Aalborg Universitet

AALBORG UNIVERSITY

Spinal nociception is facilitated during cognitive distraction

Henrich, Mauricio Carlos; Steffen Frahm, Ken; Coghill, Robert C; Kæseler Andersen, Ole

Spinal Nociception is Facilitated during Cognitive Distraction

Mauricio Carlos Henrich, [a]* **Ken Steffen Frahm,** [a] **Robert C. Coghill** [b] **and Ole Kæseler Andersen** [a]

[a] *Center for Neuroplasticity and Pain (CNAP), Integrative Neuroscience, Department of Health Science and Technology, Aalborg University, Fredrik Bajers Vej 7A, Aalborg East-9220, Aalborg, Denmark*

[b] *Pediatric Pain Research Center, Behavioral Medicine and Clinical Psychology, Cincinnati Children's Hospital Medical Center, 3333 Burnet Ave, Cincinnati, OH, 45229-3026, USA*

Abstract—The nociceptive withdrawal reflex (NWR) is a behavioral response to protect the body from noxious stimuli. The spatial characteristics of the stimulus modulate the reflex response to prevent damage to the affected tissue. Interneurons in the deep dorsal horn in the spinal cord encode the relationship between stimulus characteristics and the magnitude of the NWR and are also likely integrating spatial information of the nociceptive stimulus. The aim of this study was to use the NWR to investigate whether the spinal spatial integration of a simultaneous stimulus is modulated by shifting the attention of the participant towards (attention) or away from (distraction) the stimulus. We hypothesized that the descending activity shapes the receptive fields of the spinal neurons encoding spatial integration of nociception. Twenty healthy volunteers participated in the study. Single and simultaneous stimuli were delivered through two stimulating electrodes located in the arch and on the lateral side in the sole of the foot. The NWR was quantified by electromyography from the Tibialis Anterior and Biceps Femoris muscles during baseline and active tasks (attention and distraction). During the baseline task, spatial summation of the NWR was evoked during simultaneous stimulation. During the distraction task, the NWR was significantly larger compared to baseline, regardless of the sites being stimulated (single and simultaneous stimuli). In contrast, the NWR recorded during the attention task did not differ from baseline. These results further support that the spinal NWR pathway is under descending control which can be modulated by cognitive processes. The NWRs recorded over both proximal and distal muscles were similarly affected by the tasks, suggesting that the descending control affects the lower leg spinal system, with no discrimination between spinal segments. © 2022 The Author(s). Published by Elsevier Ltd on behalf of IBRO. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

Key words: reflex, nociception, modulation, cognitive, attention, distraction.

INTRODUCTION

The nociceptive withdrawal reflex (NWR) is a polysynaptic reflex that serves defensive purposes in humans (Kugelberg et al., 1960; Grimby, 1963; Sandrini et al., 2005; Andersen, 2007) as well as in animals (Sherrington, 1910; Schomburg, 1990; Cleland and Bauer, 2002; Clarke and Harris, 2004). Although it was first described by Sherrington as an stereotyped flexion reaction (Sherrington, 1910), a considerable amount of evidence has been accumulated in favour of a modularly organized reflex serving a functional withdrawal reflex pattern. This organization includes control over several groups of muscles across multiple joints, leading to a net movement not necessarily characterized by joint flexion (Schouenborg and Kalliomäki, 1990; Schouenborg et al., 1994; Sandrini et al., 2005; Andersen, 2007). Indeed, the motor pattern of the NWR integrates temporal (Fuhrer, 1976; Andersen et al., 2005; Bajaj et al., 2005; Perrotta et al., 2016, 2017; Sprenger et al., 2018) and spatial (Schouenborg et al., 1995; Ylioja et al., 2006; Andersen, 2007; Neziri et al., 2009; Henrich et al., 2020) characteristics of the stimulus together with proprioceptive (Serrao et al., 2014; Massé-Alarie et al., 2019), cognitive (Bjerre et al., 2011; Jure et al., 2020) and emotional states (Rhudy and Meagher, 2000; Bartolo et al., 2013; Fragiotta et al., 2019; Lannon et al., 2021), to generate the rapid, optimal motor response to any given nociceptive input under the present posture and movement. When exposed to a suprathreshold stimulus, the recruitment of synergistic muscles moves the stimulated area away from the stimulus, coordinating the recruitment of muscle groups to keep appropriate balance and maintain the current motor activity.

*Corresponding author. Address: Fredrik Bajers Vej 7A-1-206, Aalborg East 9220, Denmark.
E-mail address: mhenrich@hst.aau.dk (M. C. Henrich).
Abbreviations: ACC, anterior cingulate cortex; BF, Biceps Femoris; DDH, deep dorsal horn; NS, nociceptive specific; NWR, nociceptive withdrawal reflex; PAG, periaqueductal gray matter; RRF, reflex receptive field; RVM, rostroventral medulla; TA, Tibialis Anterior; WDR, wide dynamic range.

The spatial characteristics of the stimulus shape the reflex response to prevent damage of the exposed tissue. Among those characteristics are the location of the stimulus in the body (Grimby, 1963; Schouenborg et al., 1995; Andersen, 2007; Massé-Alarie et al., 2019), its intensity (a more intense stimulus likely recruit a larger neuronal population), the size of the stimulated area (Chan and Dallaire, 1989; Sonnenborg et al., 2000; Andersen et al., 2001; Neziri et al., 2009; von Dincklage et al., 2013; Henrich et al., 2020), and the specific sites that are simultaneously affected by the stimulation (Henrich et al., 2020). The neural substrate behind these spatial integrative processes remains to be clarified, however, dorsal horn neurons seem to play a pivotal role (Schomburg, 1990; Schouenborg et al., 1990; Coghill et al., 1991). Previous evidence has suggested that interneurons in the deep dorsal horn (DDH) encode the relationship between the intensity of the stimulus and the magnitude of the NWR (Coghill et al., 1991; Schouenborg et al., 1995; Clarke and Harris, 2004). Additionally, these neurons are likely governing the cutaneous receptive field (termed reflex receptive field, RRF) from which a NWR can be elicited (Schouenborg and Kalliomäki, 1990).

Nociceptive stimulation of the skin generates a neural drive that converges into the dorsal horn of the spinal cord (SC). The convergent information is processed and integrated by neurons that respond selectively to nociceptive stimulation (nociceptive specific, NS), and by neurons responsive to both nociceptive and innocuous stimulation (wide dynamic range, WDR). NS neurons have small receptive fields (RF). On the other hand, RFs of WDR neurons are believed to cover a significantly larger portion of the limb (Price et al., 1978). Therefore, when two simultaneous stimuli are applied in the skin, multiple RFs of both NS and WDR neurons are likely stimulated. According to previous studies in rats (Schouenborg et al., 1992, 1995), WDR neurons in the DDH of the SC are likely playing a role in the encoding of the RRF of specific muscles or group of synergistic muscles, and therefore in the spatial integration of nociceptive stimuli within the reflex circuitry. An early study in rats (Weng and Schouenborg, 1996) showed that an excitatory drive onto convergent dorsal horn neurons from multiple skin sites also triggers inhibitory mechanisms. In humans, indirect evidence has shown similar filtering effects on both perception outcomes (Quevedo et al., 2017) and on the NWR (Sonnenborg et al., 2000; Henrich et al., 2020).

The afferent information reaching the dorsal horn of the spinal cord, is processed, and projected to the ventral horn and supraspinal structures. Multiple synaptic stages within the dorsal horn of the spinal cord are subject to descending modulation (Todd, 2010). The most extensively studied descending modulatory system in relation to nociception originates in the periaqueductal gray matter and relays in the rostroventral medulla (PAG-RVM) before descending through the SC (Heinricher et al., 2009). The study of the PAG-RVM as an endogenous analgesia system was motivated by early

observations in animals showing inhibition of pain behavior when the PAG was electrically stimulated (Reynolds, 1969; Mayer et al., 1971; Liebeskind et al., 1973; Mayer and Liebeskind, 1974; Oliveras et al., 1975; Hayes et al., 1978). Human studies also reported inhibitory effects on nociception when the PAG was stimulated (Gol, 1967; Richardson and Akil, 1977). Although those early findings agreed on the anti-nociceptive nature of the PAG modulation, later studies confirmed that the PAG-RVM system can induce both spinal pro- and anti-nociception (Fields et al., 1983; Zhuo and Gebhart, 1992).

