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Stimulus predictability moderates the withdrawal strategy in response to repetitive noxious stimulation in humans

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17 **ABSTRACT**

18

19 Nociceptive withdrawal reflex (NWR) is a protective reaction to a noxious stimulus, resulting in withdrawal of

20 the affected area and thus preventing potential tissue damage. This involuntary reaction consists of neural

21 circuits, biomechanical strategies and muscle activity that ensure an optimal withdrawal. Studies of lower

22 limb NWR indicate that the amplitude of the NWR is highly modulated by extrinsic and intrinsic factors, such

23 as stimulation site, intensity, frequency, and supraspinal activity, among others. Whether the predictability of

24 the stimulus has an effect on the biomechanical strategies is still unclear. This study aimed to evaluate how

25 the predictability of impending noxious stimuli modulate the NWR reaction in the lower limb. NWR was

26 evoked on fifteen healthy participants by trains of electrical stimuli on the sole of the foot and was measured

27 in one distal (tibialis anterior) and one proximal (biceps femoris) muscle. The predictability was manipulated

28 by giving participants prior information about the onset of the stimulus trains and the number of delivered

29 stimuli per train. Results showed that the predictability of the incoming stimuli differentially modulates the

30 muscle activity involved in the NWR reaction. For the most unpredictable stimulus train, larger NWR at distal

31 muscles were evoked. Furthermore, the stereotyped temporal summation profile to repeated stimulation was

32 observed when the stimulus train was completely predictable, while it was disrupted in proximal muscles in

33 unpredictable conditions. It is inferred that the reflex response is shaped by descending control, which

34 dynamically tunes the activity of the muscles involved in the resulting reaction.

35 **NEW & NOTEWORTHY**

36 Innate defensive behaviors such as reflexes are found across all species, constituting preprogrammed

37 responses to external threats that are not anticipated. Previous studies indicated that the excitability of the

38 reflex arcs like spinal NWR pathways in humans are modulated by several cognitive factors. This study

39 assesses how the predictability of a threat affects the biomechanical pattern of the withdrawal response,

40 showing that distal and proximal muscles are differentially modulated by descending control.

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INTRODUCTION

46 The nociceptive withdrawal reflex (NWR) is a spinal reflex in response to a noxious stimulus inducing an

47 involuntary defensive reaction to withdraw the limb and protecting it from potential physical damage (Sandrini

48 et al. 2005). The NWR in humans, is typically assessed by applying an electrical stimulus to either the sural

49 nerve or the skin under the sole of the foot and by measuring the surface electromyographic (EMG)

50 responses from leg muscles (Jensen et al. 2015). The NWR is of special interest in pain research and

51 increasingly used as an objective biomarker of spinal nociceptive excitability and as an indirect measure of

52 pharmacological and non-pharmacological modulation of spinal nociceptive transmission (Von Dincklage et

53 al. 2009; Biurrun Manresa et al. 2011b, 2013; Lim et al. 2011, 2012; Lichtner et al. 2017; Alvisi et al. 2018).

54 The polysynaptic nature of the NWR open for the influence of numerous modulatory sources. Substantial

55 evidence indicates that supraspinal centers control the spinal circuits via a dynamic descending modulation.

56 Descending modulation is exerted via a number of brainstem regions, being the midbrain periaqueductal

57 gray (PAG) and the rostral ventromedial medulla (RVM) the most extensively studied (for review, Heinricher

58 et al. 2009). These descending pathways likely provide the neural substrate by which psychological factors

59 can influence spinal neural circuits. In fact, different cognitive and emotional states have been shown to

60 modulate the NWR (Rhudy et al. 2005; Bjerre et al. 2011; Hubbard et al. 2011; Roy et al. 2011, 2012; Shew

61 et al. 2011; Arsenault et al. 2013; Bartolo et al. 2013). Particularly, the stress and anxiety associated with

62 unpredictable stimuli are typical modulatory factors that can facilitate the NWR (Willer et al. 1979; Hubbard et

63 al. 2011; Quelhas Martins et al. 2015). Moreover, subjects tend to display smaller NWR responses when

64 they are aware of the upcoming stimulus intensity (Quelhas Martins et al. 2015). Similarly, subjects

65 stimulated by temporally predictable stimuli display reflexes that are smaller (Liebermann and Defrin 2009)

66 and habituate faster (Dimitrijevic et al. 1972) than those elicited by temporally unpredictable stimulations. It is

67 however unknown whether the predictability of different stimulus characteristics can have interacting effects

68 on the modulation of the NWR. Secondly, it is unknown how stimulus predictability affects the temporal

69 summation of NWR responses to repetitive stimulation (Arendt-Nielsen et al. 1994; Serrao et al. 2004; Rhudy

70 et al. 2011), since these stimulation paradigms configure a complex interaction between temporal

71 predictability and stimulus intensity.

72 The present study aimed to investigate how stimulus predictability modulates the NWR in response to

73 repetitive noxious stimulation in healthy volunteers. The predictability of the number of repeated stimuli

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74   (resembling changes in intensity) in the train and the predictability of the stimulus train onset (resembling

75   changes in timing) were manipulated during stimulation. It was hypothesized that participants would exhibit

76   larger reflexes when the incoming stimuli were unpredictable both in intensity and time, compared to when

77   they had prior knowledge about the stimulus parameters. Furthermore, it was hypothesized that

78   unpredictable stimuli would disrupt the typical temporal summation of NWR responses that is generally

79   associated to repetitive noxious stimuli.

80

81 ## METHODS

82 ## Subjects

83 The experiment was conducted on fifteen healthy subjects (eight males, mean age: 25 years, range 20-35

84 years). The study protocol was approved by the local ethics committee of Region Nordjylland (case number

85 VN 2015-0038) and performed according with the Helsinki declaration. All subjects gave their written

86 informed consent before participating in the study.

87 ## Electrical Stimulation

88 The NWR was elicited by transcutaneous electrical stimulation, delivered by a computer-controlled electrical

89 stimulator (Noxitest IES 230, Aalborg University, Denmark) through a self-adhesive surface electrode (type

90 700, 20 x 15 mm, Ambu A/S, Denmark), mounted on the arch of the foot. The anode electrode (50 x 90 mm,

91 Pals, Axelgaard Ltd., Fallbrook, California, USA) was placed on the dorsum of the foot to ensure that

92 nociceptors were activated at the arch of the foot (Frahm et al. 2013). Each stimulus consisted of a constant-

93 current burst of five 1-ms pulses delivered at 200 Hz; the subjects perceived each burst as a single stimulus.

