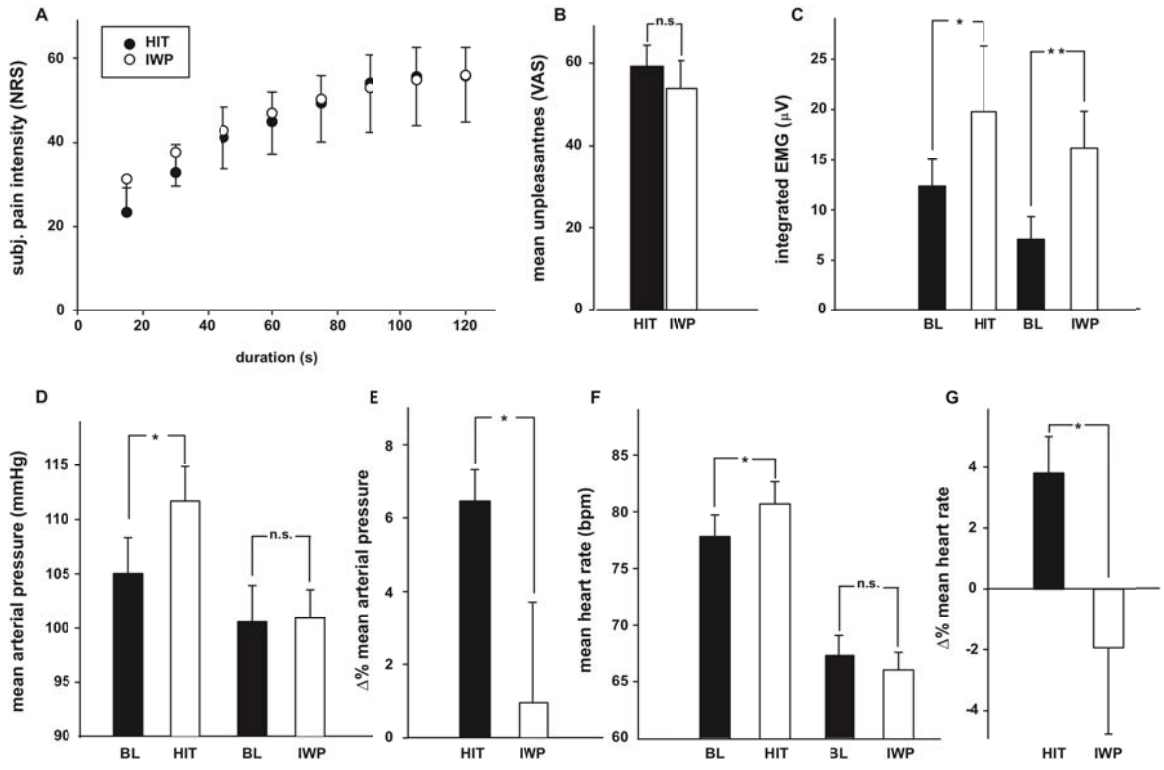

Fig. 3. Comparability and internal validity of HIT and IWP. Psychophysical data: (A) Time course of subjective pain intensity (rel. to initial pain rating). (B) Perceived overall pain unpleasantness (assessed immediately after 2-min tonic stimulation). Psychophysiological data (aggregated over 2-min stimulus duration): (C) Integrated EMG of corrugator supercilious muscle compared to baseline (BL). (D) Mean arterial blood pressure compared to BL. (E) Percent increase of mean arterial blood pressure relative to BL. (F) Mean heart rate compared to BL. (G) Percent increase of mean heart rate relative to BL. * $P < .05$, ** $P < .01$. All data expressed as *AM*±*SEM* ($N = 2 \times 12$). HIT = hot water immersion test, IWP = inter-digital web pinching.

4.4.2 Internal validity of HIT and IWP

Physiological data also revealed a relatively homogenous pattern in the sense of a higher sympathetic activity (BP, HR and HRV) during the HIT compared to IWP.

Whereas no change in BP level was seen after IWP (101±3 vs. basal values of 100±3 mmHg), the HIT engendered a significant increase in mean BP level of 7.5% relative to BL (i.e. from 105±3 to 112±3 mmHg; $t_{11} = -8.64$, $P < .0001$, effect size $d = 0.7$; Fig. 3D) and consequently to IWP ($t_{11} = 2.27$, $P = .02$, $d = 1.1$; Fig. 3E).

We did not observe any difference in mean HR during IWP (69±3 beats per min [bpm]) relative to BL (70±2 bpm), in contrast with the significant HR acceleration detected after the HIT (i.e. from 78±2 to 81±2 bpm; $t_{11} = -3.22$, $P = .004$, $d = 0.3$; Fig. 3F/G). Over and above that, the LF/HF ratio was significantly higher during both tests than during BL (IWP: 2.1±0.2 vs. 0.74±0.03, $t_{11} = -6.85$, $P < .0001$, $d = 2.4$; HIT: 2.9±0.07 vs. 0.77±0.07, $t_{11} = -29.57$, $P < .0001$, $d = 8.8$), and significantly more elevated by 40% under the HIT condition than during the IWP ($t_{11} = 3.37$, $P = .003$, $d = 0.9$).

On the other hand, both tests produced a stronger contraction of the corrugator supercilious muscle relative to BL (IWP: $t_{11} = -4.48$, $P = .0005$, $d = 1.3$; HIT $t_{11} = -1.74$, $P = .05$, $d = 0.5$), which did not significantly differ between IWP (20 μV) and the HIT (17 μV; Fig. 3C).

Taken together (see table 2 for overview), we observed a more pronounced increase in the assessed cardiovascular parameters (rel. to BL) under the HIT condition when compared to the IWP, which indicates a higher baroreceptor activity and thus stronger contamination of by BRS-related hypoalgesia during the HIT. This occurred although pain ratings and pain-related corrugator activity were—at least in tendency—higher during the IWP.

Table 2. Psychophysiological data

	IWP		HIT					
	AM±SEM	Range	*AM±SEM*	Range	Correlation IWP × HIT (*r*)	*t*-value (df = 11)	*P*-value (1-tailed)	Effect size (*d*)
Mean blood pressure (mmHg)	101±3	84-115	112±3	95-141	–.62	2.07	.03*	1.1
Mean blood pressure increase (Δ%)	1.3±1.2	–2.7-4.8	7.5±2.5	2.5-18.8	–.27	2.27	.02*	0.9
Heart rate variability (LF/HF)	2.1±0.3	0.8-3	2.8±0.1	2.5-3	–.45	3.37	.003**	0.9
Mean heart rate (BPM)	69±3	60-79	81±2	73-84	–.26	3.17	.004**	1.4
Mean heart rate increase (Δ%)	–2.2±2.1	–15-7	4.3±1.3	0.5-12	–.63	1.92	.04*	1.1
Integrated EMG (μV)	24±13	5-85	19±10	5-65	–.05	0.59	.30	Power < 10%

* $P < .05$, ** $P < .01$

4.4.3 Effectiveness of HIT and IWP

HIT and IWP were tested with regard to their capacity to suppress mechanically and thermally induced wind-up pain. Both phasic pain modalities produced marked and comparable increases in subjective pain intensity of approx. 20-35% with cumulating stimulus repeats under basal conditions. BL ratings of mechanical and thermal wind-up of pain sensation (aggregated as geometric mean averaged over stimulus presentations) were positively correlated for both test groups (r = .50-.80). Regardless of pain modality, HNCS was able to reduce temporal summation of phasic pain (see Fig. 4A-D), although this inhibition appeared to be more prominent after the HIT than after IWP (56.5% vs. 19.5%; cf. Fig. 3A/C vs. 3B/D). Analysis of slope coefficients revealed a significant suppression of both wind-up forms by both HNCS types (HIT: $F_{2,46} = 24.85$, $P < .0001$, effect size $f = 1.0$, partial $\eta^2 = .35$; IWP: $F_{2,22} = 5.89$, $P = .009$, $f = 0.7$, partial $\eta^2 = .52$). Interestingly, we were able to identify a modality effect with regard to wind-up suppression in subsequent post-hoc analyses, but not related to HNCS. While mechanical wind-up remained reduced over the whole post-HNCS test block (Fig. 4B/C), the ratings of thermally induced wind-up tended to return to BL at t_2 ($t_{11} = -3.68$, $P = .002$, $d = 1.1$ and $t_{11} = -2.99$, $P = .006$, $d = 0.86$ for HIT and IWP, respectively; Fig. 4A/D).

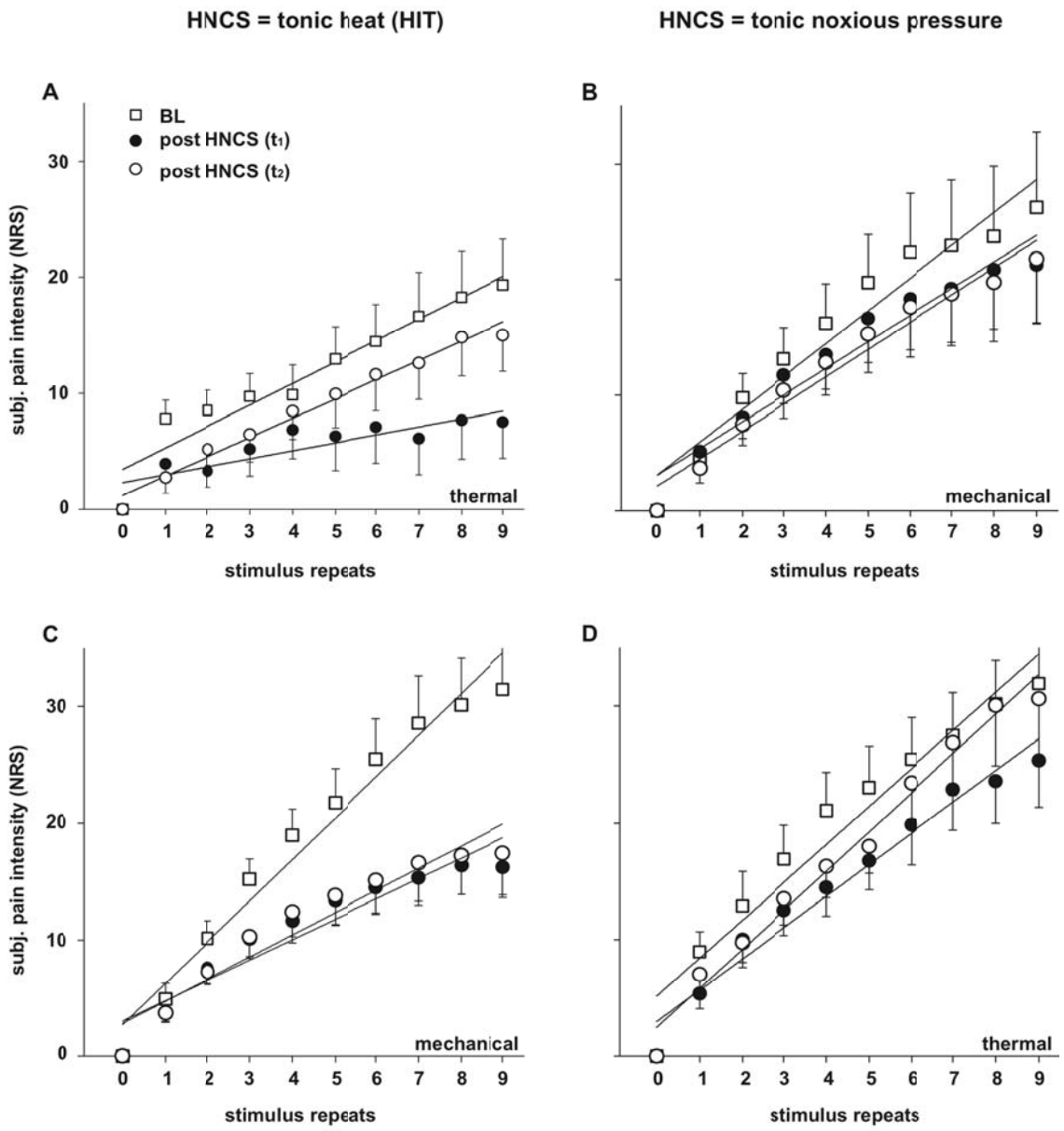

Fig. 4. Effectiveness of HIT and IWP. The graphs depict the temporal summation of subjective pain intensity ratings over stimulus repetitions (rel. to initial stimulus rating) before (BL), immediately (t_1) and 10 min after (t_2) HNCS (heterotopic noxious counter-stimulation). All data expressed as *AM*±*SEM* ($N = 4 \times 6$). The trend lines represent the linear regression functions of pain ratings over stimulus repeats. HIT = hot water immersion test, IWP = inter-digital web pinching.

