
Research Articles: Behavioral/Cognitive

Somatotopic representation of second pain in the primary somatosensory cortex of humans and rodents

Qq Jin^{a,b}, Gq Wu^{a,b}, Ww Peng^c, Xi Xia^{a,b}, L Hu^{a,b} and Gd Iannetti^d

^aCAS Key Laboratory of Mental Health, Institute of Psychology, Beijing, 100101, China

^bDepartment of Psychology, University of Chinese Academy of Sciences, Beijing, 100049, China

^cBrain Function and Psychological Science Research Center, Shenzhen University, Shenzhen, 518060, China

^dDepartment of Neuroscience, Physiology and Pharmacology, University College London, WC1E 6BT, UK.

DOI: 10.1523/JNEUROSCI.3654-17.2018

Received: 13 December 2017

Revised: 26 February 2018

Accepted: 7 April 2018

Published: 21 May 2018

Author contributions: Q.J., G.W., W.P., X.X., and L.H. performed research; Q.J., G.W., W.P., X.X., L.H., and G.I. analyzed data; Q.J., L.H., and G.I. wrote the paper; L.H. and G.I. designed research.

Conflict of Interest: The authors declare no competing financial interests.

LH is supported by the National Natural Science Foundation of China (31471082 and 31671141) and the Scientific Foundation project of Institute of Psychology, Chinese Academy of Sciences (Y6CX021008). GDI is supported by the Wellcome Trust (PAIN JLARXR) and a Consolidator Grant of the European Research Council (PAINSTRAT). GDI is additionally supported by a fellowship at the Paris Institute for Advanced Studies (France).

Corresponding author: Dr Li Hu, Key Laboratory of Mental Health, Institute of Psychology, Chinese Academy of Sciences, Beijing, China, 100101, Tel: +86 18084053555; Fax: +86-10-84249369, E-mail: huli@psych.ac.cn

Cite as: J. Neurosci ; 10.1523/JNEUROSCI.3654-17.2018

Alerts: Sign up at www.jneurosci.org/cgi/alerts to receive customized email alerts when the fully formatted version of this article is published.

Accepted manuscripts are peer-reviewed but have not been through the copyediting, formatting, or proofreading process.

Copyright © 2018 Jin et al.

This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license, which permits unrestricted use, distribution and reproduction in any medium provided that the original work is properly attributed.

Somatotopic representation of second pain in the primary somatosensory cortex of humans and rodents

QQ Jin^{a,b,*}, GQ Wu^{a,b,*}, WW Peng^c, XL Xia^{a,b}, L Hu^{a,b,#}, GD Iannetti^{d,#}

^aCAS Key Laboratory of Mental Health, Institute of Psychology, Beijing, 100101, China; ^bDepartment of Psychology, University of Chinese Academy of Sciences, Beijing, 100049, China; ^cBrain Function and Psychological Science Research Center, Shenzhen University, Shenzhen, 518060, China; ^dDepartment of Neuroscience, Physiology and Pharmacology, University College London, WC1E 6BT, UK.

* These authors contributed equally; # Shared senior authorship.

Abbreviated title: S1 somatotopy to C-fiber input in humans and rats

Corresponding author:

Dr Li Hu

Key Laboratory of Mental Health, Institute of Psychology,

Chinese Academy of Sciences, Beijing, China, 100101

Tel: +86 18084053555; Fax: +86-10-84249369

E-mail: huli@psych.ac.cn

Number of pages: 57.

Number of figures and tables: Figures (9), Tables (3).

Number of words: Abstract: (237), Introduction (642), Discussion (1455).

Conflict of Interest: The authors declare no competing financial interests.

Acknowledgements: LH is supported by the National Natural Science Foundation of China (31471082 and 31671141) and the Scientific Foundation project of Institute of Psychology, Chinese Academy of Sciences (Y6CX021008). GDI is supported by the Wellcome Trust (PAIN JLARXR) and a Consolidator Grant of the European Research Council (PAINSTRAT). GDI is additionally supported by a fellowship at the Paris Institute for Advanced Studies (France).

Abstract

There is now compelling evidence that selective stimulation of A δ -nociceptors elicits robust responses in the primary somatosensory cortex (S1). However, whether the C-fiber nociceptive input eliciting second pain has an organized projection to S1 remains an open question. Here we recorded the electrocortical responses elicited by nociceptive-specific laser stimulation of the four limbs in 202 humans (both males and females, using electroencephalography [EEG]) and 12 freely-moving rats (all males, using electrocorticography [ECoG]). Topographical analysis and source modeling revealed a clear gross somatotopy of the unmyelinated C-fiber input within the S1 contralateral to the stimulated side, in both species. In the human EEG, S1 activity could be isolated as an early-latency negative deflection (C-N1 wave at 710-730 ms) following hand stimulation, but not following foot stimulation because of the spatio-temporal overlap with the subsequent large-amplitude supramodal vertex waves (C-N2/P2). In contrast, because of the across-species difference in the representation of the body surface within S1, in rat ECoG, S1 activity could be isolated as a C-N1 following both forepaw and hindpaw stimulation. Finally, we observed a functional dissociation between the generators of the somatosensory-specific lateralized waves (C-N1) and those of the supramodal vertex waves (C-N2/P2), indicating that C-fiber unmyelinated input is processed in functionally distinct somatosensory and multimodal cortical areas. These findings demonstrated that C-fiber input conveys information about the spatial location of noxious stimulation across the body surface – a prerequisite for deploying an appropriate defensive motor repertoire.

65

66 **Significance Statement**

67 Unmyelinated C-fibers are the evolutionarily oldest peripheral afferents
68 responding to noxious environmental stimuli. Whether C-fiber input conveys
69 information about the spatial location of the noxious stimulation to the primary
70 somatosensory cortex (S1) remains an open issue. In this study, C-fibers were
71 activated by radiant heat stimuli delivered to different parts of the body in both
72 humans and rodents, while electrical brain activity was recorded. In both
73 species the C-fiber peripheral input projects to different parts of the
74 contralateral S1, coherently with the representation of the body surface within
75 this brain region. These findings demonstrate that C-fiber input conveys
76 information about the spatial location of noxious stimulation across the body
77 surface – a prerequisite for deploying an appropriate defensive motor
78 repertoire.

79

Introduction

Because of the different conduction velocity of A δ (~15 m/s) and C (~1 m/s) afferents, a single and intense cutaneous nociceptive stimulus elicits a typical double sensation: an initial A δ -fiber-related pricking 'first' pain is followed by a C-fiber-related burning 'second' pain (Lewis and Pochin, 1937; Basbaum and Bushnell, 2009).

In humans, the brain electrical responses elicited by laser heat pulses that concomitantly activate A δ - and C-fiber endings in the superficial skin layers (Bromm and Treede, 1984) show clear deflections at latencies compatible with the conduction velocity of A δ -fibers (A δ -LEPs or *late* LEPs; Treede et al., 1988). The largest deflection is a negative–positive complex (N2–P2) maximal at the scalp vertex, peaking at ~200–350 ms when stimulating the hand dorsum (Bromm and Treede, 1984). This N2-P2 complex is functionally similar to the vertex potentials elicited by sudden stimuli belonging to non-nociceptive sensory modalities (Mouraux and Iannetti, 2009), and largely reflects supramodal saliency-related neural processes, possibly consequent to the detection of behaviorally-relevant changes in the sensory environment (Downar et al., 2000). The N2-P2 vertex complex is preceded by a smaller negative deflection (N1) which, when stimulating the hand dorsum, peaks at ~160 ms and is maximal over the central-temporal region contralateral to the

stimulated side (Tarkka and Treede, 1993). The early contralateral N1 wave of the A δ -LEPs reflects somatosensory-specific activity, more related to the magnitude of the incoming nociceptive input (Lee et al., 2009). This somatosensory-specific activity is likely to subserve the high spatial acuity for first pain (Mancini et al., 2013), as indicated by the existence of fine-grained maps of A δ nociceptive input to individual digits in the human primary somatosensory cortex (Mancini et al., 2012).

In contrast, the EEG deflections related to the activation of C-fibers (C-LEPs or *ultralate* LEPs; Iannetti et al., 2003) are more difficult to detect. C-LEPs have initially been suggested to appear only when the concomitant activation of A δ -fibers is avoided or reduced (Bromm and Treede, 1983; Bragard et al., 1996; Mouraux et al., 2003), because the low intensity and high temporal predictability of the C-fiber input make it less salient than the preceding A δ -input (Iannetti et al., 2008; Mouraux and Iannetti, 2009; Ronga et al., 2013). However, we recently showed that by (1) applying advanced signal processing techniques (such as peak alignment and time-frequency decomposition) and (2) using optimal stimulus parameters (such as delivering a high number of stimuli of multiple energies within a small skin territory), the N2-P2 vertex complex can be reliably detected in single-subject C-LEPs, even when preceded by A δ -LEPs (Hu et al., 2014b).

124 Different from humans, A δ afferents are virtually insensitive to heat in rodents
125 (Lynn and Shakhaneh, 1988; Shim et al., 2005). Therefore, the same laser
126 heat pulses that coactivate A δ and C-fiber endings in primates (Treede et al.,
127 1998; Magerl et al., 1999) almost uniquely activate C-fiber afferents when
128 delivered to the rat skin (Sikandar et al., 2013; Hess, 2015; Hu et al., 2015).
129 Consequently, both pain-related behavior and central responses elicited by
130 laser pulses in rats occur at latencies only compatible with the activation of
131 C-fiber primary afferents (Sikandar et al., 2013; Xia et al., 2016).

132
133 Here we tested whether the C-fiber input has a somatotopically-organized
134 projection to the primary somatosensory cortex, in humans and rodents. We
135 recorded 64-channel EEG responses elicited by laser stimulation of the four
136 limbs in 202 healthy participants. We also recorded 14-channel
137 electrocorticographic (ECoG) responses elicited by the same laser stimulation
138 of the four paws, in 12 awake and freely-moving rats. We used topographical
139 analysis and source modeling both at group and single-subject level to test the
140 working hypothesis. Furthermore, the concomitant presence of both A δ - and
141 C-LEPs in human recordings allowed us to perform a comprehensive
142 correlation analysis of the different deflections. With this analysis we explored
143 functional relationships between somatosensory-specific and supramodal
144 generators of the brain responses evoked by C-fiber input.

Materials and methods

Experiment 1: Human EEG during hand stimulation

Subjects

EEG data were collected from 107 healthy subjects (67 females) aged 21.6 ± 1.8 years (mean $\pm$ SD, range = 18 to 26 years). All subjects gave their written informed consent and were paid for their participation. The local ethics committee approved the experiment procedures.