Among the factors that may drive descending modulation, emotional and cognitive processes have lately received considerable attention. In healthy subjects anxiety (Hubbard et al., 2011; Lannon et al., 2021) and stimuli with negative emotional valence (Rhudy et al., 2005; Roy et al., 2009; Bartolo et al., 2013; Fragiotta et al., 2019) facilitate defensive motor responses and/or self-reports of pain intensity. A cognitive manipulation of particular interest for this study is attentional shifts. In this regard, a study by Quevedo and Coghill (2007) showed that changes in attention modulated how simultaneous noxious stimuli were integrated in the perception of pain. In that study, when participants were instructed to give an overall rating for both simultaneous stimuli, spatial summation of pain was enhanced (compared to rating a single stimulus). Shifts between attention/distraction, have also shown to modulate the magnitude of the NWR (Liebermann and Defrin, 2009; Bjerre et al., 2011; Ruscheweyh et al., 2011). Particularly, Bjerre and colleagues (Bjerre et al., 2011) reported that distracting participants from the stimulus, facilitated the NWR elicited by single stimulation across different sites in the sole of the foot. On the other hand, focusing on the stimulation led to smaller NWRs. The authors interpreted those observations as an expansion/reduction of the RRF of the Tibialis Anterior (TA) muscle, produced by distraction-/attention- engaged PAG-RVM descending modulation.

Given the evidence from animal studies suggesting that WDR neurons in the DDH of the spinal cord likely encode spatial characteristics of the RRF (Schouenborg et al., 1995) one might speculate that modulation of the RRF will affect how simultaneous stimuli are integrated in the reflex pathway. The hypothesis of this study presumes that an attentional shift triggers descending modulatory drives that modulate the RRF of lower limb muscles involved in the NWR. By doing so, the integration of simultaneous stimuli is expected to be facilitated when the modulated RRF cover the two stimulation sites and inhibited when not (given the observations by Bjerre et al. 2011). Particularly, it was hypothesized that: distraction would facilitate the spatial integration of the simultaneous stimuli provoking a larger NWR magnitude when two sites in the sole of the foot within the TA RRF are simultaneously stimulated. Shifting the attention to the stimulated site was expected to shrink the RRF obstructing the integration of simultaneous stimuli -as one of the sites is no longer part of the TA RRF.

EXPERIMENTAL PROCEDURES

Subjects

Interested volunteers received written and oral information about the experiment. If they agreed to participate, and met the inclusion/exclusion criteria, written informed consent was obtained. Exclusion criteria included mental disorders or disorders of the nervous or musculoskeletal system, addiction to euphoric substances, use of medicines that may affect the results of the experiment and presence of wounds on the sole of the foot. Twenty healthy subjects were included in the experiment (12 men and 8 women, mean age 26.8). The experiment was performed according to the Declaration of Helsinki and received approval from the local Ethical Committee (North Denmark Region VN-20180047).

Participants were comfortably lying on a reclined bed in a quiet room throughout the experiment. They were instructed to remain calm, relaxed and to avoid any voluntary movement during the experiment, particularly while performing EMG recordings.

EMG recordings

Double-differential EMG recordings were acquired in Tibialis Anterior (TA) and Biceps Femoris (BF) muscles (Frahm et al., 2012) in the ipsilateral leg relative to the stimulation site. Three recording electrodes (Neuroline 720, Ambu A/S, Denmark) were mounted in each muscle site following previously published recommendations on location of recording electrodes (Hermens et al., 2000). A common reference electrode (Neuroline 720, Ambu A/S, Denmark) was placed in the ipsilateral knee over the patella bone. The EMG recordings were amplified, filtered (5–500 Hz), sampled (2 kHz), and stored for further offline analyses.

Quantification of the NWR

The NWR was elicited by applying computer-controlled electrical stimuli in the sole of the foot. The skin of the sole of the foot was prepared by a slight manual exfoliation to reduce impedance (due to the presence of thick stratum corneum layer). Two stimulating electrodes (Neuroline 700, Ambu A/S, Denmark, diameter reduced to 6 mm (Frahm et al., 2013; Henrich et al., 2020)) were mounted in the sole of the foot, medially (M) and laterally (L) (see Fig. 1). A large anodal electrode was placed on the dorsum of the foot (Pals 7.5 × 10 cm, Axelgaard Ltd., Fallbrook, California, USA) so stimuli would be perceived in the sole of the foot. The stimulus consisted of five monophasic rectangular pulses of 1 ms delivered at 200 Hz and were delivered using an automated custom-made software. Three stimulus types were used, single stimulation in M or L, and paired stimulation in both sites, M and L. The order was randomized and inter-stimulus intervals ranging between 15–30 s were used to prevent habituation (von Dincklage et al., 2013).

The NWR threshold (NWR-th) was used to set the stimulus intensity. The NWR-th was determined using an automated staircase procedure (Jensen et al., 2015a), independently for each electrode site. The staircase started with an intensity of 1 mA, increased with steps of 2 mA until a NWR was detected. Then, the intensity started decreasing until the NWR was no longer elicited. This process was repeated 2 more times with steps of 1 mA and 0.5 mA. Finally, the NWR-th was calculated as the average between the last 2 ascending and descending limits. The intensity of the stimulation was set as 150% the NWRth.

The automatic detection of the NWR was implemented in the staircase procedure described above. The criteria to decide whether a specific trial successfully elicited a NWR is based on a series of studies that attempted to standardize the NWR methodology (Rhudy and France, 2007; France et al., 2009; Jensen et al., 2015b). Specifically, the interval peak z-score (IPZ) was calculated (as in the below equation) and compared to a predefined threshold value of 12 (Rhudy and France, 2007; France et al., 2009; Jensen et al., 2015b). When the IPZ exceeds the threshold, the trial is considered as a successful NWR.

$$IPZ = \frac{(EMGpeak - baselinemean)}{baselineSD}$$

where the EMG peak is calculated in a predefined NWR window of 80–150 ms post-stimulus (Andersen, 2007). Baseline mean and standard deviation (SD) were calculated in the 70 ms pre-stimulus window.

The NWR window was defined as the interval between 80 ms and 150 ms post-stimulus as generally reported in the literature (Sandrini et al., 2005; Andersen, 2007). An electrical stimulus that is applied on the sole of the foot most likely depolarizes A and C fibers simultaneously. The difference in the conduction velocities of Aβ, Aδ and C fibers allow discriminating the observed NWR driven by Aδ nociceptive fibers. Slow conducting C fibers can be safely excluded since its contribution to the EMG in a time window before 200 ms is highly unlikely (Hugon, 1973). However, a role of Aβ fibers role cannot be completely discarded.

The NWR magnitude was quantified by calculating the root mean square value of the EMG recordings in the predefined reflex window as it is commonly reported in other studies (Terkelsen et al., 2004; Müller et al., 2016; Jure et al., 2019; Massé-Alarie et al., 2019). Offline data processing was performed in MATLAB R2019b (Mathworks, Natick, MA, USA). Values were averaged across the five repetitions of each stimulus type for further processing.

The Detection threshold (D-th) was estimated using the same staircase procedure although stimulus detection was the assessed criteria, and increasing/decreasing intensity steps were 1 mA, 0.5 mA and 0.1 mA.