4.5 Discussion

Deficient descending modulatory control has been documented in chronic pain patients (Chung and Bruehl, 2008; Staud et al., 2003, 2004), and the analysis of endogenous pain modulation is indispensable for a comprehensive understanding of the pathology of pain. In the study reported here, we compared different human pain models with regard to their

internal validity and practical usefulness for experimentally characterizing endogenous hypoalgesia.

4.5.1 Comparability of HIT and IWP

The data presented above confirm that both HNCS types are relatively similar with respect to their psychophysical characteristics. The pain experience during the two stimuli had a nearly identical time pattern and intensity, and was accompanied by sensations (i.e. unpleasantness, nervous tension) of comparable magnitude. This similarity in the subjective perception of pain is furthermore paralleled by the EMG data (i.e. autonomous corrugator muscle activity) as an objective indicator of facial expression of pain (i.e. brow lowering; Prkachin, 1992).

4.5.2 Internal validity of HIT and IWP

In contrast, and as hypothesized, sympathetic arousal associated with both tonic stimulation forms was significantly disparate. Whilst there were no identifiable or only negligible changes in cardiovascular activity (i.e. BP, HR, LF/LH) related to the pinching stimulus, significant and pronounced increases in BP and HR could be observed during hot water immersion. Analysis of HRV also suggested a stronger sympathetic regulatory activity for the latter stimulus condition. Summing up, these results corroborate the assumption that IWP is less contaminated by BRS-related hypoalgesia, a form of endogenous pain control that might also lead to a reduced central sensitization as reflected in wind-up (Chung and Bruehl, 2008).

4.5.3 Effectiveness of HIT and IWP

Both tests proved to significantly and substantially suppress thermally and mechanically induced wind-up, although this inhibitory effect was less pronounced after noxious pinching. Our data are in line with previous human studies demonstrating the reduction of thermal wind-up (Granot et al., 2006; Lautenbacher et al., 2002) or temporal summation of electrically induced nocifensive flexion reflexes by HNCS (Serrao et al., 2004). The stronger inhibition observed after the immersion test might be interpreted as a superimposition of BRS- and DNIC-related hypoalgesia (Streff et al., 2010), whereas the

reduction seen after IWP would be for the most part exclusively attributable to a more genuine DNIC-like effect (see section below).

Regarding the duration of the HNCS-induced wind-up reduction, we found that this inhibitory effect outlasted the conditioning stimulation (cf. Talbot et al., 1987), but no difference between both stimulation types could be noticed. Despite the fact that there was no modality specificity regarding both tonic tests, there was a heterogeneous time pattern depending on the quality of the stimuli used for wind-up induction. While reduction of thermally induced short-term potentiation manifested a return to BL after 10 min, mechanical wind-up was still reduced at t_2. DNIC-like effects have generally been documented to decrement within less than 10 min (Le Bars et al., 1992). Nevertheless, studies testing the temporal pattern of DNIC have usually employed thermally or electrically induced test stimuli, and data on the modulation of mechanically induced wind-up as well as on the time pattern of BRS-related hypoalgesia remain elusive.

4.5.4 Neurophysiological considerations

The subnucleus reticularis dorsalis (SRD) has been identified to be the crucial brain structure for DNIC in animals (Villanueva et al., 1996). It constitutes a part of the spinoreticular-thalamic pathway and seems to be involved in basal nociceptive transmission as well as contrast sharpening. Notwithstanding that the SRD is part of the brain structures known to be simultaneously involved in descending pain modulation and blood pressure control (Bruehl and Chung, 2004; Kubo and Misu, 1983), lesion studies have shown that DNIC is a singular form of descending control, which does not involve other nuclei of this spino-bulbo-spinal inhibitory network system like the periaqueductal grey (PAG) or the raphe nuclei for instance (Monconduit et al., 2002). In this sense, DNIC could be considered as a basal nociceptive process attributable at least in animals to SRD-transmitted descending control that might be modulated under conditions of heightened (e.g. stress-induced) cardiovascular reactivity by a more extensive neural network connecting also to the SRD. The fact that we observed a quantitatively weaker effect under IWP than HIT indicates that both HNCS tests might differentiate between BRS- and DNIC-related hypoalgesia. At the least, our data suggest that the effects observed in studies employing painful water immersion as a trigger for endogenous

descending control should strictly speaking be interpreted as the expression of BRS- and not DNIC-related hypoalgesia–even when the SRD or an analogous brain structure in humans might be indirectly involved.

We witnessed a better recovery from diffuse noxious inhibition for thermal compared to mechanical pain in the sense that mechanical wind-up was still inhibited at t_2 whereas ratings for thermal wind-up demonstrated a return to BL in the10-min observation time frame. This time effect might be attributed to a differential modulation of A- versus C-nociceptor evoked spinal responses by top-down modulatory pathways that are extraneous to DNIC. In animal pain models, it has been shown that descending inhibitory control from the lateral area of the anterior hypothalamus selectively inhibits C-fiber but not A-fiber mediated nocifensive reflexes (Simpson et al., 2008). More to the point, the PAG has been shown to exert inhibition only on pinch evoked phasic noxious responses originating from deep dorsal horn neurons with but not without C-fiber input (Waters and Lumb, 2008). Although both A- and C-fibers can be excited with qualitatively similar discharge properties by repetitive impact and heat stimuli as used in our study (Herrero et al., 2000; Koltzenburg and Handwerker, 1994), it may be postulated that the spinal activation pattern is different, and ergo differently modulated.

4.5.5 General conclusion

Our study showed that (a) both HNCS types IWP and HIT are able to produce a prominent hypoalgesia in the form of a reduced wind-up, which was (b) more pronounced after the painful immersion test. IWP-induced hypoalgesia was (c) not associated with significant cardiovascular changes (as indicator of BRS) and (d) strong enough (i.e. 20% decrease and 50% variance explanation) to be considered useful as an experimental surrogate model of endogenous hypoalgesia. To sum up, the IWP proved to be a valid paradigm for the induction of DNIC-like effects, which were non-confounded by BRS-related hypoalgesia. IWP or comparable tonic pain forms should be used as method of choice instead of immersion or ischemia when the focus lies explicitly on DNIC and not on BRS- or stress-related hypoalgesia.

4.6 Acknowledgements

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5 Article 3

5.1 Abstract

Human studies investigating sex-related differences in diffuse noxious inhibitory controls (DNIC)-induced hypoalgesia often use cardiovascular challenges as heterotopic noxious counter-stimulation (e.g. submaximal effort tourniquet or cold pressor test). Under conditions where cardiovascular parameters have not been documented, the potentially confounding impact of baroreceptor sensitivity (BRS) may explain the heterogeneity of the observed effects.

Using inter-digital web pinching (IWP) as DNIC-trigger (force 15 N), a tonic pain model previously validated to be BRS-unrelated, we investigated sex-related differences in temporal characteristics of electrically elicited (5 × 1-ms rectangular 80-Hz pulses at 20% above threshold intensity) subjective pain responses (rated on a numerical scale) and nocifensive R-III-reflex activity (assessed via electromyography [EMG]) at the contra-lateral body side in a gender-balanced sample of $N = 28$ healthy drug-free volunteers aged 21-38 (median 27) years.

HNCS using IWP produced an important and comparable reduction in pain ratings (mean $\Delta = 30\%$; $p < .001$) and EMG response (mean $\Delta = 75\%$; $p = .02$) for both sexes. We did however identify sex-related differences in the post HNCS time courses (time frame 15 min) of pain perception with women demonstrating a more rapid return to baseline compared to men ($p = .04$). Interestingly, an opposite pattern was observed regarding nociceptive reflex activity with a steeper return rate of EMG responses in males, whereas those of women remained attenuated over the entire observation period ($p = .05$).

These findings may reflect a stronger defensive (environmental rejection) response in women.

Sex-specific time course of diffuse noxious inhibitory controls-induced pain modulation and nocifensive reflex suppression in humans

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Keywords: heterotopic noxious counter-stimulation; inter-digital web pinching; nociceptive flexion reflex; psychophysics; psychophysiology; sex

5.2 Introduction

Even when leaving sexual pain disorders, menstrual and labor pain aside, epidemiological studies indicate a disproportionally high prevalence of chronic pain syndromes and multiple pain conditions among women (Berkley, 1997; Fillingim et al., 2004; Unruh, 1996). Apart from the well-documented differences in psychosocial (i.e. gender role-related) factors like dysfunctional coping styles (e.g. catastrophizing) and pain expressiveness, the higher occurrence rate of clinical pain might also be attributed to sex-specific predispositions arising from the endocrine, nociceptive or autonomic nervous systems (Fillingim, 2000; LeResche, 2005; Wiesenfeld-Hallin, 2005).

Human studies investigating the influence of sex and gender on experimentally characterized basal pain sensitivity have yielded more or less heterogeneous results with only moderate and largely modality-dependent differences insufficient to explain the variations seen in clinical pain (Riley et al., 1998). Above and beyond differences in pain detection thresholds and tolerance levels, sex-related differences in descending pain modulation might constitute a more important predictor of the female propensity for clinical pain, as suggested by studies showing a deficient endogenous analgesia (EA) in fibromyalgia patients compared to men and pain-free women, for instance (Staud et al., 2003). However, human experimental studies investigating sex- and gender-effects related to endogenous pain control have produced contradictory results (Popescu et al., in press; Pud et al., 2009). This is especially true for diffuse noxious inhibitory controls (DNIC)-induced analgesia, which can be defined as a sensory filter mechanism sharpening the contrast between the noxious input from a stimulated area and a concurrently irritated extra-segmental body region (i.e. heterotopic noxious counter-stimulation [HNCS]; Edwards, et al., 2003; Piché et al., 2009; Willer et al., 1999).

Some of the inconsistencies in the observed findings may be due to hypoalgesia elicited by confounding baroreceptor stimulation (i.e. baroreflex sensitivity [BRS]-associated hypoalgesia), since most of the studies on EA have used types of HNCS that are associated with direct cardiovascular challenges (viz. painful water immersion or ischemic pain; Bruehl and Chung, 2004; Fillingim and Maixner, 1996; McIntyre et al., 2008; Streff et al., 2010; Tuveson et al., 2006). More precisely, inter-individual variability in cardiovascular reactivity and parental history of hypertension have been

shown to be accompanied by a reduction of pain sensations induced by the cold pressor test (CPT) and thus gender effects might only be revealed when cardiovascular parameters are considered as covariates or as a quasi-experimental grouping factor (al'Absi et al., 1999, 2000, 2002). Interestingly, the few HNCS studies using physically or chemically induced muscle pain instead where the cardiovascular challenge is assumed to be negligible, have all demonstrated clear gender effects (Arendt-Nielsen et al., 2008; Ge et al., 2004; Weissman-Fogel et al., 2008).

The aim of the present study was therefore, to investigate sex differences using an experimental tonic pain model (i.e. inter-digital web pinching [IWP]) for the induction of DNIC-like hypoalgesia that has been validated in a previous psycho-physiological study to be non-confounded by baroreceptor sensitivity-related hypoalgesia (Streff et al., in press). Besides, we were interested in sex-related temporal patterns with regard to the time course of DNIC-induced hypoalgesia (Ge et al., 2004). To differentiate between gender-based responses to pain and sex-related nocifensive reflex activity, we combined subjective and objective algesimetry by using the polysynaptic and multi-segmental lower limb flexion (or RIII-)reflex (LLFR) and corrugator muscle activity as test stimuli for DNIC-efficacy and cognitively unbiased pain measures (France et al., 2002; Prkachin, 1992; Skljarevski and Ramadan, 2002). Furthermore, cardiac and electrodermal activity (EDA) were assessed as indicators of pain-related autonomic reactivity (Bromm and Treede, 1980; Dowling, 1982, 1983).

5.3 Methods

5.3.1 Subjects

The study was performed in N = 28 healthy drug-free volunteers aged 21-38 years (median age 27 yrs.) with a sex ratio of 1:1. Twenty-four of the subjects were right-handed. Health status of candidates (i.e. absence of an acute medical condition, drug abuse or history of a neurologic, psychiatric, sexual or cardiovascular disorder) was checked through an anamnestic questionnaire and sphygmanometry. All participants were normotensive with maximal to minimal values for systolic/diastolic BP of 140/60 mmHg. There was no intake of analgesics, antiphlogistics or alcohol less than 48 h before the beginning of the experimental sessions. Four of the female participants indicated to use contraceptive pills. All subjects were free of skin affections at the stimulation sites. Written informed consent was obtained and participants were awarded monetary compensation. The stimulation procedures were in accordance with the ethical guidelines of IASP and endorsed by the National Research Ethics Committee (ref. 200703/01; Charlton, 1995). There was no dropout and the stimulation procedures were well accepted.