94 Stimulation was delivered as trains of three or four of these burst stimuli (see Fig. 1), with a constant inter-

95 stimulus interval (ISI) of 1 s. The inter-train interval length was random, ranging from 10 to 15 s. Stimulation

96 intensity was set to 1.2 times the pain threshold (PTh) to a single stimulus to ensure the presence of

97 reflexes.

98 ## EMG recordings

99 Surface EMG was recorded using a single differential configuration with electrodes (type 720, Ambu A/S,

100 Denmark) placed 2 cm apart over the belly of the tibialis anterior (TA) and biceps femoris (BF) muscles,

101 along the main direction of the muscles fibers. The skin was lightly abraded before the placement of the

102 electrodes in order to decrease the impedance. EMG signals were sampled at 2400 Hz, amplified (up to

103 20000 times), band-pass filtered (5-500 Hz), displayed and stored between 500 ms of pre-stimulus and 6000

104 ms after stimulation onset.

5

Reflex and pain thresholds to electrical stimulation

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106   The NWR threshold (RTh) and the PTh to single stimulation were determined using a standardized staircase

107   procedure. The RTh was obtained by first administering an ascending staircase using steps of 2 mA until a

108   NWR was detected in the TA muscle. Afterwards, the intensity was decreased in steps of 1 mA until the

109   NWR was not elicited. The intensity was again increased in steps of 1 mA until a NWR was detected again.

110   Three ascending and three descending staircases were applied, and the RTh was defined as the average

111   intensity of the last three peaks and troughs. The NWR detection was performed in the TA and it was based

112   on the interval peak z-score criterion (Rhudy and France 2007). The interval peak z-score was calculated as

113   the difference between the peak amplitude in the NWR quantification interval (60 to 180 ms; post-stimulus

114   window) and the baseline mean amplitude (-200 to -80 ms; pre-stimulus window), divided by the standard

115   deviation of the baseline EMG amplitude. A NWR was considered as present if the interval peak z-score of

116   the EMG signal exceeded 12 (France et al. 2009).

117   The PTh was defined using the same staircase procedure, but the criterion to decrease/increase the

118   stimulus intensity was the verbal report of the evoked sensation as being painful/non-painful. The PTh was

119   also defined as the average stimulus intensity of the last three peaks and troughs.

Experimental procedure

120

121   Subjects received a verbal explanation of the experiment while being placed in supine position with back

122   support in 120°, relative to the horizontal level, and with their knees flexed approximately 30°. They were

123   familiarized with electrical stimulation by applying a single stimulus and then 3-4 stimulus trains.

124   Subsequently, the RTh and PTh were determined. The experiment consisted of a single session divided into

125   three stimulation blocks (Fig. 1). The order of the stimulation blocks was randomized across subjects and

126   separated by 5-min breaks.

127   Block A: *Predictable stimulus onset, predictable number of given stimuli per train* (**Error! Reference source**

128   **not found.**, PO-PN). In this block, 12 trains of stimuli were delivered, each train consisting of three stimuli

129   (S1, S2 and S3). Each train was preceded by a single auditory stimulus, signaling the onset of a train. The

130   auditory stimulus consisted of an 800 Hz tone (duration: 20 ms), delivered 1 s before the train onset through

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131 a speaker placed close to the site of the electrical stimulation (i.e. the foot). Subjects were verbally informed

132 that the auditory stimulus cued an incoming train of three identical stimuli.

133 Block B: *Unpredictable stimulus onset, predictable number of repeated stimuli per train* (**Error! Reference**

134 **source not found.**, UO-PN). In this block, 12 trains of stimuli were delivered, each train consisting of three

135 stimuli (S1, S2 and S3). Subjects were verbally informed that they would receive trains of three identical

136 stimuli, but the onset of each train was unpredictable, i.e. no auditory cue.

137 Block C: *Unpredictable stimulus onset, unpredictable number of repeated stimuli per train* (**Error! Reference**

138 **source not found.**, UO-UN). In this block, 24 trains of three or four stimuli were delivered. The fourth

139 stimulus was randomly present in 50% of the trials. The purpose of adding a fourth stimulus to the train was

140 to reflect changes in stimulus intensity, since longer trains are usually reported as more intense and

141 unpleasant (Arendt-Nielsen et al. 1994). Subjects were uncertain about the onset and the number of

142 repeated stimuli of each train. For this experimental condition, only the 12 trials where a train of three stimuli

143 was given were included in the analysis.

144 *- FIGURE 1 -*

145 # Data Analysis

146 The NWR size was quantified by calculating the root-mean-square (RMS) amplitude in the 60 - 180 ms post-

147 stimulus window (Biurrun Manresa et al. 2011a). The RMS was calculated for each stimulus of the train (S1,

148 S2 and S3), for each trial and for each muscle (TA and BF). In total, 3240 RMS values were obtained (3

149 stimuli × 12 trials × 3 conditions × 2 muscles × 15 subjects).

150 # Statistics

151 To examine the effect of unpredictability on the NWR size, two generalized linear mixed models (RMS TA

152 and RMS BF) were designed using SPSS 25.0 (SPSS Inc., Chicago, IL, USA). The models used a gamma

153 distribution and a log link function, since data was non-normally distributed and right skewed. The fixed

154 factors were *condition* (PO-PN, UO-PN and UO-UN), *stimulus number* (S1, S2 and S3) and their interaction.

155 The models controlled for the within-subjects variation by including a random intercept and random slopes

156 for the condition, stimulus number and trial number for each subject. A first-order autoregressive covariance

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157   structures was used, in which all variances are equal and co-variances are highest for adjacent elements

158   and decrease systematically with increasing distance.

159   The model parameters were estimated by maximum pseudo-likelihood, and the criterion for convergence

160   was that the absolute changes of the parameters estimates was less than $1 \cdot 10^{-6}$. The adjusted sequential

161   Sidak test was used for post hoc comparisons.

162   In line with current statistical trends (Wasserstein et al. 2019), no fixed threshold for statistical significance

163   was established. Instead, the results were analyzed in terms of the effect sizes and their experimental

164   relevance.