Nociceptive stimulation

Nociceptive-specific radiant-heat stimuli were generated by an infrared neodymium yttrium aluminum perovskite (Nd:YAP) laser with a wavelength of $1.34 \mu\text{m}$ (Electronical Engineering, Italy). At this wavelength, laser pulses activate directly epidermal nociceptive terminals belonging to A δ and C primary nociceptors (Iannetti et al., 2003). Laser pulses were delivered at the dorsum of left and right hands, on a squared area ($5 \times 5 \text{ cm}^2$) defined prior to the beginning of the experimental session. A He–Ne laser pointed to the area to be stimulated. The laser pulse was transmitted via an optic fiber and its diameter was set at approximately 7 mm ($\sim 38 \text{ mm}^2$) by focusing lenses. The duration of the laser pulse was 4 ms. To allow for passive skin cooling and avoid nociceptor fatigue or sensitization, the laser beam target was shifted by

167 approximately 1 cm in a random direction after each stimulus. After each
168 stimulus, subjects were instructed to rate the intensity of perceived pain on a
169 0-10 numerical rating scale (NRS), with 0 standing for “no sensation” and 10
170 standing from “pain as bad as it could possibly be”.

171

172 *Experimental design*

173 Before EEG data collection, for each subject we identify the four laser energies
174 that evoked subjective pain ratings of ~2, ~4, ~6, and ~8 on the 0-10 NRS, by
175 increasing the stimulus energy in steps of 0.25 J until the defined sensation
176 was elicited. Across the group, these energies were E1: 2.0 ± 0.2 J; E2: $2.7 \pm$
177 0.3 J; E3: 3.4 ± 0.3 J; E4: 4.1 ± 0.4 J. The experiment consisted of two blocks.
178 In each block, laser stimuli were delivered to the dorsum of either the left or the
179 right hand. In each block, 10 laser pulses of each stimulus energy were
180 delivered, for a total of 40 pulses per stimulation side (80 pulses in total). The
181 order of blocks was counterbalanced across subjects, and the order of
182 stimulus energies was pseudo-randomized. The surface temperature of the
183 stimulated hand dorsum was measured before each block using an infrared
184 thermometer, to ensure that the difference between the two blocks was
185 never >1 °C (Iannetti et al., 2004; Baumgartner et al., 2005). This temperature
186 was 33.3 ± 1.1 °C and 33.4 ± 1.1 °C before each block, respectively
187 ($t(106)=-0.87$, $P=0.39$, paired sample t-test). An auditory tone was delivered
188 randomly between 3 and 6 s after the laser stimulation, to prompt subjects to

189 rate the intensity of pain perception elicited by the laser stimulus on the same
190 0-10 NRS. The inter-stimulus interval varied randomly between 11 and 15 s
191 using a rectangular distribution.

192

193 *EEG recording*

194 Subjects were seated in a comfortable chair in a silent, temperature-controlled
195 room, and wore protective goggles. They were asked to focus their attention
196 on the stimuli, relax their muscles, and keep their eyes open and gaze slightly
197 downward. EEG data were recorded using 64 Ag-AgCl electrodes placed on
198 the scalp according to the International 10-20 system, using the nose as
199 reference (Brain Products GmbH; pass band: 0.01–100 Hz; sampling rate:
200 1000 Hz). Electrode impedances were kept <10 k Ω . To monitor ocular
201 movements and eye blinks, electro-oculographic (EOG) signals were
202 simultaneously recorded from two surface electrodes, one placed over the
203 lower eyelid, the other placed 1 cm lateral to the outer corner of the orbit.

204

205 *EEG data analysis*

206 EEG data were processed using EEGLAB (Delorme and Makeig, 2004), an
207 open source toolbox running in the MATLAB environment. Continuous EEG
208 data were band-pass filtered between 1 and 30 Hz. EEG epochs were
209 extracted using a window analysis time of 3000 ms (1000 ms pre-stimulus and
210 2000 ms post-stimulus) and baseline corrected using the pre-stimulus interval.

211 Based on visual inspection, trials contaminated by artifacts due to gross
212 movements were removed. In addition, trials contaminated by eye-blinks and
213 movements were corrected using an independent component analysis (ICA)
214 algorithm (Delorme and Makeig, 2004). In all datasets, these independent
215 components (ICs) had a large EOG channel contribution and a frontal scalp
216 distribution. After preprocessing, EEG data were re-referenced to the common
217 average.

218

219 Across four stimulus energies (E1-E4), epochs belonging to the same
220 stimulation side (left or right hand: LH or RH) were averaged, time-locked to
221 stimulus onset, thus yielding two average waveforms for each subject. Peak
222 latencies and amplitudes of A δ -N2, A δ -P2, C-N2, and C-P2 waves were
223 measured from single-subject average waveforms (Cz-average) of each
224 subject. A δ -N2 and A δ -P2 waves were defined as the most negative and
225 positive deflections between 150 and 500 ms after stimulus onset (Kunde and
226 Treede, 1993; Tu et al., 2016). C-N2 and C-P2 waves were defined as the
227 most negative and positive deflections between 700 and 1300 ms after
228 stimulus onset (Hu et al., 2014b). Single-subject average waveforms were
229 subsequently averaged to obtain group-level waveforms. Group-level scalp
230 topographies at the peak latency of the A δ -N2, A δ -P2, C-N2, and C-P2 waves
231 were computed by spline interpolation.

232

233 *Statistical analysis*

234 To explore the spatial differences between the response elicited by the
235 stimulation of the left and right hands, we plotted the scalp topographies of the
236 earliest part of A δ -LEPs (i.e., from 150 to 170 ms, corresponding to the A δ -N1)
237 and of the C-LEPs (i.e., from 710 to 730 ms, corresponding to the C-N1), in
238 steps of 10 ms. To test whether these earliest responses were lateralized with
239 respect to the stimulated hand, we compared single-subject LEP waveforms
240 elicited by the stimulation of the left and right hand, using the following
241 procedure (Hu et al., 2014a). First, for each subject and each stimulation side,
242 average LEP waveforms were normalized and expressed as Z values by
243 subtracting from each time point the mean amplitude of the waveform, and
244 then dividing the resulting value by the standard deviation of the waveform
245 amplitude. Second, a point-by-point paired sample t-test was used to compare
246 LEP responses elicited by stimulation of the left and right hand dorsum, which
247 yielded a time course of P values, representing the significance of the
248 difference between LEPs elicited by left and right hand stimulation, for each
249 electrode. Third, to account for multiple comparisons of different time points
250 and electrodes, a false discovery rate (FDR; Benjamini and Hochberg, 1995)
251 procedure was used to correct the significance level (P value). Fourth,
252 single-subject difference LEP waveforms (LH *minus* RH) were calculated to
253 display the difference of LEPs elicited by stimulation of the left and right hand
254 dorsum.

255

256 To estimate the locations of A δ -N1 and C-N1 sources, group-level LEP
257 waveforms were imported in Brain Electrical Source Analysis Software (BESA
258 5.3; MEGIS Software). Sources of LEP waveforms were modeled using
259 equivalent current dipoles from a spatiotemporal source model (Scherg and
260 Ebersole, 1993; Lopes da Silva, 2004). Based on findings of previous studies
261 (Garcia-Larrea et al., 2003; Valentini et al., 2012), a four-dipole model was
262 used. Dipole configurations were calculated within a realistic head model and
263 estimated according to the best correspondence between the recorded and
264 estimated scalp distribution. The ability of the estimated dipoles to explain
265 satisfactorily the recorded scalp topography was obtained by calculating the
266 signal residual variance (RV), i.e., the percentage of the scalp data that cannot
267 be explained by the fitted dipoles. For each dipole, its location, orientation, and
268 time course were extracted, and source locations were transformed to
269 normalized Talairach space.

270

271 Because of the contralateral scalp distribution of the early LEP activity (i.e.,
272 A δ -N1 and C-N1), to measure the amplitudes of A δ -N1 and C-N1 waves we
273 used a bipolar montage consisting in the contralateral central electrode
274 referenced to Fz (Cc-Fz; Hu et al., 2010; Valentini et al., 2012). From the
275 single-subject Cc-Fz re-referenced LEP waveform averaged across all trials
276 and all stimulus energies, peak latencies and amplitudes of A δ -N1 and C-N1

277 waves were manually measured for each subject (if a clear response cannot
278 be detected, default values 'NaN' were assigned). A δ -N1 and C-N1 were
279 defined as the negative deflections preceding the A δ -N2 and C-N2 waves,
280 respectively (Tarkka and Treede, 1993; Valentini et al., 2012). Only unique and
281 clear negative deflections preceding the A δ -N2 (C-N2) waves were classified
282 as A δ -N1 (C-N1) responses (A δ -N1: 81.3% of subjects [both LH and RH];
283 C-N1: 72.9% [LH] and 75.7% [RH]). A δ -N1 (C-N1) responses were defined as
284 absent if the A δ -N1 (C-N1) amplitude was positive or if a clear negative
285 deflection preceding the A δ -N2 (C-N2) wave could not be identified (Hu et al.,
286 2010). Single-subject LEP waveforms (Cc-Fz) were subsequently averaged to
287 obtain group-level re-referenced waveforms. Group-level scalp topographies,
288 at corresponding peak latencies of A δ -N1 and C-N1 waves, were computed by
289 spline interpolation.

290

291 To explore the functional relationship between the C-N1 wave and the other
292 dominant LEP waves, latencies and amplitudes of C-N1 wave were correlated
293 with those of other LEP waves (i.e., A δ -N1, A δ -N2, A δ -P2, C-N2, and C-P2
294 waves) across subjects, using Pearson's correlation analysis. To account for
295 multiple comparisons, P values were adjusted using the FDR correction.

296

297 Furthermore, to assess the dependence of A δ -N1 and C-N1 amplitudes on
298 stimulus energy, we calculated the averaged LEP waveform for each level of

299 stimulus energy, thus obtaining four average waveforms (E1-E4) for each
300 stimulated hand (LH and RH). Peak amplitudes of A δ -N1 and C-N1 waves at
301 each stimulus energy were measured from the averaged waveforms (Cc-Fz) of
302 each subject. A δ -N1 and C-N1 amplitudes were statistically compared using a
303 two-way repeated-measures analysis of variance (ANOVA), with 'stimulation
304 site' (two levels: LH and RH) and 'stimulus energy' (four levels: E1-E4) as
305 within-subject factors. When significant, post hoc comparisons were performed
306 using paired-sample t tests.

307
308 To assess the dependence of A δ -N1 and C-N1 amplitudes on perceived pain
309 intensity, we classified single trials in four categories (I1–I4) according to the
310 subjective pain ratings. This was achieved by first rescaling the ratings of each
311 subject between 0 and 100, defining the smallest pain rating as 0 and the
312 largest pain rating as 100 (Zhang et al., 2012). For each subject and each
313 stimulated hand (LH and RH), trials were then classified in four categories (I1:
314 ≤ 25 , I2: >25 and ≤ 50 , I3: >50 and ≤ 75 , I4: >75). EEG trials belonging to each
315 category were averaged together, thus yielding four average waveforms for
316 each subject and stimulated hand. Finally, peak amplitudes of A δ -N1 and C-N1
317 waves of each of the four categories were measured from the averaged
318 waveforms (Cc-Fz) of each subject. A δ -N1 and C-N1 amplitudes were
319 statistically compared using a two-way repeated-measures ANOVA, with
320 'stimulation site' (two levels: LH and RH) and 'pain intensity' (four levels: I1-I4)

321 as within-subject factors. When significant, post hoc comparisons were
322 performed using paired-sample t tests.