5.3.2 Experimental pain characterization

Squeeze pain induced by IWP served as HNCS type for triggering the activation of DNIC (Forster et al., 1988). In a previous study, we were able to validate this tonic pain form as a model of perceptual DNIC-induced hypoalgesia by demonstrating its potential to induce a prominent reduction of heterotopically applied pain without being accompanied by rises in blood pressure (BP; Streff et al., 2010). To obtain a subjectively unbiased measure for DNIC-effectiveness, we studied the RIII-reflex by measuring the EMG response of the biceps femoris to trains of phasic electric shocks and the corrugator (frowning muscle) response on the contralateral body side (Prkachin, 1992; Prkachin and Solomon, 2008; Rhudy et al., 2009). We opted for pressure and electric stimulation, since sex and gender differences in threshold measures have most consistently been reported for those two modalities (Greenspan et al., 2007).

5.3.2.1. Nocifensive RIII-reflex and corrugator muscle activity

The RIII-reflex was induced on the left leg by a series of five noxious electric shocks. We used rectangular constant-current pulses of 1 ms applied at a frequency of 80 Hz. An inter-stimulus interval of 4.8 s was chosen, which resulted in single pulse trains lasting 24 s. Stimulation intensity was individually adjusted before the beginning of the experimental session at 20% above pain detection threshold and kept constant throughout the experiment.

The stimuli were computer-triggered (E-Prime®; Psychology Software Tools Inc., Sharpsburg, PA, USA) and transcutaneously delivered through a bar electrode (EL350S; BIOPAC Systems Inc., Goleta, CA, USA) connected to a stimulus isolator (STMISOC; BIOPAC Systems Inc.). The stimulation electrode consisted of two convex tin electrodes with a diameter of 0.5 cm and placed 2 cm apart on an acrylic bar. It was fixed with a plaster semi-orthogonal to the retromalleolar path of the sural nerve. The EMG response of the biceps femoris muscle was recorded with two shielded 8-mm diameter Ag-AgCl electrodes (EL258S; BIOPAC Systems Inc.). Both electrodes were positioned 2.5 cm apart and parallel to the course of the muscle according the recommendations of Rainoldi et al. (2004). Electrode positions are depicted in Fig. 1B.

The activity of the corrugator supercilii muscle was monitored employing a pair of shielded Ag-AgCl electrodes (EL254S; BIOPAC Systems Inc.) with a recording diameter of 4 mm. The electrodes were separated by 1.5 cm and attached over the left eyebrow on the muscle midline.

Subjects were grounded through a surface electrode (EL258; BIOPAC Systems Inc.), which was specifically positioned on the midpoint of the left calf in order to filter out interferences between stimulation and EMG recording electrodes. Prior to electrode placement, skin was degreased with ethanol. Non-irritating conductive gel was used for all electrodes.

EMG recordings were acquired on a MP150 Data Acquisition System with an EMG100C amplifier (low and high pass filtering of 5 kHz and 1 Hz, respectively; sampling rate of 1000 Hz; BIOPAC Systems Inc.) according to the guidelines of Fridlund and Cacioppo (1986).

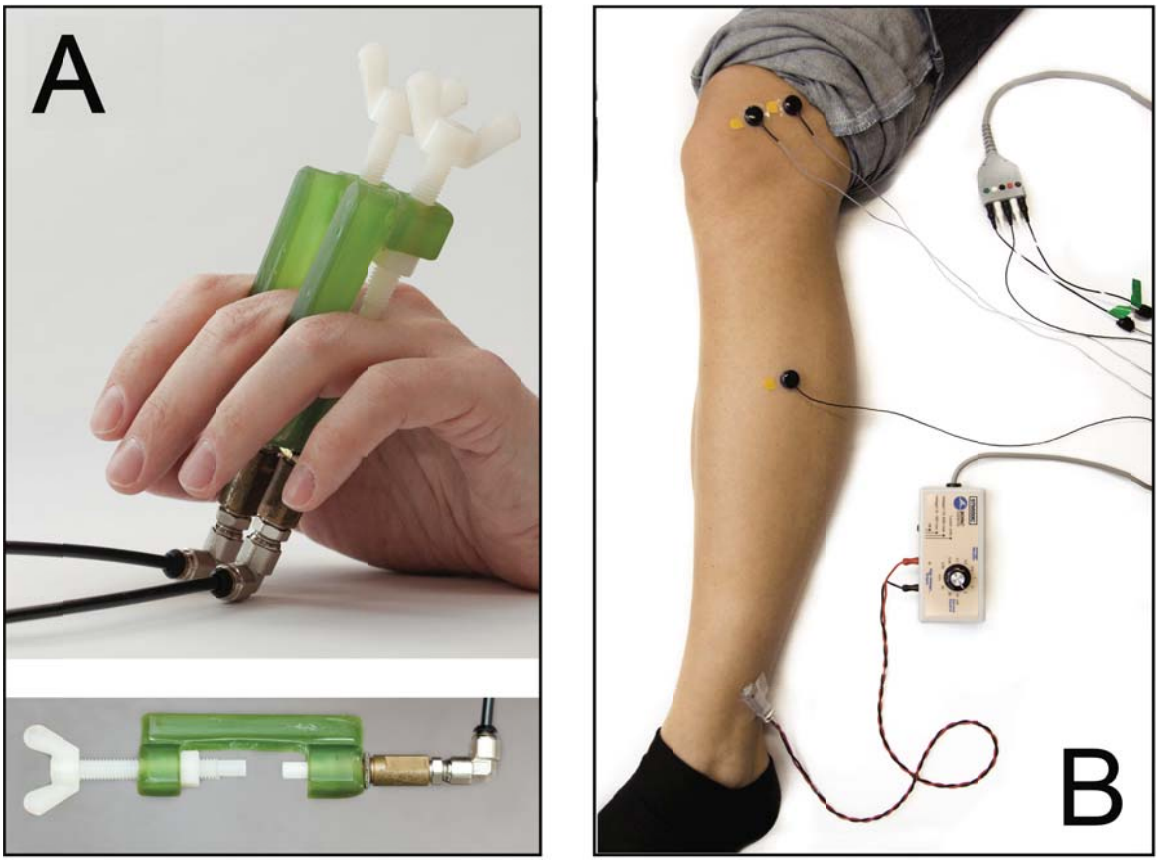

Fig. 1. Stimulation and recording setup. (A) Device used for inter-digital web pinching (IWP; after Forster et al., 1992) consisting of a pneumatically controlled plastic forceps with blunt tips (diameter 5 mm). Pressure stimuli (constant force 15 N) were applied to the inter-digital skin folds between the index, middle and ring fingers. (B) Nocifensive reflex EMG response was recorded using two shielded Ag-AgCl surface electrodes (diameter 8mm; distance 2.5 cm) in parallel to the course of the femoris muscle. The two convex Sn stimulation electrodes (diameter 0.5 cm; distance 2 cm) were positioned semi-orthogonally to the retromalleolar path of the sural nerve. The grounding electrode was placed midway between stimulation and EMG recording electrodes to avoid ground loops.

5.3.2.2. Heterotopic noxious counter-stimulation (HNCS)

IWP consisted in the application of a constant pinch pressure (force 15 N) for the duration of 2 min with two pairs of plastic forceps on the inter-digital webs between the forefinger, the middle and ring finger of the right hand. The forceps had blunt tips with a diameter of 5 mm and were pneumatically controlled by a custom-made device (after Forster et al., 1988; Fig. 1A). There was no risk of skin penetration at the stimulation force used. Forceps were disinfected with ethanol before each use to avoid any infection hazards (Putnam et al., 1992).

5.3.2.3. Subjective algesimetry

Subjective pain intensity of the phasic electric and tonic pressure stimuli was assessed on a verbally anchored numeric rating scale (NRS; 0 corresponding to *no pain* and 100 corresponding to *maximal imaginable pain*). Participants had to appraise each electric stimulus within a given pulse train, whereas the 2-min pressure stimulus was rated every 15 seconds. In addition, subjects were asked to evaluate overall pain unpleasantness as well as the amount of nervous tension sensed during the tonic pressure stimulation using a 10-cm visual analogue scale (VAS; same verbal anchors as above) and a 5-point Likert scale (1 corresponding to *minimal* and 5 to *maximal tension*), respectively.

To investigate whether DNIC might also modulate pain quality, participants filled out the sensory subscales (*temperature*, *rhythmicity* and *local penetration*) of a verbal pain descriptor scale (SES; Geissner, 1996; German version of the short form of the *McGill Pain Questionnaire*) for the phasic test stimuli.

5.3.3 Monitoring of cardiac and electrodermal activity

Heart rate (HR) was monitored through a standard pre-cordial lead II electrocardiogram (ECG100C amplifier; BIOPAC Systems Inc.; 0.5-Hz high pass and 35-Hz low pass filter) employing disposable pre-gelled Ag-AgCl electrodes placed below the right clavicle and on the left lower ribcage, respectively. To control for breathing artifacts (i.e. respiratory sinus arrhythmia), thoracic and abdominal respiration rate (RR) were recorded using strain gauge belts (Jennings et al., 1981). EDA was assessed as skin conductance (SC) with two domed Ag-AgCl electrodes (6-mm diameter; SS3LA; BIOPAC Systems Inc., USA) and processed through a constant voltage coupler (GSR100C; BIOPAC Systems Inc.; 0.5 V with 5 µS/V signal gain and 1-Hz low pass filtering).

5.3.4 Experimental protocol

The study was based on a repeated measurements design with one single session (duration approximately 60 min) consisting of two identical test blocks. Each test block (see Fig. 2) comprised the assessment of the RIII-reflex at the following time points: before (pre), during (HNCS), as well as 2, 7 and 12 min (i.e. post T1, T2 and T3, respectively) after IWP. The RIII response was elicited once per time point in a given test

block (i.e. by a series of 5 pulses as described above). Overall pain quality of the electric stimuli used to induce the RIII-reflex was assessed on the SES only for the pre and the first post HNCS time point. The two test blocks were separated by 20 min in order to avoid carry-over effects and allowing the RIII-reflex to regenerate.

Autonomic responses (HR, RR, EDA) and corrugator EMG were continuously monitored throughout the experiment. Stimulation procedures started after a short adaptation period of 5 min following electrode placement and a subsequent resting period of 2 min used for physiological stimulus-free baseline (BL) recording.

The experiments were run in a mechanically ventilated laboratory room with a constant ambient temperature of 21±0.2° C. The subjects were comfortably installed in a relaxed position onto an upholstered experimental chair (inclination of approximately 120°). All examinations were conducted by the same two (one male and one female) experimenters to control for gender-related demand characteristics and observer-/subject-expectancy effects (Robinson et al., 2001). Electrode placement was always realized by an experimenter of the opposite sex.

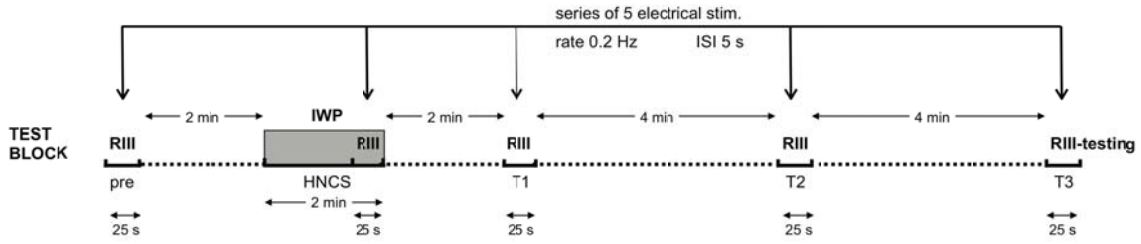

Fig. 2. Stimulation timeline. The stimulation protocol consisted of two identical test blocks, which comprised the assessment of the nocifensive flexion reflex before (pre), during (HNCS) and at three time points (T1 [2 min], T2 [7 min] and T3 [12 min]) after inter-digital web pinching (IWP). The RIII response was provoked once per time point in a given test block by a series of 5 rectangular electric shocks (0.2 Hz; inter-stimulus interval [ISI] 5 s).

5.3.5 Hypotheses

We expected a reduction of all measures (i.e. algesimetric and physiological parameters) during and after HNCS due to DNIC (confirmatory analysis). Values were supposed to return to BL within the 12-min post HNCS period. DNIC-induced hypoalgesia (in animals) has been reported to subside in less than 10 min, wherefore a time window of 12

min after IWP should have been sufficient to observe a reflex recovery from the HNCS-induced effects (Jinks et al., 2003). Sex-differences with regard to time course of pain experience and associated physiological variables were analyzed on an exploratory basis, due to the paucity of information in the current literature (see Section 1).