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166   **RESULTS**

167   **Thresholds to electrical stimulation**

168   All subjects were able to complete the experiment. The mean RTh in the TA muscle was 5.8 mA (range 3.3 -

169   9.5 mA), whereas the mean PTh was 13.8 mA (range 8.0 – 24.5 mA). Subjects reported the stimulation at

170   PTh intensity as a sharp, pinpricking sensation localized at the arch of the foot.

171   **Effects of stimulus predictability on the NWR size**

172   A strong effect of the *condition* was found for the NWR size of the TA muscle ($F_{2, 1611}$ = 8.830, *p* ≤ 0.0005,

173   **Error! Reference source not found.**A). Post hoc analysis revealed that subjects presented a larger NWR

174   size in the TA when the onset and the number of repeated stimuli of the train were unpredictable (UO-UN),

175   compared to the other two blocks (Adj. Sidak, [UO-UN compared with UO-PN] t_{1611} = 3.580, p = 0.001 and

176   [UO-UN compared with PO-PN] t_{1611} = 3.744, p = 0.001). Data suggest that the *stimulus number* ($F_{2,1611}$ =

177   2.839; p = 0.059) or the interaction between *condition* and *stimulus number* ($F_{4,1611}$ = 2.355; p = 0.052) could

178   indicate a small temporal summation effect at TA.

179   A strong effect of *condition* was found for the NWR size of the BF muscle ($F_{2, 1611}$ = 6.241; *p* = 0.002, **Error!**

180   **Reference source not found.**B). Although the *stimulus number* by itself did not show a strong effect on the

181   size of the BF muscle ($F_{2,1611}$ = 1.112, p = 0.329), there was a clear interaction between *condition* and

182   *stimulus number* ($F_{4,1611}$ = 4.592; *p* = 0.001). Post hoc analysis of the interaction revealed that subjects

183   presented a larger NWR size in the BF muscle in response to S1 when the onset of the train was

184   unpredictable (Adj. Sidak, [UO-UN compared with PO-PN], t_{1611} = 5.049, p ≤ 0.0005 and [UO-PN compared

185   with PO-PN] t_{1611} = 2.905, p = 0.01). Additionally, data suggests that, when the stimulation is predictable,

186   there is a temporal summation effect, where the NWR to S3 is larger than the S1 response at the BF muscle

187   (Adj. Sidak, [S3 compared with S1], t_{1611} = 2.342, p = 0.057).

188   *- FIGURE 2 -*

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DISCUSSION

The aim of this study was to evaluate how different degrees of predictability of impending noxious stimuli modulate the involuntary withdrawal reaction of the stimulated limb. For that purpose, healthy participants received trains of electrical stimuli on the sole of the foot to elicit the NWR, which was measured in one distal and one proximal muscle of the ipsilateral limb. In three different stimulation blocks, the predictability of the stimuli was manipulated by giving the participants prior information about the onset of the stimulus trains and the number of given stimuli in each train. When the trains of electrical stimuli were predictable both in time and intensity, participants displayed stereotypical temporal summation profiles of the NWR during the course of the stimulus train, with the last NWR response being larger than the first. This temporal summation effect was disrupted when participants lacked information about the timing of the stimulus trains. Moreover, when the trains of electrical stimuli were neither predictable in time nor in intensity, participants displayed larger NWR in the distal muscle, compared to when the stimulus trains had some degree of predictability.

Dynamic descending control of spinal defensive behaviors

Reflexes are innate defensive behaviors that are found across all members of the species, and which mainly constitute preprogrammed responses to external threats that are not anticipated in time (Ledoux and Daw 2018). Although they are considered more or less hardwired circuits, the excitability of the reflex arcs such as the spinal NWR pathways are subject to strong descending modulation from supraspinal structures Studies in spinalized animals (Schouenborg and Kalliomäki 1990; Schouenborg et al. 1992; Levinsson et al. 1999; Carlson et al. 2005; Bence and Cleland 2019) and in spinal-cord injured human patients (Shahani and Young 1971; Andersen et al. 2004; Hornby et al. 2006; Knikou 2007; Biurrun Manresa et al. 2014; Mackey et al. 2016) provide substantial evidence that a lesion to the spinal cord leads to dysfunctional NWR responses below injury, confirming that descending modulation is vital to maintain the integrity and functional organization of spinal NWR pathways (Dietz 2010).

The descending modulation is dynamic and is mainly transmitted via the PAG in the midbrain and its projections to the RVM. The PAG is a relay area that connect several limbic forebrain regions with the RVM, such as the amygdala, the anterior cingulate cortex, the dorsomedial nucleus of the hypothalamus, and the medial prefrontal cortex (Chen and Heinricher 2019). In anticipation of a forthcoming threat, the neural

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217   activity of these brain areas appears to be dependent on the degree of attention and predictability of the

218   aversive stimuli (Brown et al. 2008; Peng et al. 2019).

219   The descending control of spinal nociception pivots between facilitation and inhibition due to the activation of

220   two classes of RVM neurons called ON and OFF cells respectively, which extend their projections to primary

221   afferent terminals in the dorsal horn (Zhang et al. 2015). This top-down pathway provides a neural substrate

222   by which exteroceptive stimuli and motivational states can modulate spinal transmission (Fields 2018).

223   The balance between inhibition and facilitation highly depends on the behavioral context (Heinricher et al.

224   2009). In particular, when a threat has an element of certainty, descending modulation may provide a

225   mechanism to inhibit the briskness of innate defensive behaviors. In the present study, the results showed

226   that when participants were certain about the incoming noxious stimuli, their overall NWR responses (i.e. the

227   net withdrawal response of the limb) were generally smaller than when they were uncertain about some of

228   the stimuli characteristics. This is in line with previous reports, which found larger NWRs in response to

229   unpredictable stimuli   (Liebermann and Defrin 2009; Quelhas Martins et al. 2015). In contrast, NWR

230   responses tend to be smaller when either the timing (Dimitrijevic et al. 1972; Liebermann and Defrin 2009) or

231   the stimulus intensity (Quelhas Martins et al. 2015) are predictable. Nevertheless, predictable stimuli might

232   not always lead to smaller NWR responses. For instance, when blind-folded subjects receive an auditory cue

233   that indicates the arrival of an incoming stimulus and also the presence of a randomly approaching obstacle,

234   the synaptic transmission through NWR pathways can be facilitated (Michel et al. 2007). In the latter, the

235   certainty about the approaching obstacle given by the auditory cue could have led the participants to an

236   increased state of vigilance that enhanced their preparedness to avoid a threat in an uncertain environment.