323

324 Since A δ -N1 and C-N1 waves overlap spatially and temporally with the
325 subsequent N2-P2 complex, the signal-to-noise ratio of A δ -N1 and C-N1
326 activities can be largely enhanced if the contamination of the N2-P2 complex is
327 removed from the LEP responses (Hu et al., 2010). The removal of N2-P2
328 related activities can be achieved by performing an additional ICA, and
329 removing N2 and P2-related ICs, which are easily identified because of the
330 high signal-to-noise ratio of the N2-P2 complex (Mouraux and Iannetti, 2008).
331 Therefore, to isolate A δ -N1 and C-N1 activities, LEP responses were
332 decomposed into a series of ICs, each having a maximally independent time
333 course and a fixed scalp topography. N2- and P2-related ICs were identified
334 and removed, thus generating a new set of EEG data devoid of ICs
335 representing N2-P2 related activities. As described in Hu et al., 2010, ICs were
336 classified as "N2-P2 related" only if they satisfied the following three criteria: (1)
337 Reflecting neural activity elicited by the laser stimulus. This was tested by
338 normalizing the time course of the power of each IC as the standard deviation
339 from the mean of the pre-stimulus interval (-1000 to 0 ms, expressed as Z
340 score), and checking if the average Z score within 0 to +1500 ms post-stimulus
341 interval was larger than 5. (2) Having peaks at latency between 150 and 500
342 ms (A δ -N2/P2), and/or between 700 and 1300 ms (C-N2/P2). (3) Having a

scalp topography centrally-distributed and maximal at the vertex. After A δ -N2/P2 and C-N2/P2 related ICs were removed using this approach, A δ -N1 and C-N1 peaks were detected using the bipolar montage Cc–Fz, and their scalp topographies were plotted at the respective peak latencies.

347

Experiment 2: Human EEG during foot stimulation

349

Subjects

EEG data were collected from a different group of 95 healthy new subjects (50 females) aged 21.6 ± 1.7 years (mean $\pm$ SD, range = 18 to 25 years). All subjects gave their written informed consent and were paid for their participation. The local ethics committee approved the experimental procedures.

356

Nociceptive stimulation and Experimental design

Nociceptive stimulation and experimental design were identical to Experiment 1, with the exception that (i) laser stimuli were delivered on the dorsum of the left and right foot, and (ii) stimulation energies were as follows: E1: 2.5 J; E2: 3 J; E3: 3.5 J; E4: 4 J.

362

EEG recording and Data analysis

EEG recording and Data analysis were identical to Experiment 1, with two

exceptions, due to the fact that nociceptive stimuli were delivered to the foot. First, the C-N2 and C-P2 were defined as the most negative and positive deflections between 1,000 and 1,700 ms after stimulus onset. Second, it was not possible to measure N1 amplitude, which notoriously difficult to isolate following foot stimulation because of the spatial and temporal overlap with the N2 wave.

Experiment 3: Rat ECoG during stimulation of the four paws

Subjects

The experiment was conducted on 12 adult male Sprague-Dawley rats weighing 300-400 g (Xia et al., 2016). Rats were free-choice fed with water and food, and were housed in separate cages under temperature- and humidity-controlled conditions. They were kept in a 12-h day–night cycle (light on from 08:00 to 20:00). All experimental procedures adhered to the guidelines for animal experimentation.

Nociceptive stimulation and Experimental design

Surgical procedures and electrode coordinates are detailed in (Hu et al., 2015). After surgery, rats were kept in their cages for at least 7 days before the collection of ECoG data. During the ECoG data collection, rats were placed into a plastic chamber (length × width × height: 30×30×40 cm³), within which

387 they could freely move. The same Nd:YAP laser stimuli used in human
388 experiments were delivered on the rats' paws through holes (5-mm diameter)
389 on the floor of the chamber when the animal was spontaneously still. Ten laser
390 pulses were delivered to each of the four stimulation sites (left forepaw, right
391 forepaw, left hindpaw, and right hindpaw) using five stimulus energies (E1'-E5',
392 from 1 to 4 J, in steps of 0.75 J), for a total of 200 pulses. The order of
393 stimulation sites and stimulus energies was pseudorandomized, the
394 inter-stimulus interval was never less than 30 s, and the laser target was
395 changed after each stimulus to avoid nociceptor fatigue or sensitization.
396 Animals were video-recorded throughout the experiment, and pain-related
397 behaviors elicited by the laser stimuli were quantified using a validated 0-4
398 scale (Fan et al., 1995; Fan et al., 2009) (0: immobility; 1: head turning,
399 including shaking or elevating the head; 2: flinching; 3: withdrawal, involving
400 paw retraction from the nociceptive stimulus; 4: licking the stimulated body
401 territory and whole body movement). White noise was played throughout the
402 experiment, to avoid the activation of the auditory system by laser-generated
403 ultrasounds and thereby selectively record the cortical responses related to the
404 activation of the nociceptive system (Xia et al., 2016).

405

406 *ECoG recording and Data analysis*

407 ECoG recording and analysis were virtually identical to Experiment 1. To
408 explore the spatial differences between the response elicited by the stimulation

of the left and right paws, we plotted the scalp topographies of the earliest part of C-LEPs (i.e., from 110 to 130 ms for forepaw stimulation and from 210 to 230 ms for hindpaw stimulation, corresponding to the C-N1), in steps of 10 ms. To test whether these earliest responses were lateralized with respect to the stimulated paw, we compared single-subject LEP waveforms elicited by the stimulation of the left and right paws, using the same procedure as we did in the human Experiments 1 and 2.

To minimise the influence of widespread activities and enhance spatially-discrete activities like the C-N1 wave, we average referenced the rat ECoG data (Xia et al., 2016). We measured the peak latency and amplitude of the C-N1 waves, in single-subject LEP waveforms averaged across trials and stimulus energies. Importantly, there was a clearly detectable C-N1 response in all animals. The C-N1 wave was optimally detected from electrode RFR (following left forepaw stimulation), LFL (following right forepaw stimulation), PR1 (following left hindpaw stimulation), and PL1 (following right hindpaw stimulation). Single-subject re-referenced waveforms were subsequently averaged to obtain group-level waveforms. Group-level scalp topographies, at the peak latency of the C-N1 wave, were computed by spline interpolation.

Statistical analysis

To assess the dependence of C-N1 amplitudes on stimulus energy, we firstly

431 calculated the average LEP waveform for each level of stimulus energy, thus
432 yielding five average waveforms (E1'-E5') for each of the four stimulation sites.
433 Peak amplitude of the C-N1 wave at each stimulus energy was measured in
434 each subject. C-N1 amplitudes were compared using a two-way
435 repeated-measures ANOVA, with 'stimulation site' (four levels: left and right
436 forepaw and hindpaw) and 'stimulus energy' (five levels: E1'-E5') as
437 within-subject factors. When significant, post hoc pairwise comparisons were
438 performed using paired-sample t tests.

439

Results

Experiments 1 and 2: Human EEG

Waveforms and topographies of the early part of human C-LEPs

Figure 1 shows the grand average human LEP waveforms at Cz, and the scalp topographies at the peak latencies of A δ -N2, A δ -P2, C-N2, and C-P2 waves, for both hand and foot stimulation. Regardless of where the stimulus was delivered, the A δ -N2 and C-N2 waves were always maximal at the vertex and extended bilaterally toward the temporal regions, while the A δ -P2 and C-P2 waves were more centrally distributed (Figure 1), as also described in previous studies (Kunde and Treede, 1993; Mouraux and Iannetti, 2009; Hu et al., 2014b). Group-level latencies and amplitudes of the main peaks of both the A δ - and C-LEPs are reported in Table 1.

The top panel of Figure 2 shows the same grand average LEP waveforms, but from all recorded electrodes, in response to hand (left panel) and foot stimulation (right panel). Following hand stimulation, the scalp topographies of the earliest activity of both the A δ -LEPs (150–170 ms) and the C-LEPs (710–730 ms) displayed a clear negative maximum on the central electrodes overlying the hemisphere *contralateral* to the stimulation side. In contrast, following foot stimulation, the scalp topographies of the earliest activity in both

462 A δ -LEPs (180-200 ms) and C-LEPs (1100-1120 ms) were centrally-distributed.
 463 The bottom panels of Figure 2 show the grand average of the difference
 464 between LEP waveforms elicited by the stimulation of the left and right side, as
 465 well as the timeseries of scalp topographies in the earliest part of both the A δ -
 466 and C-LEPs (A δ -LEP: 150–170 ms [hand]; 180-200 ms [foot]; C-LEP: 710-730
 467 ms [hand]; 1100-1120 ms [foot]). In these time intervals, A δ -LEPs and the
 468 C-LEPs were significantly different when elicited by left and right hand
 469 stimulation ($P_{\text{fdr}} < 0.05$), while they were not different when elicited by left and
 470 right foot stimulation. The differences observed following hand stimulation
 471 occurred at the latencies of the A δ -N1 and of the C-N1 waves, and were
 472 localized bilaterally, on the electrodes overlying the hand area in the primary
 473 sensorimotor cortices (Figure 2).

474

475 *Source analysis of the C-N1 waves elicited by hand and foot stimulation*

476 Time courses and locations of the dipolar sources of LEPs are shown in Figure
 477 3. The source explaining the time window of the A δ -N1 and C-N1 waves was
 478 located in the contralateral primary somatosensory cortex (S1), whereas the
 479 sources explaining the time window of the A δ -N2/P2 and C-N2/P2 complex
 480 were located in the bilateral operculo-insular cortices (secondary
 481 somatosensory [S2] and/or insula) and in the anterior cingulate cortex (ACC).
 482 While the location of bilateral S2/insula and ACC were almost identical
 483 regardless of stimulated site (hand or foot) or stimulated side (right or left), the

484 generator of the N1 elicited by hand stimulation was always *contralateral* to the
 485 stimulated side (top panels): it was located in the right S1 when the left hand
 486 was stimulated, and in the left S1 when the right hand was stimulated.
 487 Importantly, this was not the case when considering the earliest part of the A δ
 488 and C-LEPs elicited by foot stimulation (bottom panels): regardless of whether
 489 the right or the left foot was stimulated, the S1 generator was always located
 490 *on the midline*, in the foot area of the primary sensorimotor cortex. At all
 491 stimulated sites, the time course of the activity of the S1 source clearly showed
 492 both A δ -N1 and C-N1 waves. The residual variance within the A δ -N1 time
 493 window (150-170 ms [hand]; 180-200 ms [foot]) was 2.8% and 1.4% for left
 494 and right hand stimulation, and 3.1% and 6.1% for left and right foot stimulation.
 495 The residual variance within the C-N1 time window (710-730 ms [hand];
 496 1110-1130 ms [foot]) was 3.9% and 2.3% for left and right hand stimulation,
 497 and 9.3% and 5.1% for left and right foot stimulation. These results indicate
 498 that the estimated S1 dipoles explained satisfactorily the scalp topography of
 499 both the A δ -N1 and the C-N1 peaks.