5.3.6 Data analysis

Pain estimates of electric and tonic pressure stimuli (IWP) were geometrically averaged for each pulse sequence or over the 2-min stimulation time, respectively. These data were then combined for each time point (i.e. pre, HNCS, post T1-3) separately by calculating the grand mean over the two test blocks. The quantitative judgments of unpleasantness and nervous tension with respect to IWP were also averaged over test blocks.

Integrated femoris and corrugator EMG-values were derived from raw EMG data with a smoothing factor of 100 and cubic-root transformed for variance stabilization (Levey, 1980). To detect stimulus-related EDA fluctuations, the standard deviation of SC amplitudes was used as indicator (Besthorn et al., 1989). Mean heart rate (HR) in beats per min (bpm) was calculated from the inter-beat RR intervals extracted from the raw ECG signals. Additionally, HR variability (HRV) was analyzed for the duration of the IWP by frequency domain analysis and the ratio of low-to-high frequency spectra power (LF/HF) computed as broad indicator of sympatho-parasympathetic balance (Berntson et al., 1997). All physiological data were relativized to the pre-stimulation BL values (i.e. percent ratios for HR and differences for EMG activity). The AcqKnowledge® 4 software package (BIOPAC Systems Inc.) was used for data acquisition and the aforementioned offline analyses.

Sex-specific changes in the time courses of physiological and psychophysical data were examined by one-tailed *t*-tests for paired or independent samples as appropriate and simple contrast analysis with the pre HNCS time points as reference based on a mixed-design analysis of variance (ANOVA) with time as repeated factor (with five levels corresponding to pre, HNCS and post T1-3 time points) and sex as independent grouping factor. Alpha level was set at .05. Variance homogeneity was verified by Levene's test. Huyn-Feldt corrections were made in case the sphericity assumption as implied by

Mauchly's test was violated. Pearson product-moment correlations coefficients (r) were calculated to estimate reliability between measures.

Statistical analyses and post hoc effect size computations were conducted using SPSS Statistics 17 (SPSS Inc., Chicago, IL, USA) and G*Power (Faul et al., 2007). Graphs were created with SigmaPlot® 11 (Systat Software Inc., Chicago, IL, USA). All data are represented as arithmetic mean (*AM*) plus standard error of the mean (*SEM*).

5.4 Results

There were no substantial differences between pain estimates for electric and pressure stimulation and sufficiently high retest reliabilities ($r \geq .60$, $p > .05$) between the two test blocks, which allowed data aggregation as described in Section 2.5.

5.4.1 RIII-reflex

Individual adjustments of electric stimulus intensity (to 1.2 × pain detection threshold) resulted in a mean stimulation magnitude of 3.4±0.3 mA and a corresponding moderate subjective pain intensity of 5.3±0.3 NRS units. Adjusted stimulation intensities, ergo pain thresholds, were practically identical for men (3.4 mA) and women (3.3 mA).

The electric pulse trains elicited a perceptible and stable LLFR in the range of 1.7-2.7 mV (values normalized to BL). The expression of the reflex response was stronger for male than for female participants, although a significant difference could not be confirmed ($p = .09$; see Fig. 3B). RIII-reflex intensity was positively correlated with adjusted stimulus intensity ($r = .36$, $p < .05$; pre HNCS), albeit for men only.

Pain thresholds were correlated with systolic BP values (assessed at the beginning of the experiments) in women ($r = .32$, $p < .05$) and diastolic BP in men ($r = .32$, $p < .05$). As expected, men had higher systolic BP values than women ($t_{13} = –2.18$, $p = .05$, Cohen's $d = .7$).

Compared to BL, the noxious electric stimulation was accompanied by rises in HR (from 69±5 to 75±5 bpm, $t_{27} = –8.97$, $p < .0001$, $d = 4.2$), more pronounced contractions of the corrugator muscle (EMG signal shifts from 1.7±0.2 to 2.9±0.5 µV, $t_{27} = –3.18$, $p = .002$, $d = 2.8$) and stronger EDA fluctuations (varying from 0.02±0.01 to 0.06±0.01 mS; $t_{27} = –6.11$, $p < .0001$, $d = 6.3$). As for the RIII-reflex, men also demonstrated a more prominent but not significantly different corrugator response (see Fig. 3D).

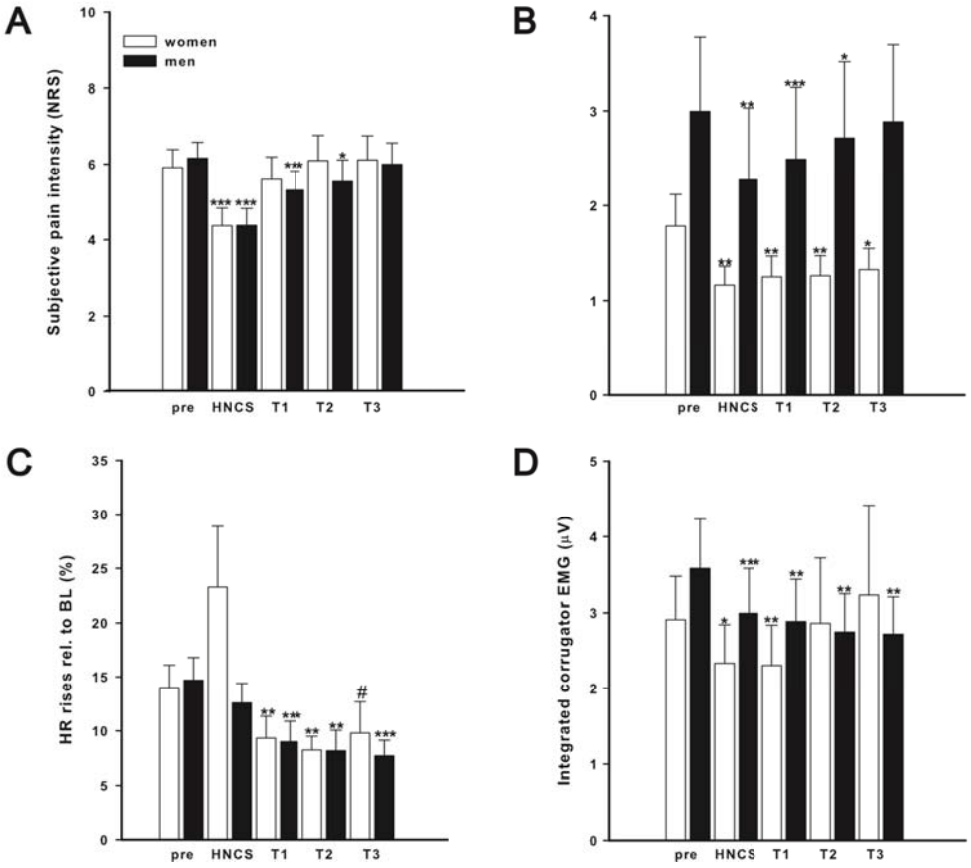

Fig. 3. Sex-dependent time course of subjective and objective algesimetric indicators of HNCS-induced hypoalgesia. (A) Subjective pain intensity of electric shocks evaluated on numeric rating scale (NRS). (B) Integrated EMG activity of the femoris muscle caused by electric stimulation and normalized to stimulation-free baseline (BL). (C) Percent increases in HR accompanying electric stimulation (relative to stimulation-free BL). (D) Integrated EMG activity of the corrugator muscle related to electric stimulation (normalized to stimulation-free BL). T1-3 correspond to 2, 7 and 12 min post HNCS, respectively. All data are represented as *AM±SEM*. # $p \leq .07$, * $p < .05$, ** $p < .01$, *** $p < .001$.

5.4.2 HNCS-induced RIII-reflex suppression

Tonic IWP caused an intense (70±0.4 and 71±0.4% NRS) and highly unpleasant (73±3 and 72±3% VAS) pain experience associated with moderate nervous tension (2.1±0.4 and 2.7±0.3 on the Likert-scale) for both sexes (data reported for men and women, respectively).

This type of HNCS induced an extensive reduction of the RIII-reflex response in the EMG signal (Δ = approximately –75%; $F_{4,52} = 15.78$, $p < .03$, Cohen's $f = 0.8$, $F_{2,26} = 28.11$, $p < .001$, $f = 0.7$) and of the related pain ratings (Δ = approximately –30%; $F_{4,52} = 9.02$, $p = .001$, $f = 1.8$, $F_{4,52} = 7.86$, $p = .003$, $f = 1.1$) for both men and women (see Fig. 3A and B). Higher pain thresholds were predictive of a smaller reflex inhibition ($r = –.31$

and –.30 for men and women, respectively; $p < .05$). The RIII-reflex suppression clearly outlasted HNCS and the EMG signal regained its initial strength not less than 12 min afterwards. This return to BL appeared to be more rapid for the pain ratings than for the RIII-reflex. We identified a sex-specific time course for both measures in the sense that pain ratings for women showed a more rapid return compared to men (see Fig. 3A), whereas an inversed trend was seen with respect to the LLFR (see Fig. 3B).

Femoris EMG signal strength and subjective pain intensity were positively correlated at all time points for both sexes ($r > .89$; $p < .05$). This was also the case for corrugator activity, albeit solely for women ($r = .34$; $p > .05$). The amount of reflex suppression was not related to systolic or diastolic BP neither for men nor women ($–.02 < r < .1$; $p > .20$).

Interestingly, HNCS also proved to modulate qualitative aspects of the pain experience in the sense that the electric shocks were discerned to be less puncturing and pulsating with reduced scores on the SES subscales *local penetration* ($t_{13} = 2.57$, $p = .01$, $d = 4.1$) and *rhythmicity* ($t_{13} = 2.88$, $p = .006$, $d = 1$) at least for men. A marginally significant change with respect to the puncturing quality of the pain stimuli was also seen in female participants ($t_{13} = 1.55$, $p = .07$, $d = 1.4$). Contrariwise, the heat sensation provoked by the painful shocks appeared to be attenuated for the female subgroup only, although this again failed to reach significance (*temperature* subscale; $t_{13} = 1.58$, $p = .07$, $d = 4.2$).

As regards peripheral autonomic functioning, we observed an intense HR increase in women (by 39% compared to men; $t_{26} = 1.81$, $p = .04$, $d = .68$), during and limited to HNCS. Post HNCS values on the other hand were all reduced compared to the pre stimulation time point ($F_{4,52} = 5.29$, $p = .025$, $f = 0.8$). More to the point, male participants even displayed a slightly decelerated HR trend over the complete course of the experiment ($F_{4,52} = 9.02$, $p < .001$, $f = 0.6$; see Fig. 3C). Frowning muscle activity was also lowered after IWP. Muscular activity stayed reduced for all post HNCS time points in males ($F_{4,52} = 10.18$, $p = .001$, $f = 0.9$) while returning to initial values in women ($F_{2,26} = 6.74$, $p = .01$, $f = 0.7$; see Fig. 3D). No differences could be identified with respect to EDA.

5.5 Discussion

With the experimental HNCS model at hand, we achieved a prominent reduction of subjective pain, nocifensive flexion reflex and corrugator activity (Streff et al., in press). As hypothesized, the suppression outlasted HNCS, which clearly indicates that the observed changes are the expression of pain inhibition processes disparate from distraction (Moont et al., 2010). RIII-reflex suppression was not related to constitutional systolic or diastolic BP, confirming previous findings and further validating the IWP model as an experimental model for DNIC (Streff et al., in press). On top of that, the study yielded interesting results as far as temporal characteristics of anti-nociceptive counter-irritation are concerned. Whereas men revealed a concordant time pattern with regard to subjective pain intensity and RIII-reflex activity, both measures diverged over the post HNCS period in women, leading to a fractionated response in terms of objective and subjective pain indicators.

5.5.1 RIII-reflex

Electric stimulation at supraliminal strength produced a strong RIII response, which was somewhat lower in women reflecting the fact that higher electric stimulation intensities are generally required to attain the withdrawal reflex threshold in men (Skljarevski and Ramadan, 2002).

Basal pain sensitivity was negatively correlated with the extent of reflex inhibition, as opposed to findings by Granot et al. (2008) who failed to find a relationship between counter-stimulation intensity and the amount of hypoalgesia produced by painful water immersion. This supports the assumption that the hypoalgesia observed in our study was genuinely DNIC-induced ergo reflecting a contrast inhibition linearly related to the amount of noxious input, contrary to a more general thermoregulatory and BRS-related response where pain inhibition is merely an epiphenomenon (see section 4.2.2).