The stimulus intensity varied between the baseline and active blocks. For the baseline block of the distraction task, it was defined as 150% of the NWR-threshold (NWR-th), while for the control block of the attention task the intensity of the stimulus was defined as 200% the detection threshold (D-th), (see Experimental protocol below).
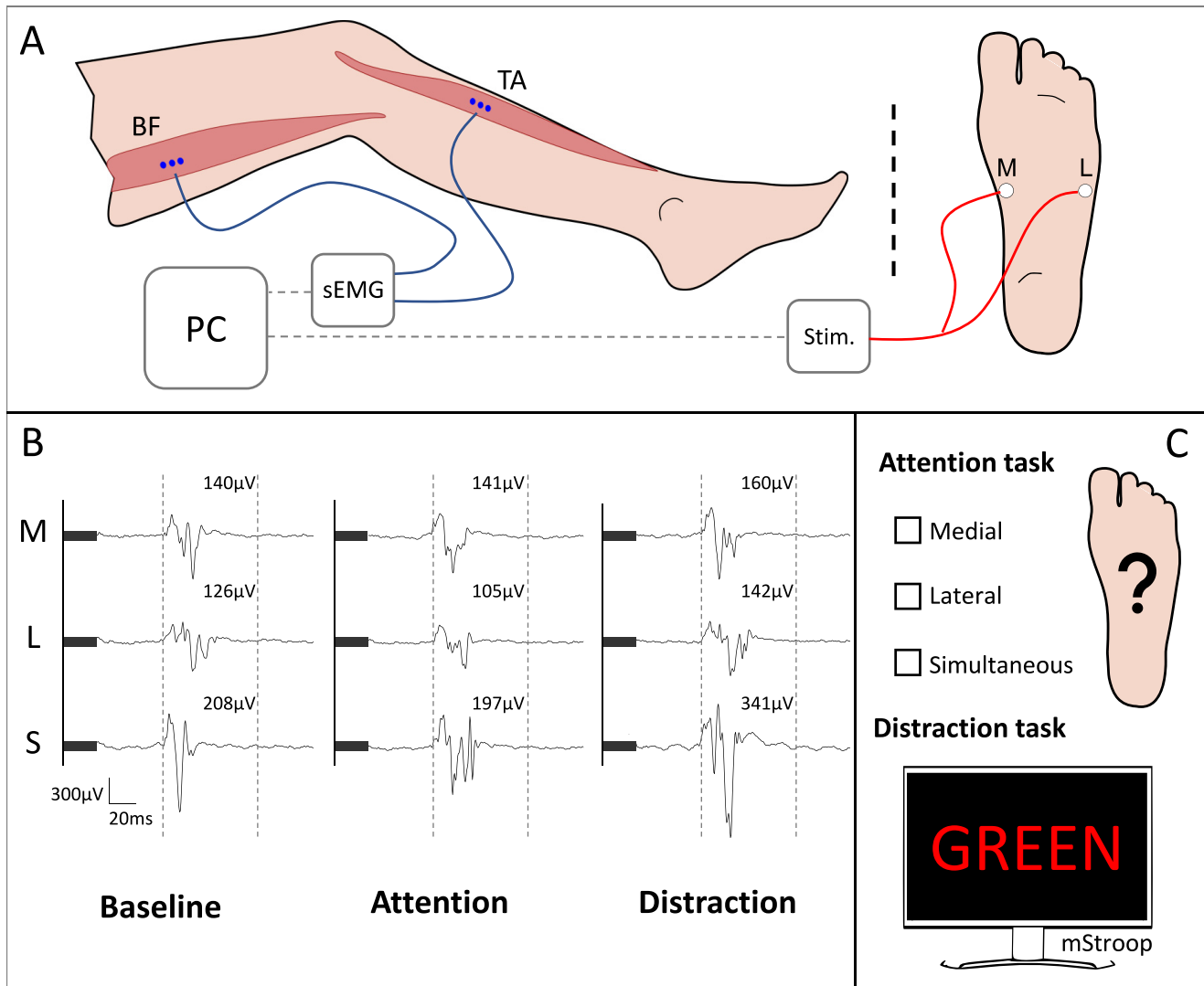
Fig. 1. Illustration indicating **(A)** elements of the experimental setup for stimulation (Stim.) of the sole of the foot and recording of the NWR via sEMG in Tibialis Anterior (TA) and Biceps Femoris muscles (BF). Stimulations were delivered at random intervals controlled by a computer (PC). **(B)** Three different types of stimuli were delivered, single stimulation in the medial side (M) or lateral side (L) of the sole of the foot, and simultaneous (S) stimulation of both sites. Representative TA EMG traces are depicted for the three stimulation sites in different conditions: Baseline, Attention and Distraction. In this panel, vertical dashed lines indicate the time window in which the NWR is quantified. The numerical value shown between these lines indicate the RMS amplitude of the signal in the analysis window. Black horizontal boxes indicate stimulation train. **(C)** Two different cognitive tasks were designed to shift the attention of the participant to the stimulus (attention) or away from it (distraction). In the attention task, subjects were asked to indicate the activated electrode. In the distraction task, subjects were instructed to state the color of the written word ignoring the written meaning while electrical stimulation at random intervals were delivered. mStroop: modified Stroop test.

Cognitive tasks: distraction (mStroop test) and attention (localization test)

The Distraction task was based on a modified version of a Stroop test (Stroop, 1935). To distract the participant from the stimulation, a series of slides were displayed showing different color names written in a black background with varying font colors. Slides changed at a frequency of 1 Hz and subjects were instructed to name the color of the font, ignoring the meaning of the written word. Missing/wrong words were counted as errors.

The Attention task consisted in identifying the site that was stimulated: M, L or both. During this task, participants were instructed to indicate the location of the applied stimulus in every trial. Missing/wrong localizations were counted as errors.

Experimental protocol

The experimental protocol started with a familiarization phase that consisted of a series of single and simultaneous stimulations, in random order and intensities, aiming at reducing effects of arousal and anxiety in the participant. Subjects were introduced to the cognitive tasks (mStroop and localization tests) before the final data collection began.

To control for the performance of the attention and distraction tasks, control blocks were performed. It consisted of two blocks, one for each cognitive task (Fig. 2). For distraction, the modified Stroop test (mStroop) was performed without delivering stimuli and counting the errors made when naming the color of the written word. For localization, a low intensity stimulation

	Familiarization	Thresholds' estimation	Control		Attention (AT)		Distraction (DI)	
			AT	DI	Baseline	Task	Baseline	Task
Cognitive test	Localization mStroop	No	Localize	mStroop	No	Localize	No	mStroop
Stimulus intensity	Random	Automated staircase	2 x Dth	No	1.5 x NWRth	1.5 x NWRth	1.5 x NWRth	1.5 x NWRth
EMG	No	TA and BF	No	No	TA and BF	TA and BF	TA and BF	TA and BF

Fig. 2. Diagram of the experimental design consisting of five blocks: Familiarization, Thresholds' estimation, Control, Attention and distraction. TA: Tibialis Anterior, BF: Biceps Femoris, Dth: detection threshold, NWRth: NWR threshold.

(2xD-th, see above) was used and localization errors were quantified.

Afterwards, four stimulation blocks were conducted in random order: Attention, Distraction, and their respective Baselines (see Fig. 2). During each of those blocks, five repetitions of each stimulus type (single in M, in L and simultaneous stimulation) were delivered in random order. No specific instructions were given to the participants during baseline, other than to remain quiet and avoid any voluntary movement during the recordings. Three to five minutes resting breaks were taken between blocks.

Statistical analyses

Due to non-normal distributed data, a nonparametric Wilcoxon signed rank test was used to compare between the size of the NWR during baseline measures and the active cognitive task. For each cognitive task, a Friedman's test was used to compare between stimulus types (single in M, single in L and paired). Wilcoxon signed rank test was performed as posthoc and corrected for multiple comparison using Bonferroni correction. Wilcoxon signed rank test was used to compare the number of errors made during the cognitive tasks vs control values. Significance level was set at $p = 0.05$. To allow better illustration, the NWR magnitudes were displayed as logarithmic.

RESULTS

Cognitive task performance

The performance (median (IQR)) of the cognitive task during the attention blocks was 100% (90%–100%) correct, while during distraction it was 97% (95%–98%) correct. During control blocks, similar performance was obtained during attention 100% (100%–100%) correct and distraction 97% (94%–99%) correct. Statistical analyses showed no differences between active and control block in either attention (Wilcoxon signed ranks,

$p > 0.05$) or distraction blocks (Wilcoxon signed ranks, $p > 0.05$).

Baseline data

The analyses of the TA NWR during baseline conditions (Fig. 3, top panel) showed a significant effect of stimulated site (Friedman's test: $\chi^2(2) = 22.8$, $p < 0.0001$). Post hoc tests showed that simultaneous stimulation elicited larger reflexes than single stimulation in the medial site (Wilcoxon signed rank test: $Z = -3.6$, $p < 0.05$), and in the lateral site (Wilcoxon signed rank test: $Z = -3.9$, $p < 0.001$). No significant difference was observed for single stimulation between M and L sites.

In contrast, analyses of the NWR in the BF muscle did not show significant effect of stimulation site (Fig. 3, bottom panel).