500

501 *Isolation of the C-N1 wave following hand stimulation*

502 The left panel of Figure 4 shows the grand average LEP waveforms elicited by
 503 the stimulation of left and right hand at electrode Cc referenced to Fz. Using
 504 this Cc-Fz montage both the A δ -N1 and C-N1 waves could be clearly detected.
 505 Their scalp topographies showed a maximum over the central electrodes

506 contralateral to the stimulation side. Across subjects, latencies and amplitudes
 507 of A δ -N1 and C-N1 waves were as follows: (1) A δ -N1: 167 ± 3 ms and $-5.2 \pm$
 508 0.3 μ V (LH stimulation; mean $\pm$ SEM); 173 ± 2 ms and -4.3 ± 0.3 μ V (RH
 509 stimulation); (2) C-N1: 736 ± 6 ms and -3 ± 0.2 μ V (LH stimulation); 737 ± 6 ms
 510 and -2.8 ± 0.2 μ V (RH stimulation). The between-subject latency variability of
 511 the A δ -N1 was 18 ± 2 ms (LH stimulation) and 19 ± 2 ms (RH stimulation). The
 512 between-subject latency variability of the C-N1 was 37 ± 4 ms (LH stimulation)
 513 and 40 ± 4 ms (RH stimulation). Two-way repeated-measures ANOVA
 514 conducted using 'afferent pathway' (two levels: A δ and C) and 'stimulated side'
 515 (two levels: left and right) only revealed a significant main effect of 'afferent
 516 pathway' ($F(1,70)=27.4$, $P<0.001$, $\eta_p^2=0.28$), most likely indicating that the
 517 latency jitter was significantly larger for C-N1 than A δ -N1 wave.

518

519 The right panel of Figure 4 shows the grand average LEP waveforms (Cc-Fz)
 520 after the removal of the N2-P2 complex using an additional ICA. The scalp
 521 topographies of both A δ -N1 and C-N1 waves show that the negative maximum
 522 over the central electrodes contralateral to the stimulated side is more clearly
 523 isolated in comparison to the LEP waveforms before N2-P2 removal.

524

525 *Relationship between C-N1 and other A δ - and C-LEP waves*

526 Results of correlation analysis between latency/amplitude of each C-LEP wave
 527 and latency/amplitude of the other A δ - and C-LEP waves are summarized in

528 Figure 5 and Table 2. Some of these results are noteworthy to understand the
529 functional significance of C-LEPs in general, and of the C-N1 wave in
530 particular.

531

532 First, the latencies of the different C-LEP waves (C-N1, C-N2, and C-P2) were
533 strongly correlated among themselves, while they were not correlated with
534 those of A δ -LEP waves (A δ -N1, A δ -N2, and A δ -P2) (Table 2). This finding
535 confirms that A δ - and C-LEPs are consequent to the arrival of somatosensory
536 volleys relayed by different populations of myelinated and unmyelinated
537 peripheral afferents.

538

539 Second, the peak amplitude of the C-N1 wave from both hands was
540 significantly and positively correlated with that of the A δ -N1 wave, while it was
541 not consistently correlated with the amplitude of the other vertex waves: i.e.,
542 C-N2, C-P2, A δ -N2, and A δ -P2 (R values and significance are reported in
543 Table 2). In contrast, amplitudes of the vertex C-N2 were significantly
544 correlated with amplitudes of the vertex A δ -N2, A δ -P2, and C-P2, but not with
545 the amplitude of either the A δ -N1 or the C-N1 wave (R values and significance
546 are reported in Table 2). This finding provides further evidence that the N1
547 waves elicited by both A δ and C afferent volleys are generated by cortical
548 structures different from those generating the vertex waves (A δ -N2/P2 and
549 C-N2/P2).

550

551 *Dependence of human C-N1 amplitudes on stimulus energy and pain intensity*

552 Amplitudes of the main A δ -LEP and C-LEP waves for different stimulus
 553 energies (E1-E4) and hand stimulation sites (LH and RH), together with their
 554 statistical comparison, are summarized in Table 3. Two-way
 555 repeated-measures ANOVA with factors 'stimulus energy' (four levels: E1-E4)
 556 and 'stimulated side' (two levels: left and right) showed that (1) A δ -N1
 557 amplitudes were significantly modulated by 'stimulus energy' ($F(3,249)=243.9$,
 558 $P<0.001$, $\eta_p^2=0.86$), 'stimulation site' ($F(1,83)=7.26$, $P=0.009$, $\eta_p^2=0.08$), and
 559 their interaction ($F(3,249)=3.40$, $P=0.02$, $\eta_p^2=0.06$), and (2) amplitudes of
 560 A δ -N2, A δ -P2, C-N1, C-N2, and C-P2 waves were significantly modulated only
 561 by 'stimulus energy'.

562

563 Post hoc pairwise comparisons indicated that (1) A δ -N1 amplitudes at E3 and
 564 E4 were significantly larger than those at E1 and E2 ($E1<E3$, $E1<E4$, $E2<E3$,
 565 $E2<E4$, $E3<E4$, $P<0.001$ for all comparisons) for both stimulation sites, and
 566 A δ -N1 amplitudes at E2 were significantly larger than those at E1 ($E1<E2$,
 567 $P<0.01$) for right hand stimulation; (2) C-N1 amplitudes were significantly
 568 larger at E3 than at E1 and E4 ($E3>E1$, $P=0.01$; $E3>E4$, $P=0.03$), at E2 than at
 569 E1 ($E2>E1$, $P=0.03$) for left hand stimulation, and at E3 than at E4 ($P=0.03$) for
 570 right hand stimulation. The results of these post hoc comparisons suggest that
 571 the higher the stimulus energy the larger the A δ -N1 amplitude (top panel of

Figure 6), while C-N1 amplitude is maximal at a moderate level of stimulus energy (i.e., E3, bottom panel of Figure 6). Similarly, post hoc pairwise comparisons revealed that the higher the stimulus energy, the larger the amplitudes of A δ -N2 and A δ -P2 waves. In contrast, C-N2 and C-P2 amplitudes were maximal at a moderate level of stimulus energy (i.e., E2 or E3), as previously described (Hu et al., 2014b).

578

Two-way repeated-measures ANOVA with factors 'pain intensity' (four levels: I1-I4) and 'stimulated side' (two levels: left and right) showed that (1) A δ -N1 amplitudes were significantly modulated by 'pain intensity' ($F(3,240)=240.8$, $P<0.001$, $\eta_p^2=0.72$) and 'stimulation site' ($F(1,80)=5.30$, $P=0.024$, $\eta_p^2=0.06$), and (2) C-N1 amplitudes waves were significantly modulated only by 'pain intensity' ($F(3,213)=3.59$, $P=0.015$, $\eta_p^2=0.05$).

585

Post hoc pairwise comparisons indicated that (1) A δ -N1 amplitudes monotonically increased with perceived pain, at both stimulation sites ($I1<I2<I3<I4$, $P<0.001$ for all comparisons); (2) C-N1 amplitudes were significantly larger at I2 and I3 than at I1 and I4 ($I1<I2$, $I4<I2$, $I1<I3$, $I4<I3$, $P<0.05$ for all comparisons) for right hand stimulation.

591

592 **Experiment 3: Rat ECoG**

593

Waveforms and topographies of the early part of rat C-LEPs

Figure 7 shows the grand average rat C-LEPs, following the stimulation of the forepaw and hindpaw, on the right and left sides. Scalp topographies of the earliest activity in the response evoked by right and left forepaw stimulation (110-130 ms, left panel) displayed a clear negative maximum on the hemisphere *contralateral* to the stimulated side. Scalp topographies of the earliest part of the response elicited by right and left hindpaw stimulation (210-230 ms, right panel) were more centrally distributed.

Isolation of the C-N1 wave

Similar to human Aδ- and C-LEP data, the widespread scalp distribution of the main vertex C-LEP components makes the detection of the early lateralized neural activities as a separate peak difficult (Xia et al., 2016). To reduce the influence of widespread activities and thus enhance spatially-discrete activities, we average referenced the data (Bertrand et al., 1985). This allowed to visualise the first two negative waves (N1, N2) and the third positive wave (P2) as separate deflections (Xia et al., 2016). The N1 wave was optimally detected from electrode RFR (following left forepaw stimulation), LFL (following right forepaw stimulation), PR1 (following left hindpaw stimulation), and PL1 (following right hindpaw stimulation) (Figure 8). The peak latency and amplitude of the N1 wave were: 125 ± 11 ms, -35.7 ± 6.2 μ V (left forepaw); 125 ± 3 ms, -29.3 ± 6.0 μ V (right forepaw); 230 ± 7 ms, -42.0 ± 6.5 μ V (left

hindpaw); 230 ± 6 ms, -34.3 ± 5.4 μ V (right hindpaw).

617

618 *Dependence of rat C-N1 amplitudes on stimulus energy*

619 In striking contrast with the human data, the amplitude of the rat C-LEP
620 response displayed a more monotonic relationship with stimulus energy
621 (Figure 9). Two-way repeated-measures ANOVA with factors 'stimulus energy'
622 and 'stimulation site' showed that amplitudes of C-N1 wave were significantly
623 modulated by stimulus energy' ($F(4,44)=41.41$, $P<0.001$, $\eta_p^2=0.79$), but not by
624 'stimulation site' ($F(3,33)=1.93$, $P=0.14$, $\eta_p^2=0.15$) or their interaction
625 ($F(12,132)=1.64$, $P=0.09$, $\eta_p^2=0.11$). Post hoc pairwise comparisons revealed
626 that C-N1 amplitudes at E3', E4', and E5' were significantly larger than those at
627 E1' and E2' ($P<0.05$; Figure 9), indicating a strong positive relationship
628 between stimulus energy and C-N1 amplitude.

Discussion

We described for the first time the somatotopical organization and the stimulus-response functions of the electrocortical responses elicited in the S1 by selective stimulation of small-myelinated A δ -fiber and unmyelinated C-fiber afferents, in humans and rats.

We obtained five main results.

First, both in human and rat recordings, the topographical distribution of the earliest part of the cortical response elicited by C-fiber input (C-LEPs) was clearly dependent on which limb the stimulus was delivered (Figures 2 and 7). When the stimulus was delivered to the hand or the forepaw, the response displayed a negative maximum in the hemisphere *contralateral* to the stimulation side. In contrast, when the stimulus was delivered to the foot or the hindpaw, the response was more centrally distributed (Figures 2 and 7).

Second, source analysis of human EEG confirmed that the cortical sources of the earliest part of C-LEPs were located in the postcentral gyrus, in positions compatible with the representations of the stimulated body district within S1 (Figure 3).