Pain thresholds for electric shocks were correlated with systolic BP values in women and diastolic BP in men, which is in line with studies reporting that pain sensitivity is differentially related to constitutional BP levels in males and females (al'Absi et al., 1999, 2002; Stewart and France, 1996). On the other hand, the strength of the RIII EMG signal and the amount of its attenuation by HNCS were unrelated to BP parameters for

both sexes. Whereas a lack of relationship between the RIII-reflex and BP under resting conditions has been documented, negative relationships between hypertension and LLFR have also been described (Edwards et al., 2007; Page and France, 1997). Thus, the test stimulus used to assess HNCS efficacy seemed not to be confounded by sex-related BP differences. However, it should be noted that the range of basal inter-individual BP differences at rest might have been too narrow to detect interdependences between BP and pain sensitivity in a healthy normotensive sample.

5.5.2 HNCS-induced RIII-reflex suppression

5.5.2.1. Time characteristics

Objective and subjective pain indicators, with the exception of the female RIII and the male corrugator response, tended to recover completely within 12 min, as hypoalgesia did not outlast the complete post HNCS observation period. The observed response recovery speaks in favor of a genuine anti-nociceptive effect not attributable to habituation or adaptation. The persisting reflex suppression in female subjects, on the contrary, could be related to a more pronounced adaptation or habituation rate to sustained and repeated pain stimulation (Hashmi and Davis, 2009).

Our data sharply contrast with findings regarding the ischemic pain model, where increased pain detection thresholds and blink RII reflex latencies are maintained at high values for at least 60 or 15 min, respectively (Pantaleo et al., 1988). Therefore, the more rapid return to BL in our model points to pain modulation processes that are different from those where a strong cardiovascular challenge is present.

5.5.2.2. Sex-specificity

Sex-related dichotomies in endogenous pain modulation have been shown to be time-dependent. Using hypertonic intramuscular saline-injection as HNCS, Ge et al. (2004) observed a significant increase of pain thresholds in referred pain areas over 15 min in men, while sensitivity returned to baseline values in females within this same time window. This is in line with our pain rating data where the pain suppression was more persistent in men than in women over a similar time period.

Extending these findings to objective pain indicators, we were able to identify a dissociation between nociceptive low-level (i.e. RIII-reflex) and higher-order (i.e. pain threshold and ratings) processing. This observation supports the idea that sex-dependent neurophysiologic mechanisms might differentially have affected spinal and cerebral nociceptive processes. This is further substantiated by the fact that the sensory characteristics of phasic pain stimuli were modulated differently in men and women. The latter finding could indicate a differential regulation at the hypothalamic level, which is the main regulatory structure in autonomic functioning (including thermoregulation) and has been shown to be capable of selectively inhibiting spinal C-fiber input via descending control of spinal processing (Robinson et al., 2001). The reduced corrugator activity in men could be attributed to an adaptation to the electric stimulation, whereas in women corrugator activity correlated positively with pain intensity and had a parallel time course. Corroborated by the finding of a higher IWP-related HR response in females, the differential response could be the expression of a stronger defensive reaction (environmental blocking versus intake) to stressors (Obrist, 1981). In agreement with this hypothesis, a relatively more pronounced stress-induced analgesia (SIA; e.g. induced by social or mental challenge) has been reported in women (al'Absi and Petersen, 2003; Girdler et al., 2005). In this sense, they also have been shown to display higher heart rates and to be more sensitive to anxiety than men who tend to express higher systolic BP during challenge (McLean and Anderson, 2009). These differences might however only become visible under conditions of mild experimental stress like public speaking, arithmetic tasks or painful stimulation. Cardiovascular challenging stressors such as CPT and submaximal effort tourniquet test (SETT) on the other hand might induce high autonomic arousal in men too, thereby overriding EA and neutralizing or even reversing sex-related differences. For instance, it has been shown that women generally display a smaller baroreflex response in models using CPT (Hogarth et al., 2007). Further support for this assumption comes from studies showing that psychological arousal might produce a dissociation between pain and the nociceptive blink reflex and that CPT and mental challenges differentially modulate perceptive and physiological correlates of phasic noxious stimuli (Koh and Drummond, 2006; Plaghki et al., 1994). More specifically, although both CPT and mental task were capable of increasing pain

thresholds and reducing pain sensation, power density of cerebral evoked potentials was only enhanced under the CPT condition.

The described sex-related findings might also depend on several psychological factors. It is well known that catastrophizing, which has been claimed to be more common in women, is a potent modulator of pain perception (Dixon et al., 2004). Experimental studies have described positive correlations with pain ratings, without any concomitant alterations in RIII-reflex threshold (France et al., 2002). Differences between men and women could however not be inferred from this study. Alternately, the flattening of the RIII response in women may be attributed to passive coping as a potential consequence of catastrophizing (Goodin et al., 2009). Rather than fight or flight reactions, the resulting resignation has been shown to trigger opioid-mediated analgesia (Frew and Drummond, 2007).

Fright reactions might constitute an additional factor as indicated by the observed HR increase. Whereas anxiety related to the anticipation of a painful stimulus leads to HR decelerations, fear induced by ongoing pain presentation is accompanied by HR accelerations, again only in women (Bradley et al., 2008; Rhudy and Meagher, 2000).

This in mind, interactions between the hypothalamus pituitary and gonadal axes might also represent important factors in the regulation of EA that warrant more detailed investigation with regard to DNIC-induced hypoalgesia and SIA (Aloisi and Bonifazi, 2006; Craft, 2007; Craft et al., 2004). Although, no effect of menstrual cycle has been identified for pain experience related to experimental pain types like ischemic and CPT, cyclic hormonal effects have been identified for descending inhibitory control mechanisms on nociceptive RIII-reflex and CPT-induced hypoalgesia (Klatzkin et al., 2010; Tassorelli et al., 2002; Tousignant-Laflamme and Marchand, 2009). Between-subject variance estimates were more or less similar for both sexes and even lower in women as far as the RIII responses were concerned, which could indicate that menstrual cycle differences were less important in our study (a normal menstrual cycle distribution assumed).

5.5.3 Conclusion

We confirmed data from a previous study showing that IWP might serve as an experimental HNCS to elicit DNIC-related hypoalgesia, at least in males. Women seem to present a propensity towards a stronger defensive reaction blocking spinal noxious input and thus moderating DNIC-effects. The findings underline the necessity to control for time effects when investigating sex or gender differences in EA, since dissimilarities in recovery rate and time course might be responsible for the observed dichotomies. Sex-related specificity in neurophysiologic (supra-/spinal) functioning and stress regulation may be presumed to underlie the differences under debate and deserve further attention.

5.6 Acknowledgements

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5.7 References

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6 Discussion and outlook

As the three studies presented in the framework of this thesis are based on each other in the sense that the second is a logical follow-up study of the first as is the third of the second, it would only be of gratuitous redundancy to discuss the same facts again in detail, this even more so since the conclusion of the third paper is quite exhaustive. I would nevertheless like to briefly reconsider the most important findings and take-home messages.

The first study analyzed the cardiovascular reactivity of two painful water immersion tests and the HIT was found to be less sympathetically confounded than the CPT (Appenzeller, 2000). Cardiovascular changes may more significantly contribute to multi-segmental hypoalgesia i.e. the DNIC effect under CPT than HIT conditions. While Granot and colleagues (2008) claimed that both tests are quite equivalent from that point of view, testing the respective pain inhibition capacities was not a main concern in this study. The HIT was even tolerated for a shorter period, despite a weaker cardiovascular challenge, i.e. weaker baroreflex reactivity and is therefore a better suited experimental pain model without producing too pronounced levels of autonomic arousal. Further research seemed however to be needed because even if the cardiovascular challenge during the HIT was less strong than during the CPT, BP increases were still identified and a remainder of hypoalgesic effects caused by the activity of baroreflexes could thus not be excluded.

IWP is a pain model commonly used in some laboratories (cf. Forster et al. 1992). Because it does not imply thermoregulatory pathways and is generally less likely to induce interfering autonomic reactions, it could produce a more selective, and hence better retraceable, form of descending pain control. That is mainly why we decided to use this pain model in our second study to trigger DNIC-like effects. An observed inhibition of the presented during and post WU-pain would be very likely to be caused by the tonic pain stimulus per se (cf. see Campbell and Stanley, 1963, for validity of causal inferences). Regarding internal validity and practical usefulness, another concern was that different pain modalities and consequently thermal and mechanical pain models were tested. Does the same modality used for tonic and phasic pain stimuli improve or impair

endogenous pain inhibition? We found a comparable pain experience for both paradigms: time pattern of subjective pain intensity ratings and inherent sensations (unpleasantness versus nervous tension) were very alike. Regarding objective pain parameters, HIT and IWP have produced similar autonomous corrugator EMG responses. Negligible changes in cardiovascular activity (BP, HR and HRV) have been observed for the pinching pain, whereas significant increases in BP and HR became visible during the HIT, indicative of a stronger sympathetic regulatory activity. Both tests were nevertheless able to substantially suppress thermal and mechanical WU, as well in an intra- as in an inter-modality manner, but this suppressive effect has generally been stronger for the HIT. The IWP being less influenced by BRS-related hypoalgesia, the efficacious working mechanism seems to be composed by BRS- and DNIC-mediated hypoalgesia. Already in this study, different time effects, depending on the nature of the WU-inducing stimulus, have been noted. Whereas the thermal WUs returned to BL values within a time period of 10 minutes, mechanical WUs stayed reduced for a prolonged period of time. DNIC inhibitions normally decay within a 10-minutes-timeframe (Le Bars et al., 1992). In this context, differential modulation of C- versus A-nociceptive fibers, extraneous to DNIC, may play a key role, because of our observation of a better recovery for thermal compared to mechanical pain after diffuse noxious inhibition. This differential modulation may be postulated because both A- and C-fibers can be excited with qualitatively similar discharge properties by repetitive impact (mechanical) and heat stimuli as we used them (Koltzenburg and Handwerker, 1994). In conclusion, IWP seems to yield a more genuine DNIC-like effect because it is not accompanied by significant cardiovascular changes and still strong enough to produce inhibition. Accordingly IWP constitutes a useful and certain experimental tool to elicit distinct endogenous hypoalgesia.

In our third study this assertion has been further corroborated because IWP has been able to reduce subjective ratings of electrical pain stimuli inducing RIII-reflex and the two objective EMG measures. Pure distraction can be excluded as a confounding factor because the pain inhibition was outlasting the HNCS duration and there was no relation between the HNCS and BP (Talbot et al., 1987). The temporal aspects of counter-irritation results reveal sex-related differences: men display a concordant time pattern as

far as pain experience and RIII-reflex muscle activity are concerned while both measures are fractionated in women. A positive correlation between systolic BP and pain thresholds in response to phasic electric shocks could only be observed in women, corroborating the differential relation between pain sensitivity and constitutional BP levels in men and women (al'Absi et al, 1999; al'Absi et al, 2000; Staud et al., 2003). Sex-related neurophysiological mechanisms might be able to differentially affect spino-cerebral nociceptive processing, because we found a dissociation between low-level and higher-order structures. The reduction of the spinal nociceptive reflexes is generally subject to low-level mechanisms, whereas for diffuse noxious inhibition there is an additional involvement of supraspinal and cortical analgesic mechanisms. Women showed a stronger defensive reaction (HR rise during IWP and electrical pain) and it has been shown that they display a more pronounced SIA (Girdler et al., 2005). A genuine DNIC-induced hypoalgesia seems to have been produced because no relationship between the amount of noxious input and the reflex inhibition has been found, an evidence for the fact that the inhibition was not only an epiphenomenon of the stimulus intensity. The return to BL within 12 minutes of the majority of pain indicators (except female RIII-reflex and male corrugator response) underlines a pure anti-nociceptive effect not attributable to adaptation or habituation (cf. distinction between our model and ischemic pain). These variables could however play a role in the persisting reflex suppression (a more sustained or repeated pain stimulation) in female subjects (Hashmi and Davis, 2009). The suppression of pain ratings is more persistent in men than in women (sex-related dichotomies have been reported in literature on pain modulation and time effects; cf. Popescu 2010, for review). The flattened RIII response in women could however have been related to passive coping as a consequence of catastrophizing. The IWP-induction added to the ongoing electrical stimulation could have caused fear reactions in female subjects. Additionally, endocrine factors may have played a role, i.e. interactions of the hypothalamus-pituitary and gonadal axes. In conclusion, the IWP constitutes an adequate experimental HNCS model, at least for males. Women may show a blocking of spinal noxious input due to a stronger defensive reaction.