237   Taken together, this suggests that the direction of descending modulation associated with stimulus

238   predictability likely depends on the behavioral circumstance in which the stimuli are received. Furthermore,

239   descending modulation triggered by stimulus predictability seems to have a strong modulatory effect on the

240   protective withdrawal associated with the NWR. Other processes that are known to modulate the NWR such

241   as systolic inhibition (Martins et al. 2009) or emotional modulation (Rhudy et al. 2006) are moderated when

242   stimuli are unpredictable. Altogether, the existing evidence suggests that stimulus predictability plays a

243   significant role in the dynamic descending modulation of the NWR pathways.

244   It might be hypothesized that unpredictable stimuli possibly produced an increased state of anxiety. It is well

245   described that the uncertainty of a potential future threat can lead anxious behaviors (Ploghaus et al. 2003).

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246   The brain areas commonly reported to be involved in anxiety-related behaviors are functionally and

247   anatomically interconnected to midbrain and brainstem areas that mediate descending control (Grupe and

248   Nitschke 2013; Tovote et al. 2015). In fact, it has been previously shown that threatening situations with

249   impending, unpredictable painful stimuli enhance the NWR responses measured at the BF muscle (Willer et

250   al. 1979; Hubbard et al. 2011; Terry et al. 2016). Yet, other studies have reported unchanged NWR

251   thresholds in the BF in subjects that had increased levels of acute anxiety (French et al. 2005) or high

252   measures of anxiety sensitivity (Terry et al. 2012). A key difference between these disagreeing findings is the

253   presence / absence of an experimental method to induce anxiety. While the studies that reported a positive

254   association between anxiety and enhanced NWR responses used the anticipation of a very painful stimulus

255   as threat, those that did not find such association only evaluated the state-trait anxiety before and after NWR

256   threshold assessment. In the present study, the psychological state was meant to be modulated by the

257   predictability of repeated trains of painful stimuli. Hence, it could be speculated that there was an induced

258   level of increased anxiety across the experimental blocks. Another important aspect to consider is that

259   previous studies estimated NWR thresholds in the BF in response to sural nerve stimulation. This procedure

260   has a high failure rate of NWR threshold estimation and involves higher stimulation intensities with higher

261   pain ratings, in comparison to foot sole stimulation (Jensen et al. 2015). Noteworthy, previous studies

262   considered only one muscle to describe the psychophysiological modulations of the withdrawal reaction. It is

263   possible that, by considering only one muscle of the kinetic chain, these studies did not observe the

264   differential modulations that may arise across the group of muscles involved in the withdrawal pattern.

265   Effects of stimulus predictability on NWR temporal summation

266   Another fundamental result from this study was the observation that stimulus unpredictability disrupted the

267   temporal summation of NWR responses to repeated stimulation. The characterization of temporal summation

268   of the NWR has important clinical implications, since temporal summation has been extensively proposed as

269   a measurement of central sensitization (Terry et al. 2011). For instance, in chronic pain patients it was

270   proposed that upregulated central integrative pain mechanisms could be one of the mechanisms that may

271   result in facilitated temporal summation of pain (Arendt-Nielsen et al. 2010; Petersen et al. 2017).

272   Interestingly, the typical temporal summation profile described in the literature (Arendt-Nielsen et al. 1994;

273   Guirimand et al. 2000), where the NWR increases gradually in response to repetitive stimuli, was clearly

274   observed only when the stimulus train were predictable in both time and intensity. Contrary to the

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275   established assumptions, subjects displayed larger NWR responses to the first stimulus of the train in the

276   proximal muscle, when the stimulus onset was unpredictable.

277   The role of descending modulation on NWR temporal summation has been highlighted in studies that

278   reported enhanced wind-up in spinalized rodents, compared to spinally intact animals (Gozariu et al. 1997).

279   Similarly, SCI patients also display facilitated NWR temporal summation (Shahani and Young 1971; Hornby

280   et al. 2006). Previous psychophysical studies, however, provide conflicting results. On one hand, studies that

281   investigated the effects of pain catastrophizing (Rhudy et al. 2011) and distraction (Ruscheweyh et al. 2011)

282   reported effects on pain perception but not on temporal summation of the NWR. On the other hand, studies

283   that investigated the mechanisms behind conditioned pain modulation (CPM) reported a concomitant

284   inhibition of both pain perception and NWR responses (Serrao et al. 2004; Lautenbacher et al. 2008). In line

285   with this, reduced pain intensity and NWR size has also been reported for the last part of an extended

286   repetitive stimulus trains (5 seconds), suggesting triggering of descending inhibition (Gozariu et al. 1997;

287   Bajaj et al. 2005). In addition, a recent psychophysical study from Holden and co-workers (Holden et al.

288   2019) have showed that CPM affects only the first stimulus in the temporal summation of pain but not the

289   temporal summation per-se, which is in agreement with the findings of present study.

Towards a differential descending modulation of defensive responses

291   The results showed that the two investigated muscles responded differently to the different levels of stimulus

292   unpredictability. Subjects displayed larger NWR responses in BF, and no noticeable changes in the TA when

293   the stimulus onset was unpredictable but the stimulus intensity was predictable. On the other hand, subjects

294   displayed larger responses on both proximal and distal muscles when both the stimulus onset and the

295   stimulus intensity were unpredictable. Since the modular organization of the NWR considers each muscle or

296   a set of synergistic muscles as isolated components in isolated scenarios, the different behavior displayed by

297   the two muscles under the same experimental conditions in this study cannot be explained only by its

298   modular organization. The biomechanical pattern of the NWR is functionally determined by its contribution to

299   the optimal withdrawal (Schouenborg and Kalliomäki 1990; Andersen et al. 1999). However, there is no

300   evidence on what 'optimal' withdrawal means in more complex scenarios, for example those involving

301   different postures, during locomotion or different degrees of predictability. Particularly, as stated above,

302   different degrees of predictability might affect cortical regions possibly influencing the descending control

303   onto the spinal cord. Studies in animal preparations have indicated that the integration of exteroceptive,

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304   proprioceptive and corticospinal projections possibly occurs in a nodal point at the laminae V and IV of the

305   spinal cord (Granmo et al. 2008; Schouenborg 2008; Tripodi et al. 2011; Levine et al. 2014), and from this

306   nodal point intermediate neurons projects neuronal connections onto the ventral area of the spinal cord

307   (Hilde et al. 2016). Findings from Levine and co-workers (2014) suggest that intermediate premotor neuronal

308   cells, coined motor synergy encoders, excite motor pools in the ventral horn, driving the activation of the

309   muscles involved in the withdrawal reaction to "optimally" disengage the affected area from the noxious

310   stimulus (see Osseward and Pfaff 2019 for a review). In this line of thought, distal and proximal muscles may

311   contribute differently to the optimal pattern. For instance, distal muscles could contribute to subtle

312   movements while proximal muscles ensure a robust withdrawal. Altogether, it could be speculated that there

313   is a differential descending control of the muscles involved in the NWR pattern that could take advantage of

314   its modular organization; this differential modulation across group of muscles would increase the efficacy of

315   the defensive responses to unpredictable threats.