651 Third, in human EEG a bipolar Cc-Fz montage allowed detecting this S1
652 activity as a distinct early deflection (C-N1 wave), peaking at 710-730 ms
653 following hand stimulation (Figure 4), whereas a C-N1 could not be clearly
654 isolated following foot stimulation, because the more medial foot
655 representation within S1 causes a strong spatial and temporal overlap with the
656 large amplitude of C-N2 (Figure 1). In contrast, in rat ECoG a C-N1 could be
657 isolated following both forepaw and hindpaw stimulation (Figure 8), because of
658 the different representation of the body surface within S1.

659

660 Fourth, stimulus-response functions were different in humans and rats. In
661 humans, C-N1 had maximal amplitude at moderate stimulus energy and pain
662 intensity (Figure 6), whereas in rats C-N1 amplitude increased
663 quasi-monotonically with stimulus energy (Figure 9). The difference in
664 stimulus-response functions was caused by the combination of the
665 across-species difference in thermal sensitivity of A δ nociceptors (Hu et al.,
666 2015) with the dependence of these electrocortical responses in the time
667 domain on stimulus relevance (Iannetti et al., 2008; Mouraux and Iannetti,
668 2009).

669

670 Finally, there was a decoupling between the activity of the S1 (indexed by
671 C-N1 in human recordings) and the activity of the supramodal generators of
672 N2-P2 vertex waves (Mouraux and Iannetti, 2009): the C-N1 amplitude was

673 positively correlated with the amplitude of the A δ -N1 wave, but was not
674 correlated with that of the other vertex waves, i.e., C-N2, C-P2, A δ -N2, and
675 A δ -P2 (Figure 5). This finding highlights the functional dissociation between
676 the generators of somatosensory-specific (C-N1 and A δ -N1) and supramodal
677 waves (A δ -N2/P2 and C-N2/P2).

678

679 *Detecting C-fiber responses in the primary somatosensory cortex of humans*
680 *and rodents*

681 Stimulus-response functions of C-fiber electrocortical responses in rats and
682 humans were clearly different. While in humans the C-N1 wave was maximal
683 at moderate levels of stimulus energy and pain intensity (Figure 6), in rats it
684 increased monotonically with stimulus energy, and only plateaued at the
685 highest stimulus energy (Figure 9). To understand this difference, it is
686 important to consider two physiological aspects: (i) the functional properties of
687 the electrocortical responses elicited by sudden and fast-rising sensory stimuli,
688 and (ii) the different heat sensitivity of A δ skin nociceptors in rodents and
689 humans.

690

691 The large brain potentials detected in EEG and ECoG recordings in response
692 to sudden sensory stimuli do not always faithfully reflect the intensity of the
693 afferent peripheral input, but are strongly determined by contextual factors,
694 such as stimulus saliency (Iannetti et al., 2008; Mouraux and Iannetti, 2009;

695 Ronga et al., 2013). This simple concept explains why in humans the C-fiber
696 response (e.g., the C-N1; Figures 1-4) is larger when preceded by an A δ -fiber
697 response elicited by stimuli of moderate energy and pain intensity (E2-E3, I2-I3,
698 Figure 6), while it becomes smaller when preceded by a large A δ -fiber
699 response elicited by stimuli of high energy (E4) (Hu et al., 2014b). For this
700 reason, the C-fiber response has been for a long time considered to be
701 detectable only when the concomitant activation of A δ -fibers is avoided or
702 reduced (Bromm and Treede, 1983; Bragard et al., 1996; Mouraux et al.,
703 2003).

704

705 This reasoning does not apply to the C-fiber ECoG responses measured in
706 rats, because A δ -nociceptors have different heat sensitivity in rodents and
707 humans. In rats, as demonstrated by recordings from peripheral axons and
708 spinal cord neurons (Devor et al., 1982; Lynn and Shakhaneh, 1988;
709 Sikandar et al., 2013), estimates of conduction velocity of peripheral afferents
710 (Jiang et al., 2007), and nocifensive behaviors (Fan et al., 2009), A δ -units are
711 virtually insensitive to heat. For this reason, in rats there is no A δ -fiber
712 response to heat, and the ECoG response elicited by the transient C-fiber
713 afferent volley is monotonically graded with the energy of the applied stimulus
714 (Figure 9).

715

716 *Unmyelinated nociceptive input elicits somatotopically-organized responses in*

717 S1

718 Although strongly debated only a few years ago (e.g., Bushnell et al., 1999;
719 Ploner et al., 2002), there is now compelling evidence that nociceptive
720 stimulation elicits robust responses in the S1. To settle the issue of whether S1
721 responds to nociceptive input, the use of somatosensory stimuli that activate
722 A δ - and C-nociceptors *selectively*, i.e., without the coactivation of
723 non-nociceptive A β -fibers (Baumgartner et al., 2005), has been decisive.
724 Psychophysically, selective stimulation of A δ -afferents has allowed revealing
725 that first-pain and touch have remarkably similar spatial acuity across the body
726 (Mancini et al., 2013). The same A δ -selective laser stimulation has allowed
727 identifying somatosensory-specific deflections in the human EEG, from the
728 seminal evidence that S1 contributes to the N1 wave (Tarkka and Treede,
729 1993) to the comprehensive characterization of N1 topography and underlying
730 sources (Valentini et al., 2012; Hu et al., 2014b). Finally, phase-encoded fMRI
731 combined with the same laser stimuli has allowed demonstrating the existence
732 of fine-grained maps of individual digits in the human S1 (Mancini et al., 2012).

733

734 Whether and how S1 also responds to unmyelinated C-fiber input remains an
735 open issue. Traditionally, second pain consequent to the arrival of the afferent
736 volley transmitted by slow-conducting C-units is less well localized than the
737 A δ -mediated first pain, although rigorous studies of spatial acuity for second
738 pain are lacking. Some authors suggest that C-input elicits responses in the

739 area 3a of S1 (Vierck et al., 2013), in contrast to the responses to A δ -input,
740 which are localized in areas 3b/1 (Whitsel et al., 2009; Vierck et al., 2013).
741 However, these studies used long thermal stimuli delivered through
742 mechanical probes that unavoidably coactivate touch-related A β -afferents,
743 and therefore it is difficult to rule out that these S1 responses are contributed
744 by the concomitant tactile input: indeed, as detailed elsewhere (Nahra and
745 Plaghki, 2005), even if the applied tactile input is constant, the heating of the
746 thermode can cause a transient response still mediated by the tactile input.
747
748 Here we used topographical analysis and source modeling of human EEG and
749 rat ECoG data to provide converging and across-species evidence that (1) the
750 earliest part of the cortical response elicited by C-fiber stimulation is generated
751 in the S1 contralateral to the stimulated side, and (2) this response follows the
752 representation of the body surface within S1 (Penfield and Boldrey, 1937;
753 Kandel et al., 2013). The observation that C-N1 amplitude is maximally
754 correlated with the amplitude of the A δ -N1 wave, but not with the amplitude of
755 the other vertex waves (A δ -N2/P2 and C-N2/P2; Figure 5) provides additional
756 evidence that the cortical generator of C-N1 is similar, if not identical, to that of
757 A δ -N1, which has been repeatedly shown to be largely contributed by the
758 contralateral S1 (Treede et al., 1988; Valentini et al., 2012; Hu et al., 2014a).
759 However, the limited spatial resolution of both scalp EEG and ECoG (Buzsaki
760 et al., 2012; Gratiy et al., 2017) does not allow excluding the possibility that the

C-N1 response (as well as the A δ -N1 response, see also Treede et al., 1988; Valentini et al., 2012) is contributed by sources in the primary motor cortex, whose topographical arrangement follows closely that of the S1 (Penfield and Boldrey, 1937). Direct recording of local field potentials is a more adequate approach to address this issue.

C-fiber afferents are phylogenetically the oldest part of the somatosensory system (Franzén et al., 1996; van Bijsterveld et al., 2003). Their main function is to inform the central nervous system of potentially noxious environmental stimuli (Julius and Basbaum, 2001). It is therefore unsurprising that the C-fiber conveys at least some information about the spatial location of noxious stimulation across the body surface, and already the lateral periaqueductal gray (PAG) receives somatotopically-organised nociceptive projections (Holstege et al., 1996; Benarroch, 2012). The somatotopically-organised projections to the primary somatosensory cortex are likely to be a prerequisite for deploying a spatially-coherent defensive motor repertoire. While the present results provide compelling evidence for a gross somatotopy within S1, the resolution of the spatial acuity of the C-fiber system remains to be fully characterized.

781 **References**

- 782 Basbaum AI, Bushnell MC (2009) Science of Pain. Oxford: Academic Press.
- 783 Baumgartner U, Cruccu G, Iannetti GD, Treede RD (2005) Laser guns and hot
784 plates. Pain 116:1-3.
- 785 Benarroch EE (2012) Periaqueductal gray: an interface for behavioral control.
786 Neurology 78:210-217.
- 787 Benjamini Y, Hochberg Y (1995) Controlling the False Discovery Rate - a
788 Practical and Powerful Approach to Multiple Testing. J Roy Stat Soc B
789 Met 57:289-300.
- 790 Bertrand O, Perrin F, Pernier J (1985) A theoretical justification of the average
791 reference in topographic evoked potential studies.
792 Electroencephalography and clinical neurophysiology 62:462-464.
- 793 Bragard D, Chen AC, Plaghki L (1996) Direct isolation of ultra-late (C-fibre)
794 evoked brain potentials by CO2 laser stimulation of tiny cutaneous
795 surface areas in man. Neurosci Lett 209:81-84.
- 796 Bromm B, Treede RD (1983) CO2 laser radiant heat pulses activate C
797 nociceptors in man. Pflugers Arch 399:155-156.
- 798 Bromm B, Treede RD (1984) Nerve fibre discharges, cerebral potentials and
799 sensations induced by CO2 laser stimulation. Hum Neurobiol 3:33-40.
- 800 Bushnell MC, Duncan GH, Hofbauer RK, Ha B, Chen JI, Carrier B (1999) Pain
801 perception: is there a role for primary somatosensory cortex? Proc Natl
802 Acad Sci U S A 96:7705-7709.
- 803 Buzsaki G, Anastassiou CA, Koch C (2012) The origin of extracellular fields
804 and currents--EEG, ECoG, LFP and spikes. Nat Rev Neurosci