In summary, a number of issues should be considered at this point:

- When using ischemic pain, CPT and HIT as DNIC-triggers, there may be a complex entanglement of baroreflex, opioid and descending pain control mechanisms (France et al., 1999).
- DNIC are under the influence of upstream structures of cortical nature (higher-order) but lower-level structures also have an impact.
- Pain suppression has been observed in all three studies (different counter-stimulation models), but it should be kept in mind that DNIC is a distinct paradigm bearing on the inhibition of phasic pain, lasting about 10 minutes, and that should not be confounded with other modulatory mechanisms (Le Bars et al., 1992).
- Sex-related differences have only been found in the third study, where the observed hypoalgesia was not related to baroreflex activity.
- Differences in the inhibition of sensory pain characteristics have also played a role in the third study: whereas the "heat" scale values (SES) were mainly reduced in women, the more rhythmic and penetrating qualities of the electrical pain stimuli seemed to be dampened in men.
- It is important to consider potential sex-related differences when studying DNIC-like inhibition (and other pain modulation pathways). Factors like differential time courses between both sexes, possibly different anatomies of pain processing systems, lower baroreflex responses in women (Hogarth et al, 2007) but a higher defensive reaction and a more pronounced SIA (Girdler et al., 2005), more catastrophizing and different hormonal reactions may play a role

A thorough analysis of endogenous pain modulation mechanisms accounting for sex-dependent effects is necessary to fully understand DNIC and pain pathology. Clinical data have accumulated, indicating that DNIC seem deficient in certain pain disorders. Dysfunctional DNIC might thus constitute a risk factor for the development of chronic pain states (cf. more important prevalence in women;'al Absi et al., 2002). Proper and adequate paradigms have to be used to identify differential nociceptive mechanisms and pain experience in men and women. These differences are well known in the clinical

setting and our aim should be to elucidate them and provide the basis for the development of new differential diagnostic and treatment approaches (Edwards et al., 2003).
In future research, a special emphasis has to be devoted to "multichannel input" studies in order to be able to answer important questions about pain processing in men and women and about differential vulnerabilities for the development of chronic pain diseases. Psychophysical and psychobiological methods have to be combined, including imaging studies performed at different time points: before, during and after exposure to experimental tonic pain stimulation. In all these studies, self-reports of pain experience should be collected at the mentioned time points.

"A combination of anatomical, neurological and neurophysiological approaches to understanding the brain mechanisms underlying sensory and affective dimensions of pain are necessary to define adequate pain models." (Price, 2002)

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8 Annexes

Annex A

Study 1: Differential physiological effects during painful tonic hand immersion tests using hot an ice water.

Probandeninformation

Sehr geehrte Probandin, sehr geehrter Proband,

Wir danken Ihnen für Ihr Interesse zur Teilnahme an einer Studie, die von der Abteilung Psychobiologie der Universität Luxemburg durchgeführt wird. In dem vorliegenden Informationsblatt werden Ihnen Inhalt und Zweck der geplanten Studie erläutert. Bitte lesen Sie diese Probandeninformation aufmerksam durch. Sollten Sie Teile dieser Aufklärung nicht genau verstehen oder darüber hinaus noch Fragen haben, sprechen Sie den/die Versuchsleiter/in bitte unmittelbar darauf an.

Ziel der Studie

Die Studie dient dazu, die Methode des Cold Pressor Testes (Eintauchen der Hand in eiskaltes Wasser) mit der Methode des Hot Water Immersion Test (Eintauchen der Hand in sehr warmes Wasser) zu vergleichen. Hierdurch soll eine besseres Verständnis der Mechanismen der chronischen Schmerzentstehung erreicht werden. Mit der Teilnahme an diesem Forschungsvorhaben ist kein individueller Gesundheitsnutzen verbunden.

Vergütung

Nach Ende der Studie, d.h. nach komplett abgeschlossener Datenerhebung, wird Ihnen ein fester Betrag von 20.- EUR ausbezahlt. Diese Vergütung stellt eine Entschädigung für Ihre Mühen und aufgewendete Zeit dar.

Dauer und Ort der Studie

Die Untersuchung besteht aus einer einmaligen Sitzung mit einer Dauer von 1 Stunde und findet an der Universität Luxemburg, Campus Limpertsberg statt.

Beschreibung der Untersuchungsverfahren

Die Untersuchungsprozedur besteht aus der Messung physiologischer Funktionen während thermischer Stimulation (Hitze- u. Kältereizung) am Unterarm bzw. der Hand sowie der Stärke und Qualität der hierdurch ausgelösten Empfindungen.
Zur Messung der Empfindlichkeit der Hautsinne kommen folgende physikalische Reize zum Einsatz:

[ ] Kälte- und Hitzereize: Reizung mittels Kontakthermode an der Innenseite des linken Unterarms (Messung von Kälte- und Hitzeempfindungen, Hitzeschmerzschwellenmessung)
[ ] Kältereize: Eintauchen der Hand in Eiswasser (ca. 5° C; max. 5 min)
[ ] Hitzereize: Eintauchen der Hand in warmes Wasser (zirka 46°C; max. 5 min)

Die eingesetzten physikalischen Reize sind nicht-invasiv und risikoarm. Als Nebenwirkung der Reize kann eine vorübergehende Rötung der Haut auftreten, die ein paar Stunden nach Abschluss der Studie wieder vollständig abgeklungen ist. Außerdem kann die Schmerzempfindlichkeit am Applikationsort und in dessen Umgebung vorübergehend gesteigert oder vermindert sein. Alle während der

Untersuchung eingesetzten Reize werden Ihnen vor Beginn des Experiments exemplarisch demonstriert.
Im Rahmen der Studie werden zudem folgende physiologische Funktionen anhand von Oberflächensensoren (d.h. nicht-invasiv) erfasst:

[ ] Herzkreislaufaktivität: Blutdruck (permanente Messung), EKG und Pulsfrequenz
[ ] Atmungsaktivität: Brust- und Bauchatmung anhand von Atemgurten
[ ] elektrodermale Aktivität anhand von Sensoren an den Fingern

Die oben beschriebenen Untersuchungsmethoden sind allgemein üblich im Rahmen der klinisch-physiologischen Diagnostik. Die Untersuchung ist und ersetzt keine ärztliche Untersuchung und liefert auch keine Informationen zum Gesundheitsstatus.

Hinweis zum Versicherungsschutz

Sollten sich aus Ihrer Teilnahme an dieser Studie nachteilige gesundheitliche Folgen ergeben, so besteht für schuldhaft durch den/die Versuchsleiter/in verursachte Gesundheitsschäden eine Haftpflichtversicherung. Für eventuelle Gesundheitsschäden oder sonstige Beeinträchtigungen (inklusive Wegunfälle) im Zusammenhang mit der Teilnahme an der Studie, die nicht auf Fehlverhalten oder Fahrlässigkeiten des/der Versuchsleiters/in zurückzuführen sind, gilt kein Versicherungsschutz.

Einwilligungserklärung

Hiermit bestätige ich, dass ich die obigen Ausführungen (Probandeninformation) aufmerksam gelesen und deren Inhalt verstanden habe. Ich habe das Ziel, den Ablauf und die Durchführung der Studie verstanden und hatte die Gelegenheit, alle mich interessierenden zusätzlichen Fragen zu stellen. Es stand mir ausreichend Bedenkzeit zur Verfügung und mir ist bewusst, dass jederzeit neu auftauchende Fragen besprochen werden können.
Mir ist bekannt, dass ich jederzeit ohne Angabe von Gründen und ohne Inkaufnahme von Nachteilen von den Untersuchungen zurücktreten kann. Im Falle eines frühzeitigen, von mir bedingten Abbruchs erkenne ich an, dass sich damit mein Anspruch auf Erstattung eines Probandenhonorars verliert.

Ich erkläre mich hiermit freiwillig bereit und damit einverstanden, an der Studie teilzunehmen.

Luxemburg, _____ _____ _____
(Datum) (Name, Vorname) (Unterschrift)

Erklärung zum Umgang mit erhobenen Daten

Die im Rahmen der Studie erhobenen Daten (einschließlich Gesundheits- und psychodiagnostischer Daten) werden ausschließlich zu Forschungszwecken weiterverwendet und Dritten nicht zugänglich gemacht. Die wissenschaftliche Verwertung (Dokumentation, Speicherung und Auswertung) und ggf. eine Veröffentlichung der Daten erfolgt ausschließlich in pseudonymisierter Form, d.h. ohne Erfassung von Name, Anschrift oder ähnlichen Angaben.

Ich habe den Inhalt der vorliegenden datenschutzrechtlichen Erklärung verstanden und bin mit der Verwendung meiner Daten in vorstehend geschilderter Weise einverstanden.

Luxemburg, _____ _____ _____
(Datum) (Name, Vorname) (Unterschrift)

Kurzanamnesebogen

Name: _____ Geburtsdatum: ___/___/_____ Code: _____

Geschlecht: m ( ) w ( ) Körpergröße: _____ cm Gewicht: _____ kg

(Zutreffendes bitte ankreuzen; alle Angaben werden streng vertraulich behandelt)	ja	nein
1) Fühlen Sie sich zurzeit vollkommen gesund?	[ ]	[ ]
2) Befinden Sie sich zurzeit in ärztlicher Behandlung?	[ ]	[ ]
_____		
3) Befanden Sie sich jemals oder befinden Sie sich zurzeit in psychotherapeutischer oder psychiatrischer Behandlung?	[ ]	[ ]
Wenn ja, wegen welcher Erkrankung wurden/werden Sie behandelt?		
_____		
4) Haben Sie jemals gelitten oder leiden Sie an:		
a) Bronchialasthma	[ ]	[ ]
b) zu hohem/niedrigem Blutdruck oder anderen Herzkreislaufbeschwerden	[ ]	[ ]
c) Lungen-, Leber- oder Nierenkrankheiten	[ ]	[ ]
d) Magendarmgeschwüren oder -blutungen	[ ]	[ ]
e) Diabetes oder Entzündungen der Bauchspeicheldrüse (Pankreatitis)	[ ]	[ ]
f) Erkrankungen der Schilddrüse	[ ]	[ ]
g) Störungen der Nebennierenfunktion (Bsp. Cushing-, Addison-Syndrom)	[ ]	[ ]
h) Knochen- (Osteoporose) oder Muskelschwund (Muskelatrophie)	[ ]	[ ]
i) rheumatischen Erkrankungen	[ ]	[ ]
j) chronischen bzw. wiederkehrenden Schmerzen	[ ]	[ ]
k) Histaminüberempfindlichkeit	[ ]	[ ]
l) Eisenmangel	[ ]	[ ]
m) Nessel-/Quaddelsucht (Urtikaria), Hautschwellungen oder Ekzemen	[ ]	[ ]
n) Bluterkrankheit (Hämophilie) oder Störungen der Blutbildung (u.a. Anämie)	[ ]	[ ]
o) Allergien oder Arzneimittelunverträglichkeiten	[ ]	[ ]
Wenn ja, an welchen (Bsp. Heuschnupfen) litten/leiden Sie?		
_____		
5) Nehmen Sie regelmäßig Drogen oder Medikamente? Wenn ja, welche?	[ ]	[ ]
_____		
6) Haben Sie in den letzten Stunden oder Tagen Drogen oder Medikamente eingenommen? Wenn ja, welche?	[ ]	[ ]
_____		

Ich versichere hiermit, alle Fragen verstanden und alle Angaben nach bestem Wissen gemacht zu haben. Ich hatte genügend Gelegenheit Fragen zu stellen. Unbekannte medizinische Begriffe wurden mir klarverständlich erläutert. Zudem bestätige ich, dass meine Angaben zur Einnahme von Medikamenten und Genussmitteln/Drogen vollständig und wahrheitsgemäß sind.

Luxemburg, _____ _____ _____
(Datum) (Name, Vorname) (Unterschrift)

Annex B

Study 2: Internal validity of inter-digital web pinching as model for perceptual diffuse noxious inhibitory controls-induced hypoalgesia in healthy humans

Probandeninformation

Sehr geehrte Probandin, sehr geehrter Proband,

Wir danken Ihnen für Ihr Interesse an dieser Studie, die von der Abteilung Psychobiologie der Universität Luxemburg durchgeführt wird, teilzunehmen. In dem vorliegenden Informationsblatt werden Ihnen Inhalt und Zweck der geplanten Studie erläutert. Bitte lesen Sie dieses aufmerksam durch. Sollten Sie Teile dieser Aufklärung nicht genau verstehen oder darüber hinaus noch Fragen haben, sprechen Sie den/die Versuchsleiter/in bitte unmittelbar darauf an.