316   To the authors' knowledge, this is the first study in which the influence of the stimulus predictability on the

317   NWR is studied in two muscles simultaneously. Based on the present observations, it is likely that

318   descending modulation acts differently across different muscles. This reinforces the view of the NWR as a

319   complex pattern across several joints, rather than a stereotyped reaction of individual muscles. Hence, it is

320   advisable that future studies consider the inclusion of several muscles when investigating the

321   psychophysiological effects over the NWR.

Limitations and future perspectives

322

323   The present study has some limitations. First, the results were obtained from a healthy population, which

324   may not translate to patients, for instance those with chronic pain. Second, the experimental protocol did not

325   include an assessment of the current psychological state of the participants during the stimulation blocks,

326   which could have helped to make better inferences about the effects of e.g. anxiety. Third, the stimulation

327   intensities used in this study were all above the pain threshold. This could have led to a ceiling effect on the

328   reflex sizes for some of the subjects.

329   The present study also has some implications. The modulatory effects of stimulus predictability can become

330   problematic in clinical examinations of the NWR, where clinicians would like to control these psychological

331   confounding factors to minimize their influence over spinal excitability. These effects pose a practical

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332   compromise since the predictability of the stimuli is a typical parameter that is manipulated to reduce the

333   habituation of the NWR (Dimitrijevic et al. 1972).

334 Conclusions

335   The results of this study suggest that the predictability of different stimulus characteristics differentially

336   affects the muscles involved in the defensive withdrawal reaction. Moreover, stimulus predictability seems to

337   disrupt the stereotyped temporal summation profile in response to repeated stimulation. These observations

338   suggest that the specific response of spinal withdrawal reflexes are strongly influenced by the prior

339   information of an incoming threat, so that the dynamic descending control may increase the efficacy of the

340   defensive responses by differentially modulating the muscle activity involved in the reaction. Finally, these

341   findings have important methodological implications for futures studies in which the NWR is used as a

342   quantitative tool to assess the response of the nociceptive system.

343

344 **ACKNOWLEDGMENTS**

347 **DISCLOSURES**

348 The authors declare that they have no conflict of interest.

349

REFERENCES

350

351 **Alvisi E**, **Serrao M**, **Conte C**, **Alfonsi E**, **Tassorelli C**, **Prunetti P**, **Cristina S**, **Perrotta A**, **Pierelli F**, **Sandrini G**. Botulinum
352 toxin A modifies nociceptive withdrawal reflex in subacute stroke patients. *Brain Behav* 8: 1–10, 2018.

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

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

357 **Arendt-Nielsen L**, **Brennum J**, **Sindrup S**, **Bak P**. Electrophysiological and psychophysical quantification of temporal
358 summation in the human nociceptive system. *Eur J Appl Physiol Occup Physiol* 68: 266–273, 1994.

359 **Arendt-Nielsen L**, **Nie H**, **Laursen MB**, **Laursen BS**, **Madeleine P**, **Simonsen OH**, **Graven-Nielsen T**. Sensitization in
360 patients with painful knee osteoarthritis. *Pain* 149: 573–581, 2010.

361 **Arsenault M**, **Piché M**, **Duncan GH**, **Rainville P**. Self-regulation of acute experimental pain with and without
362 biofeedback using spinal nociceptive responses. *Neuroscience* 231: 102–10, 2013.

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

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

368 **Bence CM**, **Cleland CL**. The nociceptive withdrawal response of the tail in the spinalized rat employs a hybrid
369 categorical–continuous spatial mapping strategy. *Exp Brain Res* 237: 1551–1561, 2019.

370 **Biurrun Manresa JA**, **Finnerup NSB**, **Johannesen IL**, **Biering-Sørensen F**, **Jensen TS**, **Arendt-Nielsen L**, **Andersen OK**.
371 Central sensitization in spinal cord injured humans assessed by reflex receptive fields. *Clin Neurophysiol* 125: 352–62,
372 2014.

373 **Biurrun Manresa JA**, **Jensen MB**, **Andersen OK**. Introducing the reflex probability maps in the quantification of
374 nociceptive withdrawal reflex receptive fields in humans. *J Electromyogr Kinesiol* 21: 67–76, 2011a.

375 **Biurrun Manresa JA**, **Neziri AY**, **Curatolo M**, **Arendt-Nielsen L**, **Andersen OK**. Test-retest reliability of the nociceptive
376 withdrawal reflex and electrical pain thresholds after single and repeated stimulation in patients with chronic low back
377 pain. *Eur J Appl Physiol* 111: 83–92, 2011b.

378 **Biurrun Manresa JA**, **Neziri AY**, **Curatolo M**, **Arendt-Nielsen L**, **Andersen OK**. Reflex receptive fields are enlarged in
379 patients with musculoskeletal low back and neck pain. *Pain* 154: 1318–1324, 2013.

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

382 **Brown CA**, **Seymour B**, **Boyle Y**, **El-Deredy W**, **Jones AKP**. Modulation of pain ratings by expectation and uncertainty:
383 Behavioral characteristics and anticipatory neural correlates. *Pain* 135: 240–250, 2008.

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

386 **Chen QL**, **Heinricher MM**. Descending Control Mechanisms and Chronic Pain. *Curr Rheumatol Rep* 21: 1–7, 2019.

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

388 **Dimitrijevic MR**, **Faganel J**, **Gregoric M**, **Nathan PW**, **Trontelj JK**. Habituation: effects of regular and stochastic
389 stimulation. *J Neurol Neurosurg Psychiatry* 35: 234–242, 1972.