Cognitive modulation of NWR sensitivity

Distraction task. When compared to baseline recordings, the TA-NWR elicited during the distracting task was significantly larger using single stimulation in the medial site (Fig. 4, top panel, gray boxes; ø: Wilcoxon signed rank test, $Z = -3.5$, $p < 0.001$), in the lateral site (Fig. 4, top panel, gray boxes; ø: Wilcoxon signed rank test, $Z = -2.2$, $p < 0.025$), and with simultaneous stimulation of both sites (Fig. 4, top panel, gray boxes; ø: Wilcoxon signed rank test, $Z = -2.9$, $p < 0.01$). Similar findings were observed in BF; NWRs were larger when stimulating through the medial electrode (Fig. 5, gray boxes; ø: Wilcoxon signed rank test, $Z = -2.2$, $p < 0.028$), through the lateral electrode (Fig. 5, gray boxes; ø: Wilcoxon signed rank test, $Z = -3.6$, $p < 0.001$), and for simultaneous stimulation (Fig. 5, gray boxes; ø: Wilcoxon signed rank test, $Z = -3.5$, $p < 0.001$). These results suggest an
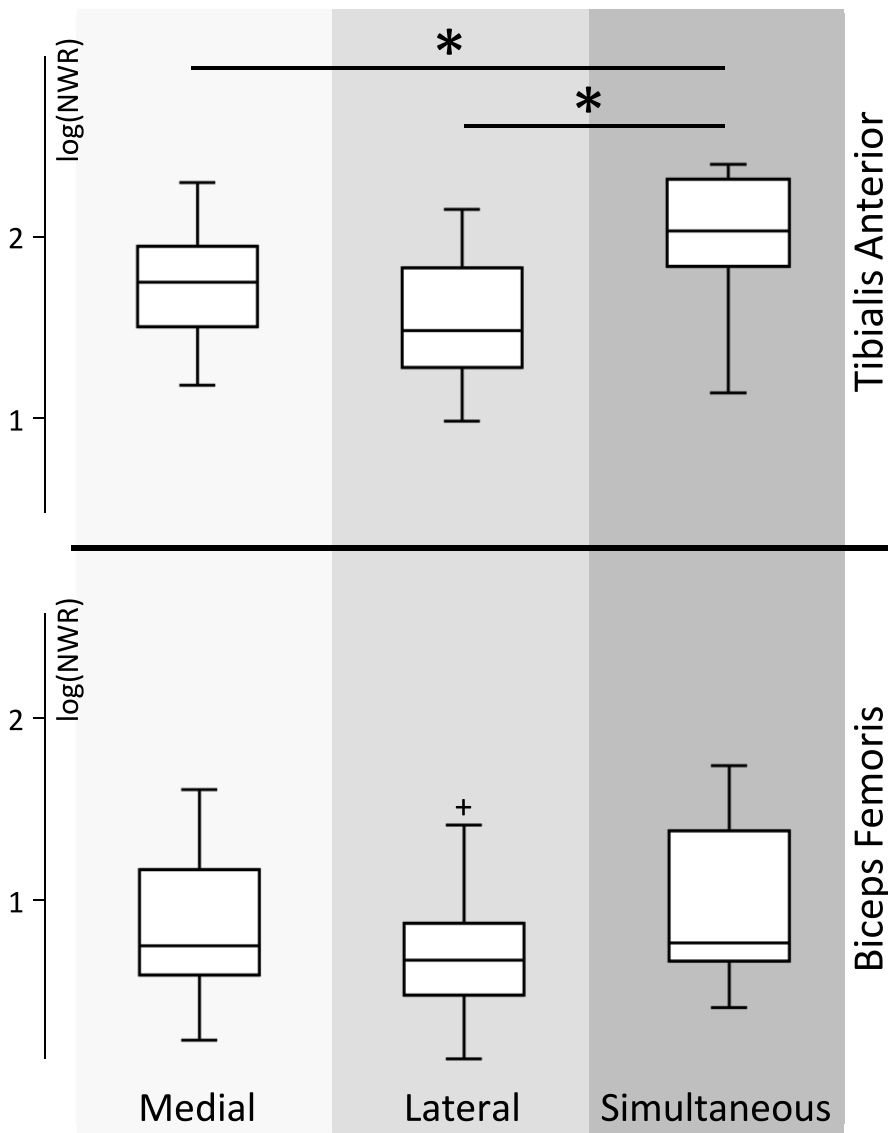
Fig. 3. Whiskers and box plot showing the size of the NWR at baseline for three different stimulus sites: Medial, Lateral and Simultaneous stimulation. The top panel shows the responses for the Tibialis Anterior (TA) Muscle, the lower panel shows the response of the Biceps Femoris (BF) muscle. For the TA muscle, simultaneous stimulation elicited larger NWRs than single stimulation (Wilcoxon signed rank test: Medial vs Lateral: *$p < 0.05$, Medial vs Simultaneous: *$p < 0.001$, Lateral vs Simultaneous: *$p < 0.001$. For the BF muscle there were no differences. Vertical axes are logarithmic for better visualization.

effective modulation of the spinal nociceptive processing when distracting the participant from the stimuli.

No significant differences were observed in the magnitude of the NWR between stimulus site (Friedman's test, $p > 0.05$), suggesting that the facilitation induced by the distraction task affect the entire NWR pathway irrespective of the spatial characteristics of the stimulus.

Attention task. Compared to baseline, there was no significant difference in the magnitude of the NWR regardless of the stimulated site, and recorded muscle (Figs. 4 and 5, white boxes; Wilcoxon signed rank test, $p > 0.05$).

Attention vs. Distraction. Direct comparison between distraction and attention showed different modulation in TA and BF. NWR recorded in TA (Fig. 4) showed facilitation during the distraction task for stimulation in M (Wilcoxon signed rank test, $Z = -3.5$, $p < 0.001$), in L (Wilcoxon signed rank test, $Z = -3.0$, $p < 0.01$), and for simultaneous stimulation (Wilcoxon signed rank test, $Z = -2.7$, $p < 0.01$). Results on BF (Fig. 5) showed a similar tendency of larger NWR during distraction, although statistical analyses revealed a non-significant tendency for stimulation in the lateral site (Wilcoxon signed rank test, $Z = -3.0$, $p = 0.067$), and a significant difference for simultaneous stimulation (Wilcoxon signed rank test, $Z = -3.5$, $p < 0.01$).

DISCUSSION

In this study, it was investigated whether cognitive tasks modulate the spinal integration of nociceptive stimuli through the NWR pathway, when delivering single and simultaneous stimulation of the arch and lateral side of the sole of the foot. In baseline conditions, spatial summation of TA-NWR was observed: simultaneous stimulation elicited significantly larger NWR than single stimulation. Regarding the cognitive modulation, as hypothesized distracting the participants from the stimulus significantly facilitated the NWR. However, the degree of summation was not altered by the cognitive tasks. The NWR facilitation observed during distraction, was not different between stimulus type (M, L and Simultaneous)... In addition, it was expected that the attention task would inhibit the NWR, an effect that could not be confirmed in this study. Finally, the NWR modulation did not differ between the Biceps Femoris and Tibialis Anterior muscles, suggesting that the descending drive affects the spinal nociceptive withdrawal reflex system with no segmental discrimination.