Ziel der Studie

Diese Studie dient dazu, ein besseres Verständnis der Mechanismen von zeitlicher Veränderung der Schmerzempfindlichkeit zu erlangen, und beschäftigt sich mit einer speziellen Form der Schmerzinhibition (DNIC) in unterschiedlichen Schmerzmodalitäten (thermisch und mechanisch). Dazu sollen verschiedene physiologische Daten erhoben werden, während sich in Zeit, Wiederholung und Qualität unterscheidenden Schmerzreize an beiden Händen dargeboten werden. Mit der Teilnahme an diesem Forschungsvorhaben ist kein individueller Gesundheitsnutzen verbunden.

Vergütung

Nach der Studie, d.h. nach komplett abgeschlossener Datenerhebung, wird Ihnen ein fester Betrag von 20.- EUR ausgezahlt. Diese Vergütung stellt eine Entschädigung für Ihre Mühen und aufgewendete Zeit dar.

Dauer und Ort der Studie

Die Untersuchung besteht aus einer einmaligen Sitzung mit einer Dauer von zirka 1½ Stunden und findet an der Universität Luxemburg, Campus Limpertsberg, in der Abteilung für Psychophysiologie statt.

Beschreibung der Untersuchungsverfahren

Die Untersuchungsprozedur besteht aus der Messung physiologischer Funktionen während thermischer Stimulation (Hitze) am Mittelfinger der linken Hand, oder Eintauchen der rechten Hand während 2 Minuten in warmes Wasser, sowie mechanischer Stimulation an der linken Hand, anhand eines Impact Stimulators oder durch Interdigitalquetschen.

Zur Messung der Empfindlichkeit der Hautsinne kommen folgende physikalische Reize zum Einsatz:

- Darbietung von Hitzereizen über eine Kontakthermode am Mittelfinger der linken Hand (Hitzeschmerz-schwellenmessung und perzeptueller Wind-up)
- Darbietung von mechanischen Reizen durch den Impact Stimulator (Schwellenmessung und perzeptueller Wind-up) an den Fingern der linken Hand
- Eintauchen der rechten Hand in heißes (zirka 46/47°C) Wasser oder Interdigitalquetschen an der rechten Hand, jeweils während 2 Minuten

Die eingesetzten physikalischen Reize sind nicht-invasiv und risikoarm. Als Nebenwirkung der Reize kann eine vorübergehende Rötung der Haut auftreten, die spätestens ein paar Stunden nach Abschluss der Untersuchung wieder vollständig abklingt. Außerdem kann die Schmerzempfindlichkeit am Applikationsort und in dessen Umgebung vorübergehend gesteigert oder vermindert sein. Alle während der Untersuchung eingesetzten Reize werden Ihnen vor Beginn des Experiments exemplarisch demonstriert.

Im Rahmen der Studie werden zudem folgende physiologische Funktionen anhand von Oberflächensensoren (d.h. nicht-invasiv) permanent erfasst:

- Herzkreislaufaktivität: kontinuierlicher Blutdruck und EKG
- Atemaktivität
- Elektromyographische Aktivität (Muskelaktivität des Stirnmuskels „corrugator supercilii“)
- Hauttemperatur (der Hand)

Die oben beschriebenen Untersuchungsmethoden sind im Rahmen der klinisch-physiologischen Diagnostik allgemein üblich. Die Untersuchung ersetzt keine ärztliche Untersuchung und liefert auch keine Informationen zum Gesundheitsstatus.

Hinweis zum Versicherungsschutz

Sollten sich aus Ihrer Teilnahme an dieser Studie nachteilige gesundheitliche Folgen ergeben, so besteht für schuldhaft durch den/die Versuchsleiter/in verursachte Gesundheitsschäden eine Haftpflichtversicherung. Für eventuelle Gesundheitsschäden oder sonstige Beeinträchtigungen (inklusive Wegunfälle) im Zusammenhang mit der Teilnahme an der Studie, die nicht auf Fehlverhalten oder Fahrlässigkeiten des/der Versuchsleiters/in zurückzuführen sind, gilt kein Versicherungsschutz.

Einwilligungserklärung

Hiermit bestätige ich, dass ich die obigen Ausführungen (Probandeninformation) aufmerksam gelesen und deren Inhalt verstanden habe. Ich habe das Ziel, den Ablauf und die Durchführung der Studie verstanden und hatte die Gelegenheit, alle mich interessierenden zusätzlichen Fragen zu stellen. Es stand mir ausreichend Bedenkzeit zur Verfügung und mir ist bewusst, dass jederzeit neu auftauchende Fragen besprochen werden können. Mir ist bekannt, dass ich jederzeit ohne Angabe von Gründen und ohne Inkaufnahme von Nachteilen von den Untersuchungen zurücktreten kann. Im Falle eines frühzeitigen, von mir bedingten Abbruchs erkenne ich an, dass sich damit mein Anspruch auf Erstattung eines Probandenhonorars verliert.

Ich erkläre mich hiermit freiwillig bereit und damit einverstanden, an der Studie teilzunehmen.

Luxemburg, _____ _____ _____
(Datum) (Name, Vorname) (Unterschrift)

Erklärung zum Umgang mit erhobenen Daten

Die im Rahmen der Studie erhobenen Daten (einschließlich Gesundheits- und psychodiagnostischer Daten) werden ausschließlich zu Forschungszwecken weiterverwendet und Dritten nicht zugänglich gemacht. Die wissenschaftliche Verwertung (Dokumentation, Speicherung und Auswertung) und ggf. eine Veröffentlichung der Daten erfolgt ausschließlich in anonymisierter Form, d.h. ohne Erfassung von Name, Anschrift oder ähnlichen Angaben.

Ich habe den Inhalt der vorliegenden datenschutzrechtlichen Erklärung verstanden und bin mit der Verwendung meiner Daten in vorstehend geschilderter Weise einverstanden.

Luxemburg, _____ _____ _____
(Datum) (Name, Vorname) (Unterschrift)

Kurzanamnesebogen

Name: _____ Geburtsdatum: ___/___/_____ Code: _____

Geschlecht: m ( ) w ( ) Körpergröße: _____ cm Gewicht: _____ kg

(Zutreffendes bitte ankreuzen; alle Angaben werden streng vertraulich behandelt)	ja	nein
1) Fühlen Sie sich zurzeit vollkommen gesund?	[ ]	[ ]
2) Befinden Sie sich zurzeit in ärztlicher Behandlung? Wenn ja, wegen welcher Erkrankung werden Sie behandelt? _____	[ ]	[ ]
3) Befanden Sie sich jemals oder befinden Sie sich zurzeit in psychotherapeutischer oder psychiatrischer Behandlung? Wenn ja, wegen welcher Erkrankung wurden/werden Sie behandelt? _____	[ ]	[ ]
4) Haben Sie jemals gelitten oder leiden Sie an:		
a) Herzkreislauferkrankungen	[ ]	[ ]
b) rheumatischen Erkrankungen	[ ]	[ ]
c) chronischen bzw. wiederkehrenden Schmerzen	[ ]	[ ]
d) Durchblutungsstörungen (z.B. Morbus Raynaud, Morbus Meunière, Tinnitus)	[ ]	[ ]
e) Hauterkrankungen (z.B. Ekzeme, Schuppenflechte, allergische Hautreaktionen)	[ ]	[ ]
f) neurologischen Erkrankungen	[ ]	[ ]
g) Anämie (Eisen,…)	[ ]	[ ]
h) gastrointestinalen Blutungen	[ ]	[ ]
i) Magengeschwüren	[ ]	[ ]
5) Nehmen Sie regelmäßig Drogen oder Medikamente? Wenn ja, welche? _____	[ ]	[ ]
6) Haben Sie in den letzten Stunden oder Tagen Drogen oder Medikamente eingenommen? Wenn ja, welche? _____	[ ]	[ ]

Ich versichere hiermit, alle Fragen verstanden und alle Angaben nach bestem Wissen gemacht zu haben. Ich hatte genügend Gelegenheit Fragen zu stellen. Unbekannte medizinische Begriffe wurden

mir klarverständlich erläutert. Zudem bestätige ich, dass meine Angaben zur Einnahme von Medikamenten und Genussmitteln/Drogen vollständig und wahrheitsgemäß sind.

Luxemburg, _____ _____ _____
(Datum) (Name, Vorname) (Unterschrift)

Annex C

Study 3: Sex-specific time course of diffuse noxious inhibitory controls-induced pain modulation and nocifensive reflex suppression in humans

Probandeninformation

Sehr geehrte Probandin, sehr geehrter Proband,

Wir danken Ihnen für Ihr Interesse an dieser Studie, die von der Abteilung Psychobiologie der Universität Luxemburg durchgeführt wird, teilzunehmen. In dem vorliegenden Informationsblatt werden Ihnen Inhalt und Zweck der geplanten Studie erläutert. Bitte lesen Sie dieses aufmerksam durch. Sollten Sie Teile dieser Aufklärung nicht genau verstehen oder darüber hinaus noch Fragen haben, sprechen Sie den/die Versuchsleiter/in bitte unmittelbar darauf an.

Ziel der Studie

Diese Studie dient dazu, ein besseres Verständnis der Mechanismen von zeitlicher Veränderung der Schmerzempfindlichkeit zu erlangen, und beschäftigt sich mit einer speziellen Form der Schmerzinhibition (DNIC) in unterschiedlichen Schmerzmodalitäten (mechanisch und elektrisch). Dazu sollen verschiedene physiologische Daten erhoben werden, während sich in Zeit, Wiederholung und Qualität unterscheidenden Schmerzreize an der rechten Hand und am linken Bein dargeboten werden. Mit der Teilnahme an diesem Forschungsvorhaben ist kein individueller Gesundheitsnutzen verbunden.

Vergütung

Nach der Studie, d.h. nach komplett abgeschlossener Datenerhebung, wird Ihnen ein fester Betrag von 30.- EUR ausgezahlt. Diese Vergütung stellt eine Entschädigung für Ihre Mühen und aufgewendete Zeit dar.

Dauer und Ort der Studie

Die Untersuchung besteht aus einer einmaligen Sitzung mit einer Dauer von zirka 1 Stunde und findet an der Universität Luxemburg, Campus Limpertsberg, in der Abteilung für Psychophysiologie statt.

Beschreibung der Untersuchungsverfahren

Die Untersuchungsprozedur besteht aus der Messung physiologischer Funktionen während mechanischer Stimulation durch Interdigitalquetschen an der rechten Hand oder elektrischer Stimulation und Auslösung des RIII-Reflexes am linken Bein.
Zur Messung der Empfindlichkeit der Hautsinne kommen folgende physikalische Reize zum Einsatz:

- Darbietung von elektrischenen Reizen anhand von 2 Elektroden, durch den Voltage Stimulator (Schwellenmessung RIII-Reflexauslösung) an der linken Wade.
- Interdigitalquetschen an der rechten Hand, während 2 Minuten

Die eingesetzten physikalischen Reize sind nicht-invasiv und risikoarm. Als Nebenwirkung der Reize kann eine vorübergehende Rötung der Haut auftreten, die spätestens ein paar Stunden nach Abschluss der Untersuchung wieder vollständig abklingt. Außerdem kann die Schmerzempfindlichkeit am Applikationsort und in dessen Umgebung vorübergehend gesteigert oder vermindert sein.

Im Rahmen der Studie werden zudem folgende physiologische Funktionen anhand von Oberflächensensoren (d.h. nicht-invasiv) permanent erfasst:

- Herzkreislaufaktivität: Elektrokardiogramm (EKG)
- Atemaktivität (abdominale und thorakale Atmung gemessen mit 2 Atmungsriemen)
- Elektromyographische Aktivität (Muskelaktivität des Stirnmuskels „corrugator supercilii“ und des Beinmuskels „biceps femoris“)
- Elekrtodermale Aktivität (EDA) (an 2 Fingern der linken Hand abgeleitet)

Die oben beschriebenen Untersuchungsmethoden sind im Rahmen der klinisch-physiologischen Diagnostik allgemein üblich. Die Untersuchung ersetzt keine ärztliche Untersuchung und liefert auch keine Informationen zum Gesundheitsstatus.

Hinweis zum Versicherungsschutz

Sollten sich aus Ihrer Teilnahme an dieser Studie nachteilige gesundheitliche Folgen ergeben, so besteht für schuldhaft durch den/die Versuchsleiter/in verursachte Gesundheitsschäden eine Haftpflichtversicherung. Für eventuelle Gesundheitsschäden oder sonstige Beeinträchtigungen (inklusive Wegunfälle) im Zusammenhang mit der Teilnahme an der Studie, die nicht auf Fehlverhalten oder Fahrlässigkeiten des/der Versuchsleiters/in zurückzuführen sind, gilt kein Versicherungsschutz.