390 **Von Dincklage F**, **Send K**, **Hackbarth M**, **Rehberg B**, **Baars JH**. Comparison of the nociceptive flexion reflex threshold
391 and the bispectral index as monitors of movement responses to noxious stimuli under propofol mono-anaesthesia. *Br*
392 *J Anaesth* 102: 244–250, 2009.

393 **Fields HL**. How expectations influence pain. *Pain* 159: S3–S10, 2018.

17

394 **Frahm KS**, **Mørch C**, **Grill WM**, **Lubock NB**, **Hennings K**, **Andersen OK**. Activation of peripheral nerve fibers by
395 electrical stimulation in the sole of the foot. *BMCNeurosci* 14:116. do: 114–116, 2013.

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

398 **French DJ**, **France CR**, **France JL**, **Arnott LF**. The influence of acute anxiety on assessment of nociceptive flexion reflex
399 thresholds in healthy young adults. *Pain* 114: 358–363, 2005.

400 **Gozariu M**, **Bragard D**, **Willer J**, **Le Bars D**. Temporal Summation of C-Fiber Afferent Inputs: Competition Between
401 Facilitatory and Inhibitory Effects on C-Fiber Reflex in the Rat. *J Neurophysiol* 78: 3165–3179, 1997.

402 **Granmo M**, **Petersson P**, **Schouenborg J**. Action-based body maps in the spinal cord emerge from a transitory floating
403 organization. *J Neurosci* 28: 5494–5503, 2008.

404 **Grillon C**, **Baas JMP**, **Cornwell B**, **Johnson L**. Context Conditioning and Behavioral Avoidance in a Virtual Reality
405 Environment : Effect of Predictability. (2006). doi: 10.1016/j.biopsych.2006.03.072.

406 **Grupe DW**, **Nitschke JB**. Uncertainty and anticipation in anxiety: An integrated neurobiological and psychological
407 perspective. *Nat Rev Neurosci* 14: 488–501, 2013.

408 **Guirimand F**, **Dupont X**, **Brasseur L**, **Chauvin M**, **Bouhassira D**. The Effects of Ketamine on the Temporal Summation
409 (Wind-Up) of the RIII Nociceptive Flexion Reflex and Pain in Humans. *Anesth Analg* 90: 408, 2000.

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

412 **Hilde KL**, **Levine AJ**, **Hinckley CA**, **Hayashi M**, **Montgomery JM**, **Gullo M**, **Driscoll SP**, **Grosschedl R**, **Kohwi Y**, **Kohwi-**
413 **Shigematsu T**, **Pfaff SL**. Satb2 Is Required for the Development of a Spinal Exteroceptive Microcircuit that Modulates
414 Limb Position. *Neuron* 91: 763–776, 2016.

415 **Holden S**, **Petersen KK**, **Arendt-Nielsen L**, **Graven-Nielsen T**. Conditioning pain modulation reduces pain only during
416 the first stimulation of the temporal summation of pain paradigm in healthy participants. *Eur J Pain (United Kingdom)* :
417 1390–1396, 2019.

418 **Hornby TG**, **Rymer WZ**, **Benz EN**, **Schmit BD**. Windup of Flexion Reflexes in Chronic Human Spinal Cord Injury: A
419 Marker for Neuronal Plateau Potentials? *J Neurophysiol* 89: 416–426, 2006.

420 **Hubbard CS**, **Ornitz E**, **Gaspar JX**, **Smith S**, **Amin J**, **Labus JS**, **Kilpatrick LA**, **Rhudy JL**, **Mayer EA**, **Naliboff BD**.
421 Modulation of nociceptive and acoustic startle responses to an unpredictable threat in men and women. *Pain* 152:
422 1632–1640, 2011.

423 **Jensen MB**, **Biurrun Manresa JA**, **Andersen OK**. Reliable estimation of nociceptive withdrawal reflex thresholds. *J*
424 *Neurosci Methods* 253: 110–115, 2015.

425 **Knikou M**. Plantar cutaneous input modulates differently spinal reflexes in subjects with intact and injured spinal cord.
426 *Spinal Cord* 45: 69–77, 2007.

427 **Lautenbacher S**, **Kunz M**, **Burkhardt S**. The effects of DNIC-type inhibition on temporal summation compared to single
428 pulse processing: Does sex matter? *Pain* 140: 429–435, 2008.

429 **Ledoux J**, **Daw ND**. Surviving threats: Neural circuit and computational implications of a new taxonomy of defensive
430 behaviour. *Nat Rev Neurosci* 19: 269–282, 2018.

431 **Levine AJ**, **Hinckley CA**, **Hilde KL**, **Driscoll SP**, **Poon TH**, **Montgomery JM**, **Pfaff SL**. Identification of a cellular node for
432 motor control pathways. *Nat Neurosci* 17: 586–593, 2014.

433 **Levinsson A**, **Luo X-L**, **Holmberg H**, **Schouenborg J**. Developmental Tuning in a Spinal Nociceptive System: Effects of
434 Neonatal Spinalization. *J Neurosci* 19: 10397–10403, 1999.

435 **Lichtner G**, **Rehberg B**, **Jakuscheit A**, **Weth J**, **Jurth C**, **von Dincklage F**. Intraoperative monitoring of analgesia using
436 nociceptive reflexes correlates with delayed extubation and immediate postoperative pain. *Eur J Anaesthesiol* 34:
437 297–305, 2017.

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

440 **Lim ECW**, **Sterling M**, **Pedler A**, **Coombes BK**, **Vicenzino B**. Evidence of spinal cord hyperexcitability as measured with

18

441 nociceptive flexion reflex (NFR) threshold in chronic lateral epicondylalgia with or without a positive neurodynamic
442 test. *J Pain* 13: 676–684, 2012.

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

445 **Mackey AS**, **Uttaro D**, **McDonough MP**, **Krivis LI**, **Knikou M**. Convergence of flexor reflex and corticospinal inputs on
446 tibialis anterior network in humans. *Clin Neurophysiol* 127: 706–715, 2016.

447 **Martins AQ**, **Ring C**, **McIntyre D**, **Edwards L**, **Martin U**. Effects of unpredictable stimulation on pain and nociception
448 across the cardiac cycle. *Pain* 147: 84–90, 2009.

449 **Michel J**, **Van Hedel HJA**, **Dietz V**. Facilitation of spinal reflexes assists performing but not learning an obstacle-
450 avoidance locomotor task. *Eur J Neurosci* 26: 1299–1306, 2007.