-
- 805 13:407-420.
- 806 Delorme A, Makeig S (2004) EEGLAB: an open source toolbox for analysis of
807 single-trial EEG dynamics including independent component analysis. J
808 Neurosci Methods 134:9-21.
- 809 Devor M, Carmon A, Frostig R (1982) Primary afferent and spinal sensory
810 neurons that respond to brief pulses of intense infrared laser radiation: a
811 preliminary survey in rats. Exp Neurol 76:483-494.
- 812 Downar J, Crawley AP, Mikulis DJ, Davis KD (2000) A multimodal cortical
813 network for the detection of changes in the sensory environment. Nat
814 Neurosci 3:277-283.
- 815 Fan RJ, Shyu BC, Hsiao S (1995) Analysis of nocifensive behavior induced in
816 rats by CO₂ laser pulse stimulation. Physiology & behavior
817 57:1131-1137.
- 818 Fan RJ, Kung JC, Olausson BA, Shyu BC (2009) Nocifensive behaviors
819 components evoked by brief laser pulses are mediated by C fibers.
820 Physiology & behavior 98:108-117.
- 821 Franzén O, Johansson R, Terenius L (1996) Somesthesia and the
822 neurobiology of the somatosensory cortex. Basel: Birkhäuser.
- 823 Garcia-Larrea L, Frot M, Valeriani M (2003) Brain generators of laser-evoked
824 potentials: from dipoles to functional significance. Neurophysiol Clin
825 33:279-292.
- 826 Gratiy SL, Hanes G, Denman D, Hawrylycz MJ, Koch C, Einevoll GT,
827 Anastassiou CA (2017) From Maxwell's equations to the theory of
828 current-source density analysis. Eur J Neurosci 45:1013-1023.
- 829 Hess A (2015) Importance of knowing your stimulation paradigm:

-
- 830 methodological considerations on laser-evoked pain responses in rats
831 and humans. *Pain* 156:2404-2405.
- 832 Holstege G, Bandler R, Saper CB (1996) The emotional motor system. *Prog*
833 *Brain Res* 107:3-6.
- 834 Hu L, Mouraux A, Hu Y, Iannetti GD (2010) A novel approach for enhancing the
835 signal-to-noise ratio and detecting automatically event-related
836 potentials (ERPs) in single trials. *Neuroimage* 50:99-111.
- 837 Hu L, Valentini E, Zhang ZG, Liang M, Iannetti GD (2014a) The primary
838 somatosensory cortex contributes to the latest part of the cortical
839 response elicited by nociceptive somatosensory stimuli in humans.
840 *Neuroimage* 84:383-393.
- 841 Hu L, Cai MM, Xiao P, Luo F, Iannetti GD (2014b) Human brain responses to
842 concomitant stimulation of Aδ and C nociceptors. *J Neurosci*
843 34:11439-11451.
- 844 Hu L, Xia XL, Peng WW, Su WX, Luo F, Yuan H, Chen AT, Liang M, Iannetti G
845 (2015) Was it a pain or a sound? Across-species variability in sensory
846 sensitivity. *Pain* 156:2449-2457.
- 847 Iannetti GD, Hughes NP, Lee MC, Mouraux A (2008) Determinants of
848 laser-evoked EEG responses: pain perception or stimulus saliency? *J*
849 *Neurophysiol* 100:815-828.
- 850 Iannetti GD, Leandri M, Truini A, Zambreau L, Cruccu G, Tracey I (2004)
851 Aδ nociceptor response to laser stimuli: selective effect of stimulus
852 duration on skin temperature, brain potentials and pain perception. *Clin*
853 *Neurophysiol* 115:2629-2637.
- 854 Iannetti GD, Truini A, Romaniello A, Galeotti F, Rizzo C, Manfredi M, Cruccu G

-
- 855 (2003) Evidence of a specific spinal pathway for the sense of warmth in
856 humans. *J Neurophysiol* 89:562-570.
- 857 Jiang N, Cooper BY, Nemenov MI (2007) Non-invasive diode laser activation
858 of transient receptor potential proteins in nociceptors. *Proc SPIE Int Soc*
859 *Opt Eng* 6428.
- 860 Julius D, Basbaum AI (2001) Molecular mechanisms of nociception. *Nature*
861 413:203-210.
- 862 Kandel ER, Schwartz JH, Jessell TM, Siegelbaum S, Hudspeth AJ (2013)
863 Principles of neural science, 5th Edition. New York, NY ; London:
864 McGraw-Hill.
- 865 Kunde V, Treede RD (1993) Topography of middle-latency somatosensory
866 evoked potentials following painful laser stimuli and non-painful
867 electrical stimuli. *Electroencephalogr Clin Neurophysiol* 88:280-289.
- 868 Lee MC, Mouraux A, Iannetti GD (2009) Characterizing the cortical activity
869 through which pain emerges from nociception. *J Neurosci*
870 29:7909-7916.
- 871 Lewis T, Pochin EE (1937) The double pain response of the human skin to a
872 single stimulus. *Clin Sci* 3:37-67.
- 873 Lopes da Silva F (2004) Functional localization of brain sources using EEG
874 and/or MEG data: volume conductor and source models. *Magn Reson*
875 *Imaging* 22:1533-1538.
- 876 Lynn B, Shakhaneh J (1988) Properties of A delta high threshold
877 mechanoreceptors in the rat hairy and glabrous skin and their response
878 to heat. *Neurosci Lett* 85:71-76.
- 879 Magerl W, Ali Z, Ellrich J, Meyer RA, Treede RD (1999) C- and A delta-fiber

-
- 880 components of heat-evoked cerebral potentials in healthy human
881 subjects. *Pain* 82:127-137.
- 882 Mancini F, Haggard P, Iannetti GD, Longo MR, Sereno MI (2012) Fine-grained
883 nociceptive maps in primary somatosensory cortex. *J Neurosci*
884 32:17155-17162.
- 885 Mancini F, Sambo CF, Ramirez JD, Bennett DL, Haggard P, Iannetti GD (2013)
886 A fovea for pain at the fingertips. *Curr Biol* 23:496-500.
- 887 Mouraux A, Iannetti GD (2008) Across-trial averaging of event-related EEG
888 responses and beyond. *Magn Reson Imaging* 26:1041-1054.
- 889 Mouraux A, Iannetti GD (2009) Nociceptive laser-evoked brain potentials do
890 not reflect nociceptive-specific neural activity. *J Neurophysiol*
891 101:3258-3269.
- 892 Mouraux A, Guerit JM, Plaghki L (2003) Non-phase locked
893 electroencephalogram (EEG) responses to CO₂ laser skin stimulations
894 may reflect central interactions between A partial partial differential- and
895 C-fibre afferent volleys. *Clin Neurophysiol* 114:710-722.
- 896 Nahra H, Plaghki L (2005) Innocuous skin cooling modulates perception and
897 neurophysiological correlates of brief CO₂ laser stimuli in humans. *Eur*
898 *J Pain* 9:521-530.
- 899 Penfield W, Boldrey E (1937) Somatic Motor and Sensory Representation in
900 the Cerebral Cortex of Man As Studied By Electrical Stimulation. *Brain*
901 60:389-443.
- 902 Ploner M, Gross J, Timmermann L, Schnitzler A (2002) Cortical representation
903 of first and second pain sensation in humans. *Proc Natl Acad Sci U S A*
904 99:12444-12448.

-
- 905 Ronga I, Valentini E, Mouraux A, Iannetti GD (2013) Novelty is not enough:
 906 laser-evoked potentials are determined by stimulus saliency, not
 907 absolute novelty. *J Neurophysiol* 109:692-701.
- 908 Scherg M, Ebersole JS (1993) Models of brain sources. *Brain Topogr*
 909 5:419-423.
- 910 Shim B, Kim DW, Kim BH, Nam TS, Leem JW, Chung JM (2005) Mechanical
 911 and heat sensitization of cutaneous nociceptors in rats with
 912 experimental peripheral neuropathy. *Neuroscience* 132:193-201.
- 913 Sikandar S, Ronga I, Iannetti GD, Dickenson AH (2013) Neural coding of
 914 nociceptive stimuli-from rat spinal neurones to human perception. *Pain*
 915 154:1263-1273.
- 916 Tarkka IM, Treede RD (1993) Equivalent electrical source analysis of
 917 pain-related somatosensory evoked potentials elicited by a CO2 laser. *J*
 918 *Clin Neurophysiol* 10:513-519.
- 919 Treede RD, Meyer RA, Campbell JN (1998) Myelinated mechanically
 920 insensitive afferents from monkey hairy skin: heat-response properties.
 921 *Journal of neurophysiology* 80:1082-1093.
- 922 Treede RD, Kief S, Holzer T, Bromm B (1988) Late somatosensory evoked
 923 cerebral potentials in response to cutaneous heat stimuli.
 924 *Electroencephalogr Clin Neurophysiol* 70:429-441.
- 925 Tu Y, Zhang Z, Tan A, Peng W, Hung YS, Moayed M, Iannetti GD, Hu L (2016)
 926 Alpha and gamma oscillation amplitudes synergistically predict the
 927 perception of forthcoming nociceptive stimuli. *Hum Brain Mapp*
 928 37:501-514.
- 929 Valentini E, Hu L, Chakrabarti B, Hu Y, Aglioti SM, Iannetti GD (2012) The

930 primary somatosensory cortex largely contributes to the early part of the
931 cortical response elicited by nociceptive stimuli. *Neuroimage*
932 59:1571-1581.

933 van Bijsterveld OP, Kruize AA, Bleys RL (2003) Central nervous system
934 mechanisms in Sjogren's syndrome. *Br J Ophthalmol* 87:128-130.

935 Vierck CJ, Whitsel BL, Favorov OV, Brown AW, Tommerdahl M (2013) Role of
936 primary somatosensory cortex in the coding of pain. *Pain* 154:334-344.

937 Whitsel BL, Favorov OV, Li Y, Quibrera M, Tommerdahl M (2009) Area 3a
938 neuron response to skin nociceptor afferent drive. *Cereb Cortex*
939 19:349-366.

940 Xia XL, Peng WW, Iannetti GD, Hu L (2016) Laser-evoked cortical responses
941 in freely-moving rats reflect the activation of C-fibre afferent pathways.
942 *Neuroimage* 128:209-217.

943 Zhang ZG, Hu L, Hung YS, Mouraux A, Iannetti GD (2012) Gamma-band
944 oscillations in the primary somatosensory cortex--a direct and obligatory
945 correlate of subjective pain intensity. *J Neurosci* 32:7429-7438.