Top-down modulation of the NWR

Descending pathways from the brainstem support top-down modulation of spinal nociceptive pathways (Tracey
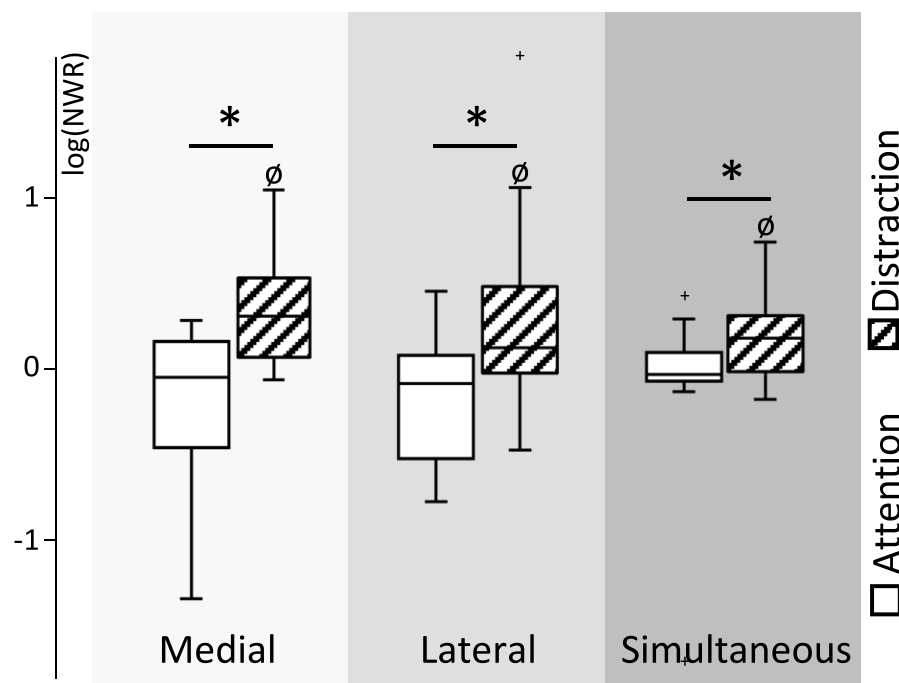
Fig. 4. Whisker and box plot showing the magnitude of the NWR in TA during both the distraction and the attention tasks. The distraction task effectively modulated the size of the NWR compared to baseline (ø: Wilcoxon signed rank test, $p < 0.05$) and compared to the attention task (*: Wilcoxon signed rank test, $p < 0.05$), regardless of the stimulated site. No significant effect of site was observed during distraction or attention (Friedman's test, $p > 0.05$). Vertical axes are logarithmic for better visualization.
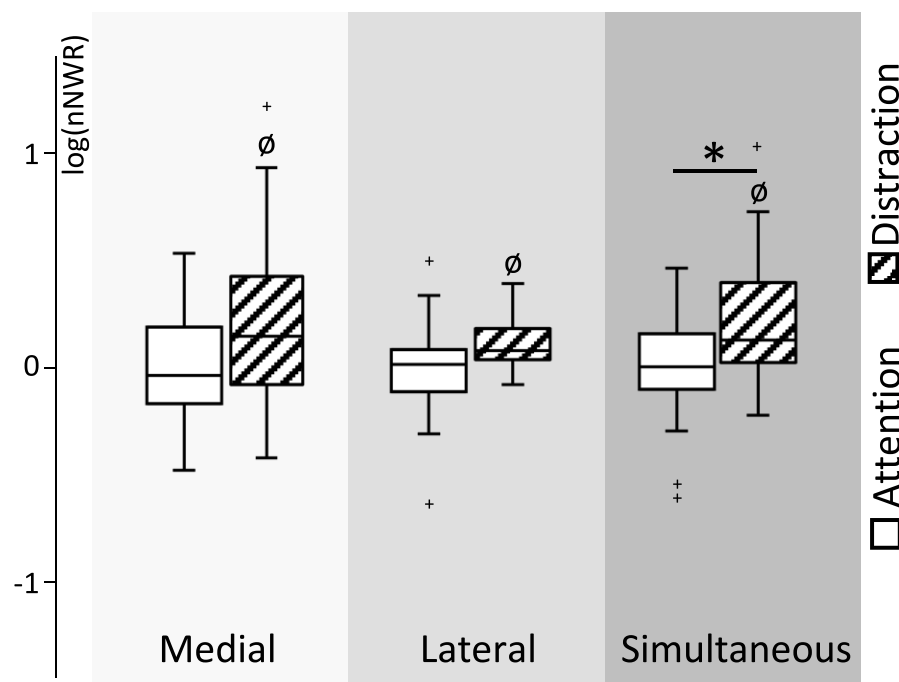

Fig. 5. Whisker and box plot showing the magnitude of NWR in the BF. The NWR was elicited by stimulation in different sites (M, L and Simultaneous) and under two different cognitive tasks (Attention and Distraction). The distraction task (compared to baseline) facilitated the NWR regardless of the stimulated site (ø: Wilcoxon signed rank test, $p < 0.05$). Direct comparison between distraction and attention showed that the former condition elicited larger NWR, only for simultaneous stimulation (*: Wilcoxon signed rank test, $p < 0.05$). Vertical axes are logarithmic for better visualization.

and Mantyh, 2007). Animal and human studies have provided evidence for facilitatory and inhibitory influences originating from supraspinal structures (such as the PAG, dorsal reticular nucleus and ventrolateral medulla) onto spinal nociceptive circuits (see Heinricher et al., 2009 for a review). The polysynaptic spinal reflex pathway has its first synaptic connections at dorsal horn neurons exposed to descending supraspinal modulation. Specifically, PAG neurons projecting to the dorsal horn via the RVM are believed to play a key role in depressing/enhancing the nociceptive inflow at spinal level, consequently inhibiting (Zhuo and Gebhart, 1997; Danziger et al., 1998; Tracey et al., 2002; Quevedo and Coghill, 2007) or facilitating (Zhuo and Gebhart, 1997; Danziger et al., 1998; Defrin et al., 2007; Quevedo and Coghill, 2007) behavioral responses and the perception of pain.

Attempts to characterize the link between cognitive processes and descending modulation of spinal nociceptive processes are abundant in the literature. Particularly, emotions engage brain structures that likely affect the gain of the spinal nociceptive system. Fear, stress, anxiety, relaxation have been shown to modulate nociceptive responses, such as the NWR (Hayes et al., 1978; Rhudy and Meagher, 2000; Rhudy et al., 2005, 2008; Roy et al., 2012; Lannon et al., 2021). Similarly, the role of cognitive processes in the modulation of pain have been extensively studied. A study by Hadjipavlou and colleagues in healthy humans has provided evidence that support the link between the PAG and many structures, more rostrally located, that are known to be active in cognitive tasks, such as prefrontal cortex, amygdala, thalamus hypothalamus and rostroventral medial medulla (Hadjipavlou et al., 2006). The presence of such anatomical connections may constitute the neural basis that support the existence of a cognitive-brainstem loop with the potential to control descending modulation in

the nociception system. Consistent with this evidence, functional imaging studies have shown that noxious-stimulation induced activation of the spinal cord is modulated during attention tasks (Sprenger et al., 2018). However, studies manipulating participant's attention have reported discrepant results, particularly on the direction of the modulatory effect (inhibition vs facilitation). The mechanisms behind the modulation still remain elusive to characterize.

Evidence regarding the modulatory effect of attentional shifts on pain ratings generally agrees on that distracting subjects from the noxious stimuli reduces the intensity of the perceived pain (Devine and Spanos, 1990; Lautenbacher et al., 1998; Bantick et al., 2002; Tracey et al., 2002; Valet et al., 2004; Quevedo and Coghill, 2007). On the other hand, studies that reported the effect of distraction on reflex responses have shown contradictory results. An early study made by Miller and colleagues has shown inhibition of pain and reflex responses when subjects performed a demanding mental task consisting of arithmetic subtraction (Miller et al., 1979). Kiernan and colleagues reported that distracting subjects from the noxious event by the induction of hypnosis inhibit intensity of pain perception and the magnitude of the reflex (Kiernan et al., 1995). In agreement with the latter, Zachariae and colleagues also reported inhibition of the NWR when hypnotic analgesia was induced (Zachariae et al., 1998). By using a similar hypnotic methodology, Danziger and colleagues reported that eleven out of eighteen of their participants had inhibited reflex responses while in the other seven subjects the reflex was facilitated (Danziger et al., 1998). A study by Liebermann and Defrin also showed evidence supporting that cognitive tasks might induce facilitation of the NWR (Liebermann and Defrin, 2009). In the latter, when participants were unaware of the upcoming noxious stimulation, the latency of the NWR was decreased significantly (Liebermann and Defrin, 2009). In line with more recent studies (Bjerre et al., 2011; Arguissain et al., 2014), our results showed that the magnitude of the NWR was significantly facilitated during the distraction condition (Fig. 3), compared to baseline.

Contradictory results regarding modulation of the reflex might be based on inconsistencies in the methodology for manipulating attention across studies. Hypnotic analgesia studies (Miller et al., 1979; Danziger et al., 1998) may be associated with increased activation of the same brain structures (i.e.: anterior cingulate cortex (ACC), dorsolateral and orbitofrontal prefrontal cortices) involved in descending modulation of pain (Kupers et al., 2005). Studies using the Stroop test as a distracting method, similar as the one used in the present study, have shown enhanced activity in ACC and orbitofrontal areas and inhibited insula, thalamus and mid-cingulate areas involved in the codification of pain intensity (Bantick et al., 2002; Valet et al., 2004; Schumann et al., 2018).