451 **Osseward PJ**, **Pfaff SL**. Cell type and circuit modules in the spinal cord. *Curr Opin Neurobiol* 56: 175–184, 2019.

452 **Peng W**, **Huang X**, **Liu Y**, **Cui F**. Predictability modulates the anticipation and perception of pain in both self and others.
453 *Soc Cogn Affect Neurosci* 14: 747–757, 2019.

454 **Petersen KK**, **Arendt-Nielsen L**, **Finocchietti S**, **Hirata RP**, **Simonsen O**, **Laursen MB**, **Graven-Nielsen T**. Age
455 Interactions on Pain Sensitization in Patients with Severe Knee Osteoarthritis and Controls. *Clin J Pain* 33: 1081–1087,
456 2017.

457 **Ploghaus A**, **Becerra L**, **Borras C**, **Borsook D**. Neural circuitry underlying pain modulation: Expectation, hypnosis,
458 placebo. *Trends Cogn Sci* 7: 197–200, 2003.

459 **Quelhas Martins A**, **Mcintyre D**, **Ring C**. Aversive event unpredictability causes stress-induced hypoalgesia.
460 *Psychophysiology* 52: 1066–1070, 2015.

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

463 **Rhudy JL**, **Martin SL**, **Terry EL**, **France CR**, **Bartley EJ**, **DelVentura JL**, **Kerr KL**. Pain catastrophizing is related to
464 temporal summation of pain but not temporal summation of the nociceptive flexion reflex. *Pain* 152: 794–801, 2011.

465 **Rhudy JL**, **Williams AE**, **McCabe KM**, **Nguyên MAT V**, **Rambo P**. Affective modulation of nociception at spinal and
466 supraspinal levels. *Psychophysiology* 42: 579–587, 2005.

467 **Rhudy JL**, **Williams AE**, **McCabe KM**, **Rambo PL**, **Russell JL**. Emotional modulation of spinal nociception and pain: The
468 impact of predictable noxious stimulation. *Pain* 126: 221–233, 2006.

469 **Roy M**, **Lebuis a**, **Hugueville L**, **Peretz I**, **Rainville P**. Spinal modulation of nociception by music. *Eur J Pain* 16: 870–7,
470 2012.

471 **Roy M**, **Lebuis A**, **Peretz I**, **Rainville P**. The modulation of pain by attention and emotion: a dissociation of perceptual
472 and spinal nociceptive processes. *Eur J Pain* 15: 641.e1–10, 2011.

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

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

477 **Schouenborg J**. Action-based sensory encoding in spinal sensorimotor circuits. *Brain Res Rev* 57: 111–117, 2008.

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

480 **Schouenborg J**, **Kalliomäki J**. Functional organization of the nociceptive withdrawal reflexes. I. Activation of hindlimb
481 muscles in the rat. *Exp Brain Res* 83: 67–78, 1990.

482 **Serrao M**, **Rossi P**, **Sandrini G**, **Parisi L**, **Amabile GA**, **Nappi G**, **Pierelli F**. Effects of diffuse noxious inhibitory controls
483 on temporal summation of the RIII reflex in humans. *Pain* 112: 353–360, 2004.

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

485 **Shew WL**, **Yang H**, **Yu S**, **Roy R**, **Plenz D**. Information capacity and transmission are maximized in balanced cortical
486 networks with neuronal avalanches. *J Neurosci* 31: 55–63, 2011.
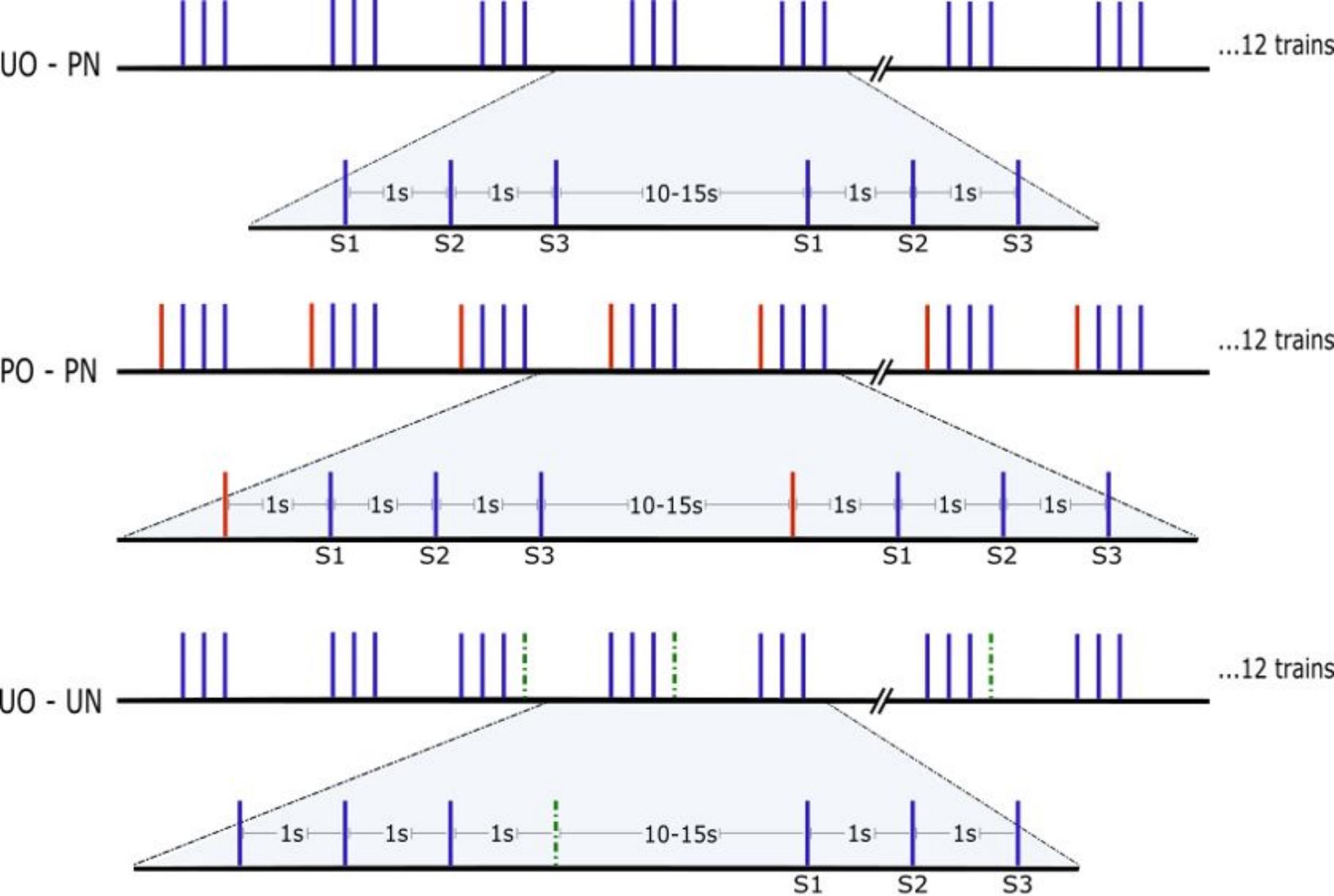
19