946

Figure legends

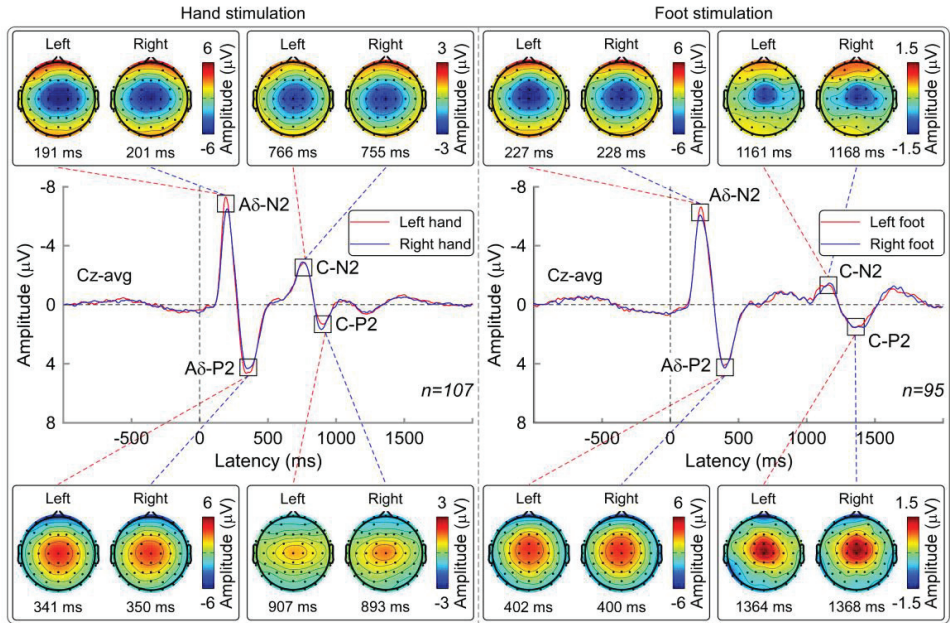
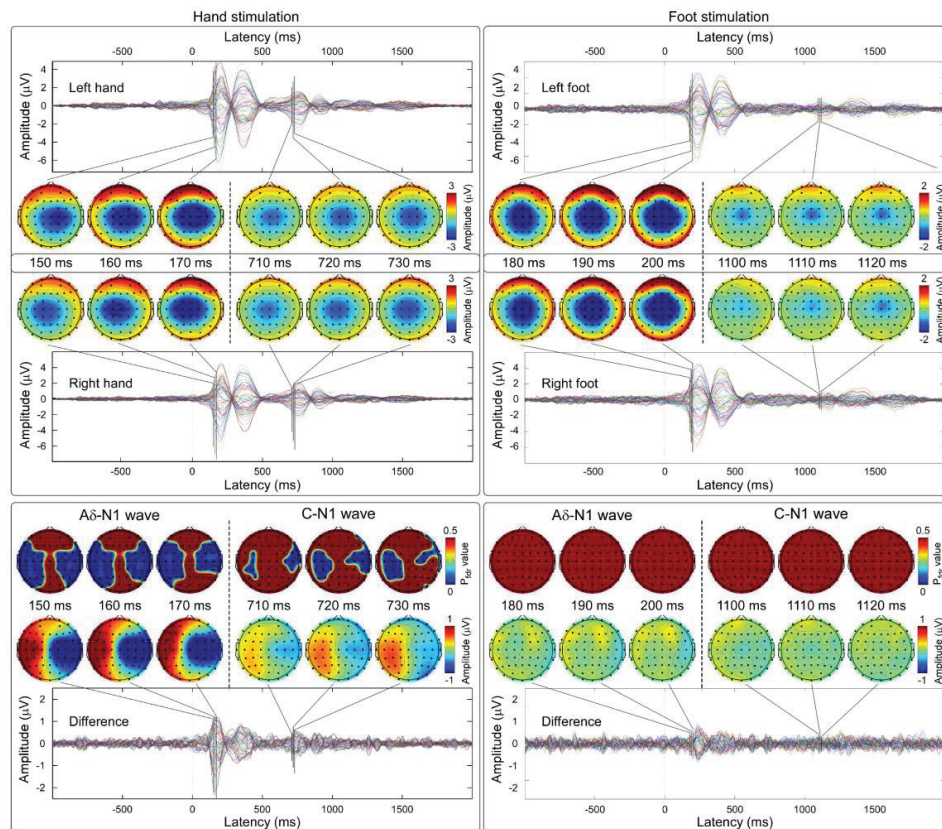


Figure 1. Grand average LEP waveforms and scalp topographies of Aδ-N2, Aδ-P2, C-N2, and C-P2 waves.

LEP responses were elicited by the stimulation of the hand (*left panel*) and foot (*right panel*) dorsum on the left (red waveforms) and right (blue waveforms) sides. Data were collected from 64 channels, in 107 subjects (hand stimulation) and 95 subjects (foot stimulation). Displayed signals were recorded from the vertex (Cz vs average reference). Scalp topographies are displayed at the peak latency of Aδ-N2, Aδ-P2, C-N2, and C-P2 waves.



960

961

962 **Figure 2.** Topographies and statistical comparisons of the earliest part of Aδ-
 963 and C-LEPs elicited by left and right stimulation of hand and foot.

964 *Top panels:* Grand averages from all electrodes are plotted in different colors
 965 and superimposed. Series of scalp topographies of the earliest part of
 966 Aδ-LEPs and C-LEPs (Aδ-LEP: 150–170 ms [hand]; 180–200 ms [foot]; C-LEP:
 967 710–730 ms [hand]; 1100–1120 ms [foot]) are displayed with a 10-ms interval.
 968 Note that the scalp topographies of the earliest part of the Aδ-LEPs and
 969 C-LEPs elicited by hand stimulation show a negativity contralateral to the
 970 stimulated side, while the scalp topographies of the earliest part of the
 971 Aδ-LEPs and C-LEPs elicited by foot stimulation are centrally-distributed. This
 972 observation is compatible with the somatotopical organization of the primary
 973 somatosensory cortex.

974 *Bottom panels:* Grand averages and scalp topographies of the difference
975 waveforms obtained by subtracting the LEPs elicited by right and left
976 stimulation, for hand (left panel) and foot (right panel). The top row shows the
977 scalp topography of the statistical comparisons between left and right LEPs at
978 the latencies of A δ -N1 and C-N1 waves. The earliest part of both A δ -LEPs and
979 C-LEPs elicited by the stimulation of the hand shows significant differences
980 ($P_{\text{tdr}} < 0.05$), localized bilaterally on the electrodes overlying the hand area in
981 the primary somatosensory cortices. In contrast, the earliest part of both
982 A δ -LEPs and C-LEPs elicited by the stimulation of the left and right foot was
983 not significantly different.

984

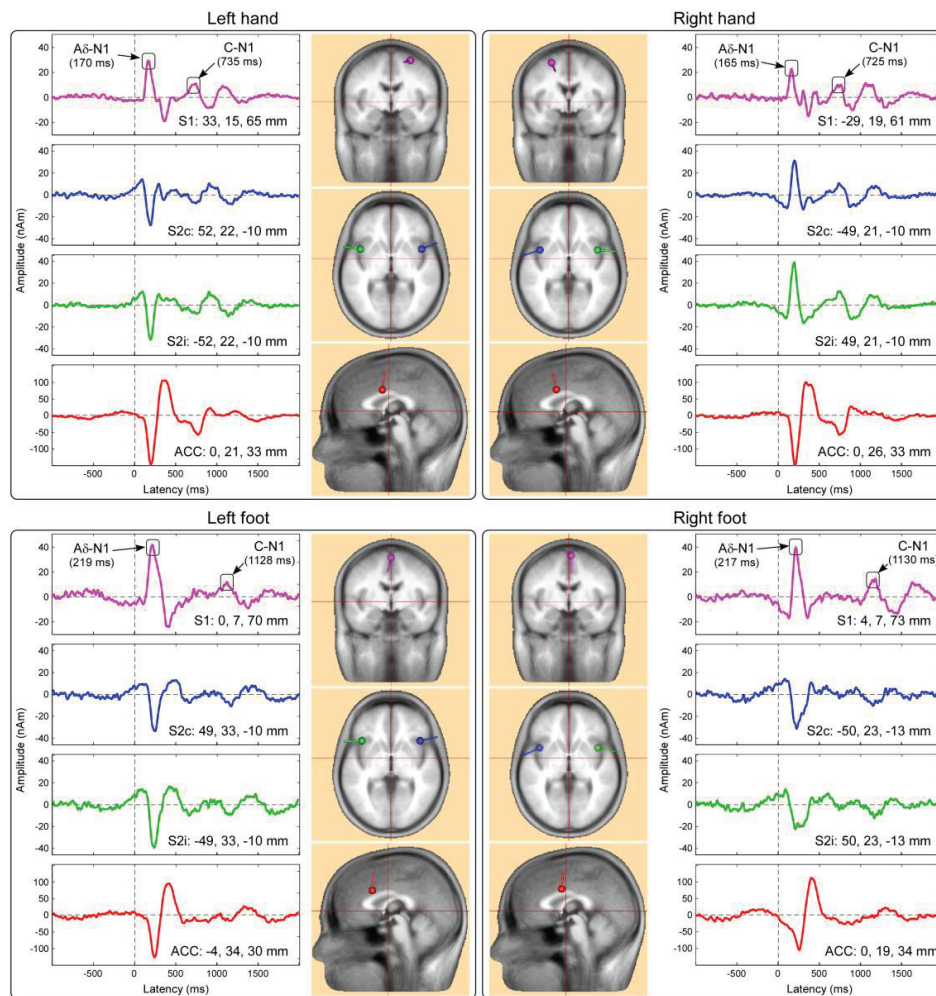


Figure 3. Dipolar sources of group average LEP waveforms elicited by hand (top) and foot (bottom) stimulation.

The scalp LEP responses were explained by four equivalent dipoles, located in the primary somatosensory cortex (S1, pink), the bilateral secondary somatosensory cortex (S2, green and blue), and the anterior cingulate cortex (ACC, red). Source time courses are displayed in the lateral part of each panel, and the locations and orientations of dipole sources are displayed in the medial part of each panel. Note that the spatial locations of S2 and ACC dipoles are similar in the responses elicited by stimuli delivered on the right and left sides. In contrast, the spatial location of the S1 dipole varies according

997 to the stimulated side, but only when stimulating the hand (*top*): it was located
998 in the right S1 when the left hand was stimulated, and vice versa. Note also
999 that the peaks of activity of the S1 timecourse occur at the latency of the A δ -N1
1000 and C-N1 waves.
1001

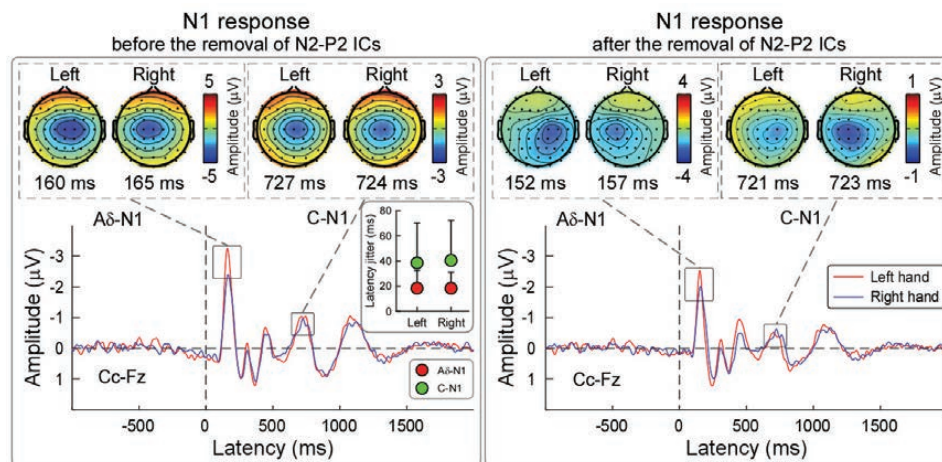


Figure 4. Detection of Aδ-N1 and C-N1 waves.