Regarding the attention condition, no significant modulation of the NWR was observed (see Fig. 3) in this study. The attention task consisted of identifying the stimulated site. Since only three different spatial configurations were used (M, L and simultaneous), a possible explanation of the lack of modulation is that the task was not as cognitively demanding as expected. This is also reflected in the low number of errors made during the task (7%). Therefore, we speculate that since strong attentional focus was not necessary for the subject to correctly complete the task, no reflex modulation was induced.

According to our results and those discussed above, distracting the subject from a noxious stimulus may produce a differential modulation between the spinally mediated NWR and the perception of pain. Meaning a net facilitation of the NWR but inhibition of perceived pain. From a body protection perspective, it can be speculated that this modulation is advantageous for evasive behavior when a potentially dangerous stimulus is applied to the body. Indeed, the motor response is more rapid (facilitation of spinally mediated responses) while the perception of pain intensity is dampened.

Modulation of the spatial integration of nociception

The exact neural basis behind the spatial integration of nociception remains to be elucidated, challenged by the fact that direct recordings of spinal neurons are not possible in human studies. Indirect evidence has suggested that simultaneous stimuli applied in the sole of the foot in healthy humans are integrated in the spinal cord and produce an enhanced motor response from a functional perspective (Henrich et al., 2020, 2021). The spatial summation of reflexes during simultaneous stimulation in the present investigation is consistent with a spinal integration of spatially distinct nociceptive inputs.

Electrophysiological studies in rats have provided evidence supporting that a set of wide dynamic range neurons (WDR), located in the DDH of the spinal cord, encode the spatial characteristics of the skin area from which a stimulus can trigger a NWR (Schouenborg et al., 1995; Morgan, 1998). The NWR circuit is likely organized in functional modules that allow the recruitment of muscles that would produce the optimal defensive behavior (e.g. flexion/extension of specific joints) while preserving balance (Schouenborg and Kalliomäki, 1990; Andersen et al., 1999; Sandrini et al., 2005). Those WDR neurons likely govern the RRFs in human subjects. For instance, TA and BF were chosen since in baseline conditions TA-RRF is restricted to the medial side of the sole of the foot while BF-RRF extends to the entire sole (Andersen et al., 2001). The extent and spatial sensitivity of the RRF can be dynamically altered by top-down modulation, as it has been shown in studies on spinal transected animals and spinal cord injured patients (Schouenborg et al., 1992; Andersen et al., 2004).

A previous study on human spinal nociception showed that distracting the subject from the noxious stimulation enlarge the RRF of the TA muscle (Bjerre et al., 2011). That dynamic enlargement of the TA-RRF was interpreted as a descending modulation affecting WDR neurons located in the DDH of the spinal cord. The cognitive activity might have rendered the sensitivity of the somatosensory system as a whole, but also shaped

the extension of skin that is covered by neuronal RFs. If the spatial extent of the RRF is indeed modulated by the distraction task, it is likely that multiple stimuli applied within the expanded RRF will be integrated into a larger NWR response. However, the degree of summation when using simultaneous stimulation did not differ with single stimulation in any site. These results might suggest that the spatial integration of simultaneous stimuli *per se* is not under cognitive descending control, and that the modulation is inducing a facilitation of the entire NWR pathway. Moreover, the similar effects observed in TA and BF (Fig. 3) suggest that the cognitive task affect the entire spinal system with no discrimination of spinal segment.

The NWR plays a protective role in the nociceptive system. As such, the spatial characteristics of the RRF of those muscles involved in the reflex are of utmost importance, meaning that the area that is first withdrawn from the noxious stimuli correspond to the stimulated area (Schouenborg et al., 1992, 1994; Andersen et al., 1999; Sandrini et al., 2005; Biurrun Manresa et al., 2014). Since this spatial organization of the reflex has a strong protective role, it might be advantageous from an evolutionary role, that the NWR encoding remains robust despite any cognitive modulation, which might disrupt its protective significance. This is at least partially supported by electrophysiological evidence in rats. A study has shown that DDH neurons that putatively encode the spatial characteristics of the RRF and the muscles of the NWR cannot be antidromically activated from the upper cervical spinal cord (Schouenborg et al., 1995). Although a ceiling effect that limits the summation of the simultaneous stimuli cannot be completely discarded, the most likely explanation of our results seems to be based on the functional modular organization of the NWR.

The direct comparison between the distraction and attention tasks (Figs. 4 and 5) confirmed that cognitive manipulation produces different modulatory effects onto the spinal integration of the NWR. The difference in the magnitude of the NWR between distraction and attention was significant for all stimulation sites in TA (Fig. 4), but only for simultaneous stimulation in BF. We previously argued that the localization test used to manipulate subject's attention was not demanding enough to induce a significant modulation of the NWR, compared to baseline conditions. Since the attention manipulation alone did not effectively modulate the NWR, it seems likely that the observed modulation in the direct comparison was produced by the distracting task.

LIMITATIONS

Animal studies that investigate mechanisms of spinal nociception generally use methodologies involving direct assessment of specific populations of neurons (Le Bars et al., 2001; Mogil, 2009). The direct translation of those findings to human studies is not possible and indirect methodologies to assess spinal nociception must be used. One of such methods is the quantification of the NWR as used in the present study.

To elicit the NWR, stimulation of different nature can be applied in the skin (Mørch et al., 2007). Electrical stimulation has the advantage that the stimulus onset/offset, and the stimulus intensity, can be easily controlled. Electrical stimulation of the skin is not a natural stimulus, and it bypasses the peripheral receptors and artificially depolarizes the cell membrane. Since A and C fibers are simultaneously activated, one can speculate on the partial contribution of different fiber types based on their conduction velocity (Hugon, 1973; Andersen, 2007). The definition of the NWR window in the present project is based on the $A\delta$ fiber conduction velocity. Therefore, the discussion of the results assumes that the observed NWR represents nociceptive processing particularly driven by $A\delta$ fibers. Most C fiber contribution can be excluded due to their slow conduction velocity. On the other hand, faster conducting $A\beta$ fibers cannot be completely discarded. However, it is important to note that the use of small diameter stimulating electrodes, as in the present experiment, seem to be more preferential to $A\delta$ fiber activation (Mørch et al., 2011; Frahm et al., 2013).

The present results indicate that the distraction task induces a pronociceptive state within the spinal cord that facilitates the NWR, regardless of the stimulated skin region and which joints are involved in the reflex. We hypothesized that the distracting task would expand the RRF to cover both stimulation sites (M and L), thus favoring spatial summation of simultaneous stimuli. However, the gain in the magnitude of the NWR when using simultaneous stimulus was not different to single stimulation during the distraction condition. This might be reflecting that the direction of attention to non-somatosensory stimuli has minimal impact on spatial tuning of nociceptive processes. These results also confirm previous evidence of spatial summation within the spinal NWR circuitry. The mechanisms behind spatial integration of nociception in the spinal cord are likely playing a role in encoding/decoding spatial characteristics of pain. For instance, SSP is widely reported in the literature for simultaneous stimulation, and animal studies provided evidence of such spinal mechanisms (Coghill et al., 1993). This study provided novel evidence using the NWR to assess human spinal nociception. Based on the present results and complemented by studies on the perception of pain, it seems that the sensory integration in the spinal cord constitutes the basis of a spinal spatial filtering phenomenon that support the optimal defensive motor behavior and the corresponding perceptual experience. The NWR is then a useful objective tool to complement future research on the effect of descending cognitive modulation on spinal nociception.

REFERENCES

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

Andersen OK, Sonnenborg FA, Arendt-Nielsen L (1999) Modular organization of human leg withdrawal reflexes elicited by electrical stimulation of the foot sole. Muscle Nerve 22:1520–1530.

Andersen OK, Sonnenborg FA, Arendt-Nielsen L (2001) Reflex receptive fields for human withdrawal reflexes elicited by non-

painful and painful electrical stimulation of the foot sole. Clin Neurophysiol 112:641–649.

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

Andersen OK, Spaich EG, Madeleine P, Arendt-Nielsen L (2005) Gradual enlargement of human withdrawal reflex receptive fields following repetitive painful stimulation. Brain Res 1042:194–204.

Arguissain FG, Biurrun Manresa JA, Mørch CD, Andersen OK (2014) Modulatory Effect on Spinal and Supraspinal Responses during Cognitive Attentional and Distraction Tasks. Cham): (Springer. p. 271–277.