487 **Terry EL**, **France CR**, **Bartley EJ**, **DelVentura JL**, **Kerr KL**, **Vincent AL**, **Rhudy JL**. Standardizing procedures to study
488 sensitization of human spinal nociceptive processes: Comparing parameters for temporal summation of the
489 nociceptive flexion reflex (TS-NFR). *Int J Psychophysiol* 81: 263–274, 2011.

490 **Terry EL**, **Kerr KL**, **DelVentura JL**, **Rhudy JL**. Anxiety Sensitivity Does Not Enhance Pain Signaling at the Spinal Level. *Clin*
491 *J Pain* 28: 505–510, 2012.

492 **Terry EL**, **Thompson KA**, **Rhudy JL**. Does pain catastrophizing contribute to threat-evoked amplification of pain and
493 spinal nociception? *Pain* 157: 456–465, 2016.

494 **Tovote P**, **Fadok JP**, **Lüthi A**. Neuronal circuits for fear and anxiety. *Nat Rev Neurosci* 16: 317–31, 2015.

495 **Tripodi M**, **Stepien AE**, **Arber S**. Motor antagonism exposed by spatial segregation and timing of neurogenesis. *Nature*
496 479: 61–66, 2011.

497 **Wasserstein RL**, **Schirm AL, Lazar NA**. Moving to a World Beyond " p < 0.05." *Am Stat* 73: 1–19, 2019.

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

500 **Zhang Y**, **Zhao S**, **Rodriguez E**, **Takatoh J**, **Han BX**, **Zhou X**, **Wang F**. Identifying local and descending inputs for primary
501 sensory neurons. *J Clin Invest* 125: 3782–3794, 2015.
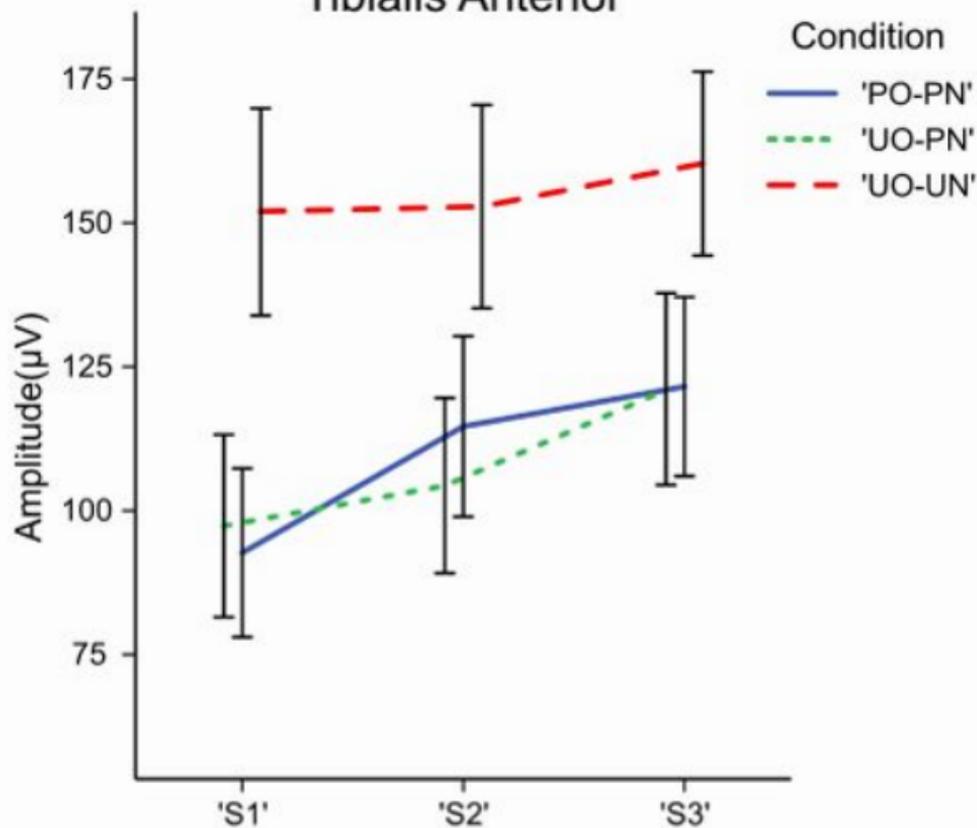
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505 **FIGURE CAPTIONS**

506 Fig. 1. Experimental design. Each stimulation block corresponded to a different experimental condition. The order of

507 blocks was randomized across subjects. Details are explained in the text.

508

509 Fig. 2. Averaged root-mean squared nociceptive withdrawal reflex (NWR) responses to the three stimuli of the train, for

510 the three different experimental conditions. The NWR responses were measured at the tibialis anterior muscle (A) and

511 biceps femoris muscle (B). (PO-PN: *predictable stimulus onset, predictable number of given stimuli per train;* UO-PN:

512 *unpredictable stimulus onset, predictable number of repeated stimuli per train;* UO-UN*: unpredictable stimulus onset,*

513 *unpredictable number of repeated stimuli per train).* Error bars represent 95% confidence intervals.

21

A

Tibialis Anterior

Condition
— 'PO-PN'
····· 'UO-PN'
- - - 'UO-UN'

Amplitude(μV)

175
150
125
100
75

'S1'   'S2'   'S3'

B

Biceps Femoris

Amplitude(μV)

40
35
30
25
20
15

'S1'   'S2'   'S3'

Stimulus