Einwilligungserklärung

Hiermit bestätige ich, dass ich die obigen Ausführungen (Probandeninformation) aufmerksam gelesen und deren Inhalt verstanden habe. Ich habe das Ziel, den Ablauf und die Durchführung der Studie verstanden und hatte die Gelegenheit, alle mich interessierenden zusätzlichen Fragen zu stellen. Es stand mir ausreichend Bedenkzeit zur Verfügung und mir ist bewusst, dass jederzeit neu auftauchende Fragen besprochen werden können.
Mir ist bekannt, dass ich jederzeit ohne Angabe von Gründen und ohne Inkaufnahme von Nachteilen von den Untersuchungen zurücktreten kann. Im Falle eines frühzeitigen, von mir bedingten Abbruchs erkenne ich an, dass sich damit mein Anspruch auf Erstattung eines Probandenhonorars verliert.

Ich erkläre mich hiermit freiwillig bereit und damit einverstanden, an der Studie teilzunehmen.

Luxemburg, _____ _____ _____
(Datum) (Name, Vorname) (Unterschrift)

Erklärung zum Umgang mit erhobenen Daten

Die im Rahmen der Studie erhobenen Daten (einschließlich Gesundheits- und psychodiagnostischer Daten) werden ausschließlich zu Forschungszwecken weiterverwendet und Dritten nicht zugänglich gemacht. Die wissenschaftliche Verwertung (Dokumentation, Speicherung und Auswertung) und ggf. eine Veröffentlichung der Daten erfolgt ausschließlich in anonymisierter Form, d.h. ohne Erfassung von Name, Anschrift oder ähnlichen Angaben.

Ich habe den Inhalt der vorliegenden datenschutzrechtlichen Erklärung verstanden und bin mit der Verwendung meiner Daten in vorstehend geschilderter Weise einverstanden.

Luxemburg, _____ _____ _____
(Datum) (Name, Vorname) (Unterschrift)

Kurzanamnesebogen

Name: _____ Geburtsdatum: ___/___/_____ Code: _____

Geschlecht: m ( ) w ( ) Körpergröße: _____ cm Gewicht: _____ kg

(Zutreffendes bitte ankreuzen; alle Angaben werden streng vertraulich behandelt)	ja	nein
1) Fühlen Sie sich zurzeit vollkommen gesund?	[ ]	[ ]
2) Befinden Sie sich zurzeit in ärztlicher Behandlung? Wenn ja, wegen welcher Erkrankung werden Sie behandelt? _____	[ ]	[ ]
3) Befanden Sie sich jemals oder befinden Sie sich zurzeit in psychotherapeutischer oder psychiatrischer Behandlung? Wenn ja, wegen welcher Erkrankung wurden/werden Sie behandelt? _____	[ ]	[ ]
4) Haben Sie jemals gelitten oder leiden Sie an:		
a) Herzkreislauferkrankungen	[ ]	[ ]
b) rheumatischen Erkrankungen	[ ]	[ ]
c) chronischen bzw. wiederkehrenden Schmerzen	[ ]	[ ]
d) Durchblutungsstörungen (z.B. Morbus Raynaud, Morbus Meunière, Tinnitus)	[ ]	[ ]
e) Hauterkrankungen (z.B. Ekzeme, Schuppenflechte, allergische Hautreaktionen)	[ ]	[ ]
f) neurologischen Erkrankungen	[ ]	[ ]
5) Nehmen Sie regelmäßig Drogen oder Medikamente? Wenn ja, welche? _____	[ ]	[ ]
6) Haben Sie in den letzten Stunden oder Tagen Drogen oder Medikamente eingenommen? Wenn ja, welche? _____	[ ]	[ ]

Ich versichere hiermit, alle Fragen verstanden und alle Angaben nach bestem Wissen gemacht zu haben. Ich hatte genügend Gelegenheit Fragen zu stellen. Unbekannte medizinische Begriffe wurden mir klarverständlich erläutert. Zudem bestätige ich, dass meine Angaben zur Einnahme von Medikamenten und Genussmitteln/Drogen vollständig und wahrheitsgemäß sind.

Luxemburg, _____ _____ _____
(Datum) (Name, Vorname) (Unterschrift)

Annex D

Used questionnaires

BIS/BAS questionnaire

	Trifft gar nicht zu	Trifft eher nicht zu	Trifft eher zu	Trifft genau zu
1. Eine eigene Familie ist die wichtigste Sache im Leben	☐	☐	☐	☐
2. Sogar wenn mir etwas Schlimmes bevorsteht, bin ich selten nervös oder ängstlich	☐	☐	☐	☐
3. Ich strenge mich besonders an, damit ich erreiche, was ich möchte	☐	☐	☐	☐
4. Wenn mir etwas gut gelingt, bleibe ich sehr gern bei der Sache	☐	☐	☐	☐
5. Ich bin immer bereit, etwas Neues zu versuchen, wenn ich denke, dass es Spaß machen wird	☐	☐	☐	☐
6. Es ist wichtig für mich, wie ich gekleidet bin	☐	☐	☐	☐
7. Wenn ich erreiche, was ich will, bin ich voller Energie und Spannung	☐	☐	☐	☐
8. Kritik oder Beschimpfungen verletzen mich ziemlich stark	☐	☐	☐	☐
9. Wenn ich etwas haben will, tue ich gewöhnlich alles um es zu bekommen	☐	☐	☐	☐
10. Ich werde oft Dinge nur deshalb tun, weil sie Spaß machen könnten	☐	☐	☐	☐
11. Es ist schwierig für mich, Zeit für solche Dinge wie Friseurbesuche zu finden	☐	☐	☐	☐
12. Wenn ich eine Chance sehe, etwas Erwünschtes zu bekommen, versuche ich sofort mein Glück	☐	☐	☐	☐
13. Ich bin ziemlich besorgt oder verstimmt, wenn ich glaube oder weiß, dass jemand wütend auf mich ist	☐	☐	☐	☐
14. Wenn ich eine Gelegenheit für etwas sehe, das ich mag, bin ich sofort voller Spannung	☐	☐	☐	☐
15. Ich handle oft so, wie es mir gerade in de Sinn kommt	☐	☐	☐	☐
16. Wenn ich glaube, dass mir etwas Unangenehmes bevorsteht, bin ich gewöhnlich ziemlich unruhig	☐	☐	☐	☐
17. Ich wundere mich oft über das menschliche Verhalten	☐	☐	☐	☐
18. Wenn mir was Schönes passiert, berührt mich das oft sehr stark	☐	☐	☐	☐
19. Ich bin besorgt, wenn ich glaube, das ich eine wichtige Sache schlecht gemacht habe	☐	☐	☐	☐

20. Ich brauche Abwechslung und neue Erfahrungen	☐ ☐ ☐ ☐
21. Wenn ich etwas erreichen will, verfolge ich hartnäckig mein Ziel	☐ ☐ ☐ ☐
22. Verglichen mit meinen Freunden habe ich sehr wenig Ängste	☐ ☐ ☐ ☐
23. Ich fände es sehr aufregend einen Wettbewerb zu gewinnen	☐ ☐ ☐ ☐
24. Ich habe Angst, Fehler zu machen	☐ ☐ ☐ ☐

SES questionnaire (part 2):

Bitte machen Sie ein Kreuz *auf die Zahl* die am besten zutrifft.

„Ich empfinde meine Schmerzen als...

Teil B		trifft genau zu	trifft weitgehend zu	trifft ein wenig zu	trifft gar nicht zu
15.	...schneidend	4	3	2	1
16.	...klopfend	4	3	2	1
17.	...brennend	4	3	2	1
18.	...reiβend	4	3	2	1
19.	...pochend	4	3	2	1
20.	...glühend	4	3	2	1
21.	...stechend	4	3	2	1
22.	...hämmernd	4	3	2	1
23.	...heiβ	4	3	2	1
24.	…durchstoβend	4	3	2	1

Annex E

Instructions of the used apparati during the studies

E.1 Instruktionen für die Bestimmung der Kälte/Wärmewahrnehmung

Anhand dieses Tests möchten wir herauszufinden, ob Sie die präsentierten Testtemperaturen (kalt oder warm, in einer zufälligen Abfolge) auf altergemäßem Niveau wahrnehmen. Dabei werden mittels einer Thermode, die an Ihrem linken Unterarm angebracht wurde, Wärme- bzw. Kältereize appliziert.

Während dieses Tests wird die Temperatur der Thermode von einer neutralen Ausgangstemperatur aus kontinuierlich an- oder absteigen. Drücken Sie eine der beiden Maustasten, sobald Sie eine Veränderung der Temperatur der Thermode (kalt oder warm) wahrnehmen. Lassen Sie bitte Ihre Finger an einer der beiden Maustasten, sodass Sie jederzeit bereit sind, schnell zu antworten. Es ist sehr wichtig, dass Sie schnell antworten, sobald Sie eine Temperaturveränderung wahrnehmen.

Bleiben Sie bitte wachsam und konzentriert während der gesamten Testzeit.
Antworten Sie erst, wenn Sie sicher sind einen kalten/warmen Stimulus zu empfinden.

Um konsistente Messwerte zu erzielen, wird dieses Verfahren einige Male wiederholt.

E.2 Instruktionen für die Schmerzschwellenbestimmung

Bei diesem Test sind wir an Ihrer Wahrnehmungssensibilität für Schmerzreize interessiert. Wir messen, ab wann ein Hitzereiz für Sie unangenehm wird. Dazu werden mittels einer Thermode, die an Ihrem linken Unterarm angebracht wird, Wärme- bzw. Hitzereize appliziert.

Sie sollten **nach** jedem Reiz (der ungefähr 3 Sekunden andauert) eine der beiden Maustasten betätigen und zwar die Taste **„N“** (für „no“), wenn der Reiz für Sie **nicht schmerzhaft** war, und die Taste **„Y“** (für „yes“), wenn er leicht **schmerzhaft** war. Es geht hierbei nicht darum, wieviel Schmerz Sie aushalten können. Sie sollten die Taste „Y“ drücken, wenn Sie eine leichte schmerzhafte Empfindung verspüren bzw. sobald Sie das Gefühl haben, dieser Reiz würde für Sie sehr unangenehm werden, wenn Sie ihn länger ertragen müssten.

Das Programm appliziert solange Hitzereize, bis es Ihre Schmerzschwelle bestimmen konnte. Wenn Sie vorher an irgendeinem Punkt des Verfahrens das Experiment stoppen möchten, betätigen Sie bitte eine der beiden Tasten, oder sagen Sie „Stopp“. Das Betätigen der Taste stellt die thermische Vorrichtung ab.

Bitte bleiben Sie wachsam und konzentriert während des gesamten Tests.

E.3 Instruktionen zum heißen und kaltem Wasser

Bei dem nun folgenden Test geht es darum, die rechte Hand bis zum Handgelenk in eines der beiden Wasserbecken einzutauchen. Eines der Becken enthält kaltes Wasser, das mit einer Pumpe konstant auf 5-6°C gehalten wird, während das andere Becken warmes Wasser enthält, das mittels eines Heizstabs und ggf. Hinzufügen von erhitztem Wasser auf 47-48°C reguliert wird.

Der Versuchsleiter gibt an, in welches Becken Sie Ihre Hand zuerst eintauchen müssen. Ihre Aufgabe ist es, die Hand solange eingetaucht zu lassen, bis Sie den dabei auftretenden Schmerz nicht mehr aushalten können, und dabei die auftretenden Schmerzempfindungen subjektiv einzuschätzen.
Gleich nach dem ersten Test wird die Hauttemperatur der Hand gemessen. Anschließend wird die Hand in ein neutral temperiertes Becken (32°C) eingetaucht. Nach einer weiteren 7-minütigen Wartezeit wird die Hauttemperatur noch einmal bestimmt. Daraufhin erfolgt ein identischer Test, bei dem die Hand in das zweite Becken eingetaucht wird.

Der Versuchsleiter wird Sie während den beiden Tests wiederholt fragen, wie Sie die Wassertemperatur empfinden: Dazu sollten Sie jeweils eine Zahl zwischen 0 und 100 angeben, die widerspiegelt, wie schmerzhaft es sich für Sie anfühlt. 0 bedeutet dabei kein Schmerz, 1 der leichteste Schmerz, den Sie sich vorstellen können, 50 ein Schmerz mittlerer Intensität und 100 der stärkste Schmerz, den Sie ertragen können.

Parallel dazu sollten Sie dem Versuchsleiter den Moment angeben, ab dem Sie eine **erstmalige Schmerzempfindung** verspüren. Lassen Sie dabei aber trotzdem die Hand im Wasser!