Bajaj P, Arendt-Nielsen L, Andersen OK (2005) Facilitation and inhibition of withdrawal reflexes following repetitive stimulation: Electro- and psychophysiological evidence for activation of noxious inhibitory controls in humans. Eur J Pain 9:25–31.

Bantick SJ, Wise RG, Ploghaus A, Clare S, Smith SM, Tracey I (2002) Imaging how attention modulates pain in humans using functional MRI. Brain 125:310–319.

Bartolo M, Serrao M, Gamgebeli Z, Alpaidze M, Perrotta A, Padua L, Pierelli F, Nappi G, Sandrini G (2013) Modulation of the human nociceptive flexion reflex by pleasant and unpleasant odors. Pain 154:2054–2059.

Biurrun Manresa JA, Finnerup NSB, Johannesen IL, Biering-Sørensen F, Jensen TS, Arendt-Nielsen L, Andersen OK (2014) Central sensitization in spinal cord injured humans assessed by reflex receptive fields. Clin. Neurophysiol 125:352–362.

Bjerre L, Andersen AT, Hagelskjær MT, Ge N, Mørch CD, Andersenl OK (2011) Dynamic tuning of human withdrawal reflex receptive fields during cognitive attention and distraction tasks. Eur J Pain 15:816–821.

Chan CWY, Dallaire M (1989) Subjective pain sensation is linearly correlated with the flexion reflex in man. Brain Res 479:145–150.

Clarke RW, Harris J (2004) The organization of motor responses to noxious stimuli. Brain Res Rev 46:163–172.

Cleland CL, Bauer RE (2002) Spatial transformations in the withdrawal response of the tail in intact and spinalized rats. J Neurosci 22:5265–5270.

Coghill RC, Price DD, Hayes RL, Mayer DJ (1991) Spatial distribution of nociceptive processing in the rat spinal cord. J Neurophysiol 65:133–140.

Coghill RC, Mayer DJ, Price DD (1993) The roles of spatial recruitment and discharge frequency in spinal cord coding of pain: a combined electrophysiological and imaging investigation. Pain 53:295–309.

Danziger N, Fournier E, Bouhassira D, Michaud D, De Broucker T, Santarcangelo E, Carli G, Chertock L, Willer JC (1998) Different strategies of modulation can be operative during hypnotic analgesia: a neurophysiological study. Pain 75:85–92.

Defrin R, Peleg S, Weingarden H, Heruti R, Urca G (2007) Differential effect of supraspinal modulation on the nociceptive withdrawal reflex and pain sensation. Clin Neurophysiol 118:427–437.

Devine DP, Spanos NP (1990) Effectiveness of maximally different cognitive strategies and expectancy in attenuation of reported pain. J Pers Soc Psychol 58:672–678.

Fields HL, Bry J, Hentall I, Zorman G (1983) The activity of neurons in the rostral medulla of the rat during withdrawal from noxious heat. J Neurosci 3:2545–2552.

Fragiotta G, Pierelli F, Coppola G, Conte C, Perrotta A, Serrao M (2019) Effect of phobic visual stimulation on spinal nociception. Physiol Behav 206:22–27.

Frahm KS, Jensen MB, Farina D, Andersen OK (2012) Surface EMG crosstalk during phasic involuntary muscle activation in the nociceptive withdrawal reflex. Muscle Nerve 46:228–236.

Frahm KS, Mørch CD, Grill WM, Lubock NB, Hennings K, Andersen OK (2013) Activation of peripheral nerve fibers by electrical stimulation in the sole of the foot. BMC Neurosci 14:1.

France CR, Rhudy JL, McGlone S (2009) Using normalized EMG to define the nociceptive flexion reflex (NFR) threshold: Further evaluation of standardized NFR scoring criteria. Pain 145:211–218.

Fuhrer MJ (1976) Interstimulus interval effects on habituation of flexor withdrawal activity mediated by the functionally transected human spinal cord. Arch Phys Med Rehabil 57:577–582.

Gol A (1967) Relief of pain by electrical stimulation of the septal area. J Neurol Sci 5:115–120.

Grimby L (1963) Normal plantar response: integration of flexor and extensor reflex components. J Neurol Neurosurg Psychiat 26:39–50.

Hadjipavlou G, Dunckley P, Behrens TE, Tracey I (2006) Determining anatomical connectivities between cortical and brainstem pain processing regions in humans: A diffusion tensor imaging study in healthy controls. Pain 123:169–178.

Hayes RL, Bennett GJ, Newlon PG, Mayer DJ (1978) Behavioral and physiological studies of non-narcotic analgesia in the rat elicited by certain environmental stimuli. Brain Res 155:69–90.

Heinricher MM, Tavares I, Leith JL, Lumb BM (2009) Descending control of nociception: Specificity, recruitment and plasticity. Brain Res Rev 60:214–225.

Henrich MC, Frahm KS, Andersen OK (2020) Spinal spatial integration of nociception and its functional role assessed via the nociceptive withdrawal reflex and psychophysical measures in healthy humans. Physiol Rep 8:11–20.

Henrich MC, Frahm KS, Andersen OK (2021) Tempo-spatial integration of nociceptive stimuli assessed via the nociceptive withdrawal reflex in healthy humans. J Neurophysiol 126:373–382.

Hermens HJ, Freriks B, Disselhorst-Klug C, Rau G (2000) Development of recommendations for SEMG sensors and sensor placement procedures. J Electromyogr Kinesiol 10:361–374.

Hubbard CS, Ornitz E, Gaspar JX, Smith S, Amin J, Labus JS, Kilpatrick LA, Rhudy JL, Mayer EA, Naliboff BD (2011) Modulation of nociceptive and acoustic startle responses to an unpredictable threat in men and women. Pain 152:1632–1640.

Hugon M (1973). In: Human Reflexes, Pathophysiology of Motor Systems, Methodology of Human Reflexes. S. Karger AG. p. 713–729.

Jensen MB, Manresa JB, Andersen OK (2015a) A new objective method for acquisition and quantification of reflex receptive fields. Pain 156:555–564.

Jensen MB, Manresa JB, Andersen OK (2015b) Reliable estimation of nociceptive withdrawal reflex thresholds. J Neurosci Methods 253:110–115.

Jure FA, Arguissain FG, Biurrun Manresa JA, Andersen OK (2019) Conditioned pain modulation affects the withdrawal reflex pattern to nociceptive stimulation in humans. Neuroscience 408:259–271.

Jure FA, Arguissain FG, Biurrun Manresa JA, Graven-Nielsen T, Andersen OK (2020) Stimulus predictability moderates the withdrawal strategy in response to repetitive noxious stimulation in humans. J Neurophysiol 123:2201–2208.

Kiernan Brian D, Dane Joseph R, Philips Lawrence H, Price Donald D (1995) Hypnotic analgesia reduces R-III nociceptive reflex: further evidence concerning the multifactorial nature of hypnotic analgesia. Pain 60(1):39–47. https://doi.org/10.1016/0304-3959 (94)00134-Z.

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

Kupers R, Faymonville ME, Laureys S (2005) The cognitive modulation of pain: Hypnosis- and placebo-induced analgesia. Prog Brain Res 150:251–269.

Lannon EW, Jure FA, Andersen OK, Rhudy JL (2021) Does threat enlarge nociceptive reflex receptive fields? J Pain 22:487–497.

Lautenbacher S, Pauli P, Zaudig M, Birbaumer N (1998) Attentional control of pain perception: the role of hypochondriasis. J Psychosom Res 44:251–259.

Le Bars D, Gozariu M, Cadden SW (2001) Animal models of nociception. Pharmacol Rev 53:597–652.

Liebermann DG, Defrin R (2009) Characteristics of the nociceptive withdrawal response elicited under aware and unaware conditions. J Electromyogr Kinesiol 19:e114–e122.

Liebeskind JC, Guilbaud G, Besson J-M, Oliveras J-L (1973) Analgesia from electrical stimulation of the periaqueductal gray matter in the cat: behavioral observations and inhibitory effects on spinal cord interneurons. Brain Res 50:441–446.

Massé-Alarie H, Salomoni SE, Hodges PW (2019) The nociceptive withdrawal reflex of the trunk is organized with unique muscle receptive fields and motor strategies. Eur J Neurosci 50:1932–1947.

Mayer DJ, Liebeskind JC (1974) Pain reduction by focal electrical stimulation of the brain: An anatomical and behavioral analysis. Brain Res 68:73–93.

Mayer DJ, Wolfle TL, Akil H, Carder B, Liebeskind JC (1971) Analgesia from Electrical Stimulation in the Brainstem of the Rat. Science (80- 174:1351–1354.

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

Mogil JS (2009) Animal models of pain: progress and challenges. Nat Rev Neurosci 10:283–294.

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

Mørch CD, Hennings K, Andersen OK (2011) Estimating nerve excitation thresholds to cutaneous electrical stimulation by finite element modeling combined with a stochastic branching nerve fiber model. Med Biol Eng Comput 49:385–395.

Morgan, M.M. (1998). Direct comparison of heat-evoked activity of nociceptive neurons in the dorsal horn with the hindpaw withdrawal reflex in the rat. https://doiorg/101152/Jn199879117479, 174–180.

Müller M, Biurrun Manresa JA, Treichel F, Agten CA, Heini P, Andersen OK, Curatolo M, Jüni P (2016) Discriminative ability of reflex receptive fields to distinguish patients with acute and chronic low back pain. Pain 157:2664–2671.

Neziri AY, Curatolo M, Bergadano A, Petersen-Felix S, Dickenson A, Arendt-Nielsen L, Andersen OK (2009) New method for quantification and statistical analysis of nociceptive reflex receptive fields in humans. J Neurosci Methods 178:24–30.

Oliveras JL, Redjemi F, Guilbaud G, Besson JM (1975) Analgesia induced by electrical stimulation of the inferior centralis nucleus of the raphe in the cat. Pain 1:139–145.

Perrotta A, Bolla M, Anastasio MG, Serrao M, Sandrini G, Pierelli F (2016) Modulation of temporal summation threshold of the nociceptive withdrawal reflex by transcutaneous spinal direct current stimulation in humans. Clin Neurophysiol 127:755–761.

Perrotta A, Chiacchiaretta P, Anastasio MG, Pavone L, Grillea G, Bartolo M, Siravo E, Colonnese C, De Icco R, Serrao M, Sandrini G, Pierelli F, Ferretti A (2017) Temporal summation of the nociceptive withdrawal reflex involves deactivation of posterior cingulate cortex. Eur J Pain 21:289–301.

Price DD, Hayes RL, Ruda M, Dubner R (1978) Spatial and temporal transformations of input to spinothalamic tract neurons and their relation to somatic sensations. J Neurophysiol 41:933–947.

Quevedo AS, Coghill RC (2007) Attentional modulation of spatial integration of pain: Evidence for dynamic spatial tuning. J Neurosci 27:11635–11640.

Quevedo AS, Mørch CD, Andersen OK, Coghill RC (2017) Lateral inhibition during nociceptive processing. Pain 158:1046–1052.

Reynolds DV (1969) Surgery in the rat during electrical analgesia induced by focal brain stimulation. Science (80-) 164:444–445.

Rhudy JL, France CR (2007) Defining the nociceptive flexion reflex (NFR) threshold in human participants: A comparison of different scoring criteria. Pain 128:244–253.

Rhudy JL, Meagher MW (2000) Fear and anxiety: Divergent effects on human pain thresholds. Pain 84:65–75.

Rhudy JL, Williams AE, McCabe KM, Nguyên MATV, Rambo P (2005) Affective modulation of nociception at spinal and supraspinal levels. Psychophysiology 42:579–587.

Rhudy JL, Williams AE, McCabe KM, Russell JL, Maynard LJ (2008) Emotional control of nociceptive reactions (ECON): Do affective valence and arousal play a role? Pain 136:250–261.

Richardson DE, Akil H (1977) Pain reduction by electrical brain stimulation in man. Part 1: Acute administration in periaqueductal and periventricular sites. J Neurosurg 47:178–183.

Roy M, Piché M, Chen J-I, Peretz I, Rainville P (2009) Cerebral and spinal modulation of pain by emotions. Proc Natl Acad Sci U S A 106:20900–20905.

Roy M, Lebuis A, Hugueville L, Peretz I, Rainville P (2012) Spinal modulation of nociception by music. Eur J Pain 16:870–877.

Ruscheweyh R, Kreusch A, Albers C, Sommer J, Marziniak M (2011) The effect of distraction strategies on pain perception and the nociceptive flexor reflex (RIII reflex). Pain 152:2662–2671.

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

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

Schouenborg J, Holmberg H, Weng H-R-R (1992) Functional organization of the nociceptive withdrawal reflexes. II. Changes of excitability and receptive fields after spinalization in the rat. Exp Brain Res 90:469–478.

Schouenborg J, Kalliomäki J, Weng HR (1990) Identification of putative reflex interneurones in the nociceptive withdrawal reflex paths. Acta Physiol Scand 1:25.

Schouenborg J, Kalliomäki J (1990) Functional organization of the nociceptive withdrawal reflexes. Exp Brain Res 83:67–78.

Schouenborg J, Weng H-R, Holmberg H (1994) Modular organization of spinal nociceptive reflexes: a new hypothesis. Physiology 9:261–265.

Schouenborg J, Weng HR, Kalliomäki J, Holmberg H (1995) A survey of spinal dorsal horn neurones encoding the spatial organization of withdrawal reflexes in the rat. Exp Brain Res 106:19–27.

Schumann A, Köhler S, de la Cruz F, Güllmar D, Reichenbach JR, Wagner G, Bär K-J (2018) The use of physiological signals in brainstem/midbrain fMRI. Front Neurosci:718.

Serrao M, Cortese F, Fragiotta G, Pastore AL, Palleschi G, Coppola G, Carbone A, Pierelli F (2014) Bladder filling attenuates spinal cord nociceptive reflexes in humans. Clin Neurophysiol 125:2271–2276.

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

Sonnenborg FA, Andersen OK, Arendt-Nielsen L (2000) Modular organization of excitatory and inhibitory reflex receptive fields elicited by electrical stimulation of the foot sole in man. Clin Neurophysiol 111:2160–2169.

Sprenger C, Stenmans P, Tinnermann A, Büchel C (2018) Evidence for a spinal involvement in temporal pain contrast enhancement. Neuroimage 183:788–799.

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

Terkelsen AJ, Andersen OK, Molgaard H, Hansen J, Jensen TS (2004) Mental stress inhibits pain perception and heart rate variability but not a nociceptive withdrawal reflex. Acta Physiol Scand 180:405–414.

Todd AJ (2010) Neuronal circuitry for pain processing in the dorsal horn. Nat Rev Neurosci 11:823–836.

Tracey I, Mantyh PW (2007) The cerebral signature for pain perception and its modulation. Neuron 55:377–391.

Tracey I, Ploghaus A, Gati JS, Clare S, Smith S, Menon RS, Matthews PM (2002) Imaging attentional modulation of pain in the periaqueductal gray in humans. J Neurosci 22:2748–2752.

Valet M, Sprenger T, Boecker H, Willoch F, Rummeny E, Conrad B, Erhard P, Tolle TR (2004) Distraction modulates connectivity of the cingulo-frontal cortex and the midbrain during pain—an fMRI analysis. Pain 109:399–408.

von Dincklage F, Olbrich H, Baars JH, Rehberg B (2013) Habituation of the nociceptive flexion reflex is dependent on inter-stimulus interval and stimulus intensity. J Clin Neurosci 20:848–850.

Weng HR, Schouenborg J (1996) Cutaneous inhibitory receptive fields of withdrawal reflexes in the decerebrate spinal rat. J Physiol 493:253–265.

Ylioja S, Carlson S, Raij TT, Pertovaara A (2006) Localization of touch versus heat pain in the human hand: A dissociative effect of temporal parameters on discriminative capacity and decision strategy. Pain 121:6–13.

Zachariae R, Andersen OK, Bjerring P, Jørgensen MM, Arendt-Nielsen L (1998) Effects of an opioid antagonist on pain intensity and withdrawal reflexes during induction of hypnotic analgesia in high- and low-hypnotizable volunteers. Eur J Pain 2:25–34.

Zhuo M, Gebhart GF (1992) Characterization of descending facilitation and inhibition of spinal nociceptive transmission from the nuclei reticularis gigantocellularis and gigantocellularis pars alpha in the rat. J Neurophysiol 67:1599–1614.

Zhuo M, Gebhart GF (1997) Biphasic modulation of spinal nociceptive transmission from the medullary raphe nuclei in the rat. J Neurophysiol 78:746–758.