Grand averages and scalp topographies of Aδ-N1 and C-N1 waves elicited by stimulating the hand dorsum on the left (red waveforms) and the right sides (blue waveforms). *Left panel:* Aδ-N1 and C-N1 waves are measured from the contralateral central electrode (Cc vs Fz). Note that the Cc–Fz montage allows to isolate Aδ-N1 and C-N1 peaks, whose scalp topography has a maximum contralateral to the stimulated side. Histograms in the inset show the absolute difference (mean±SD) between single-subject and group-average latency of Aδ-N1 and C-N1 waves: the C-N1 wave has a larger jitter than the Aδ-N1 wave. *Right panel:* after the removal of the independent components capturing the activity of the partially overlapping N2-P2 vertex waves, the topography of both the Aδ-N1 and C-N1 is more lateralized.

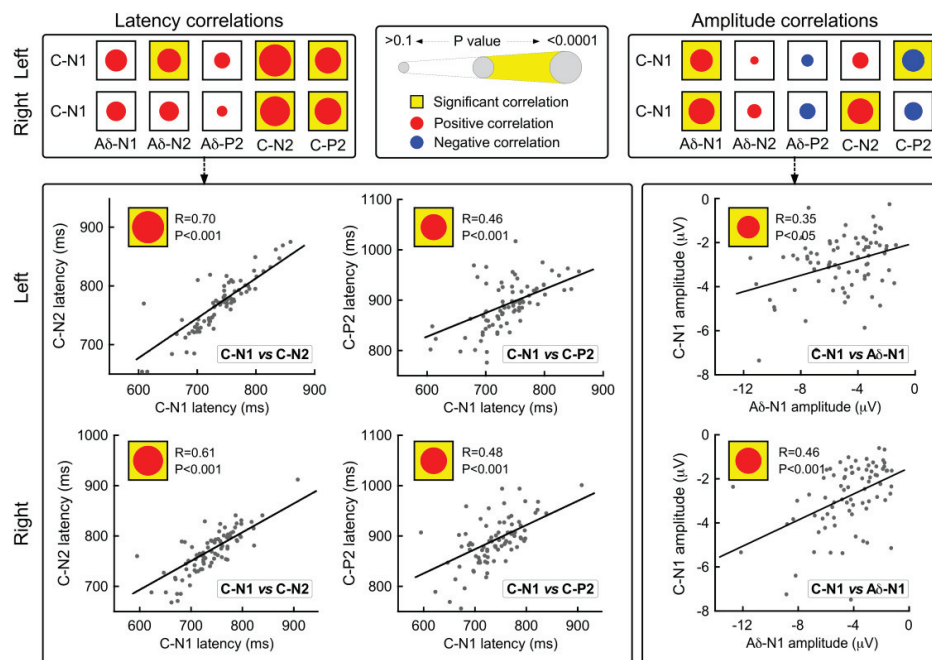
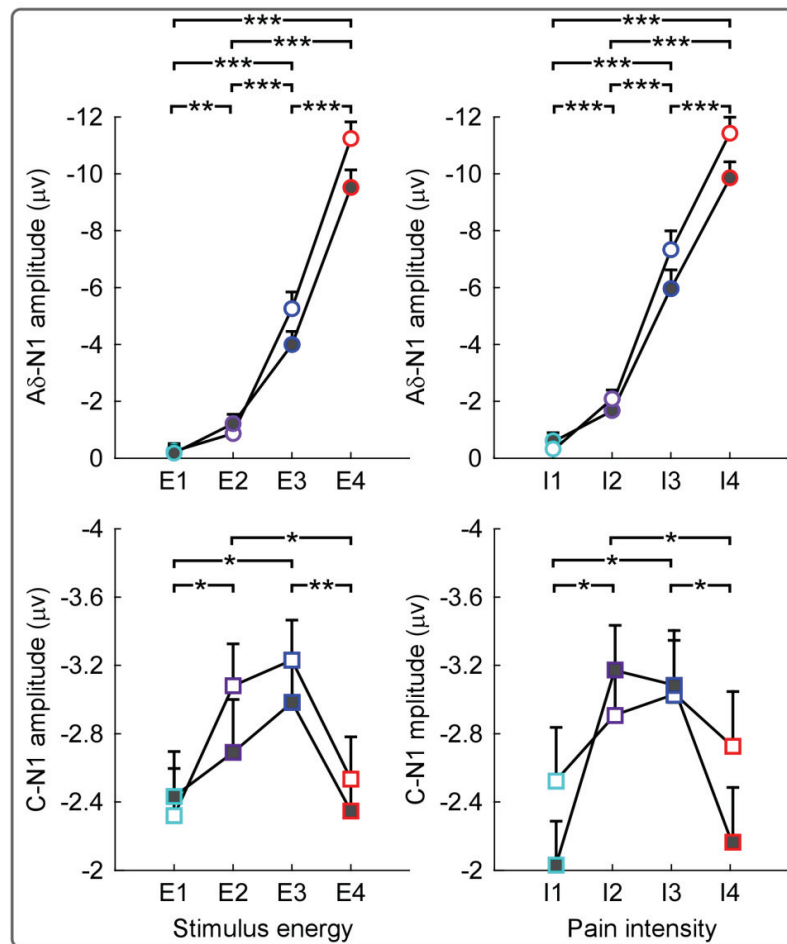


Figure 5. Correlations between C-N1 and other LEP waves.

The *top panel* shows all possible latency and amplitude correlations between the C-N1 and other LEP waves (i.e., Aδ-N1, Aδ-N2, Aδ-P2, C-N2, and C-P2). Red and blue dots represent positive and negative correlations, and the dot size reflect the significance of the correlation. When a correlation was significant, the corresponding box was marked in yellow. Note that for both left and right hand dorsum stimulations, C-N1 amplitudes were most strongly correlated with Aδ-N1 amplitudes. The *bottom panel* shows single-subject data for the significant correlations: each dot represents a subject, and black lines represent the best linear fit.

1029



1030

1031

1032 **Figure 6.** Dependence of human Aδ-N1 and C-N1 amplitudes on stimulus
1033 energy and perceived pain.

1034 Group-level amplitudes of Aδ-N1 (*top*, circles) and C-N1 (*bottom*, squares)
1035 waves elicited by stimulation of the left (empty symbols) and right (solid
1036 symbols) hand dorsum at different levels of stimulus energy (*left*: E1-E4) and
1037 different categories of pain intensity (*right*: I1-I4). Error bars represent SEM
1038 across subjects. Note that Aδ-N1 amplitudes increase monotonically with
1039 stimulus energy or pain intensity, whereas C-N1 amplitudes are maximal at a
1040 moderate levels of stimulus energy (E2 and E3) or pain intensity (I2 and I3).

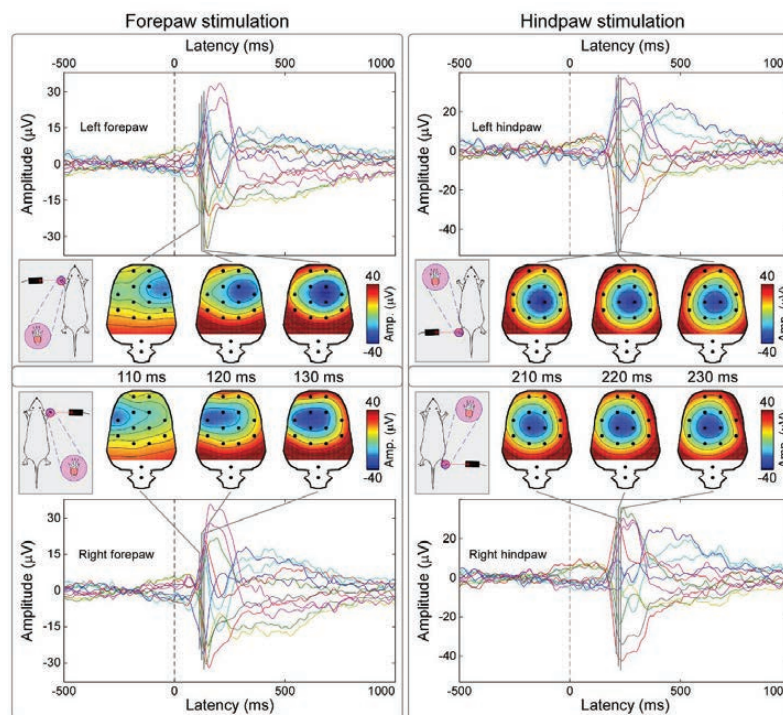


Figure 7. S1 contribution to the earliest part of the rat C-LEPs elicited by stimulation of the left and right (*top and bottom panels*) forepaw and hindpaw (*left and right panels*).

Grand averages waveforms from all electrodes are plotted in different colors and superimposed. Series of scalp topographies of the earliest part of C-LEPs (110-130 ms [forepaw]; 210-230 ms [hindpaw]) are displayed with a 10-ms interval. Note that the scalp topographies of the earliest part of the C-LEP response elicited by forepaw stimulation display a negativity strongly contralateral to the stimulated side, while the scalp topographies of the earliest part of C-LEP response elicited by hindpaw stimulation, albeit contralateral, is more centrally distributed. These topographies are compatible with the somatotopical organization of the primary somatosensory cortex, and indicate that S1 contributes to the early part of the brain response evoked by C-fiber stimulation.

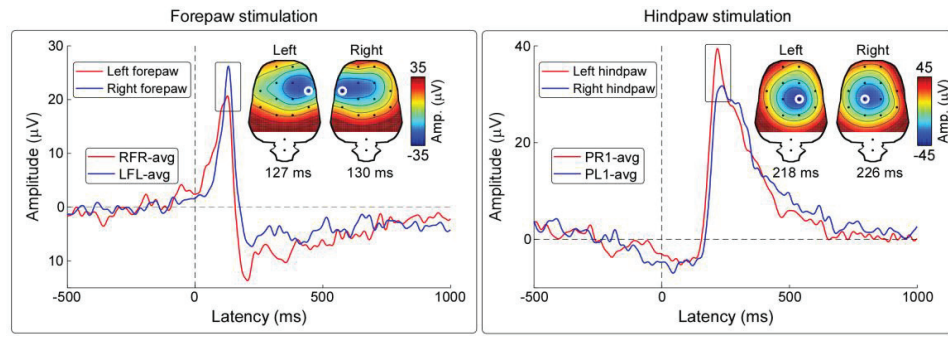


Figure 8. Isolation of C-N1 waves in rats.

Grand-average C-N1 waves elicited by the stimulation of the left (red waveforms) and right (blue waveforms) forepaw (*left panel*) and hindpaw (*right panel*). The C-N1 was optimally detected from the electrodes highlighted in white: RFR following left forepaw stimulation, LFL following right forepaw stimulation, PR1 following left hindpaw stimulation, and PL1 following right hindpaw stimulation. Scalp topographies at peak latency show a maximum contralateral to the stimulated side, clearly lateralized in the response elicited by forepaw stimulation, and less lateralized in the response elicited by hindpaw stimulation.

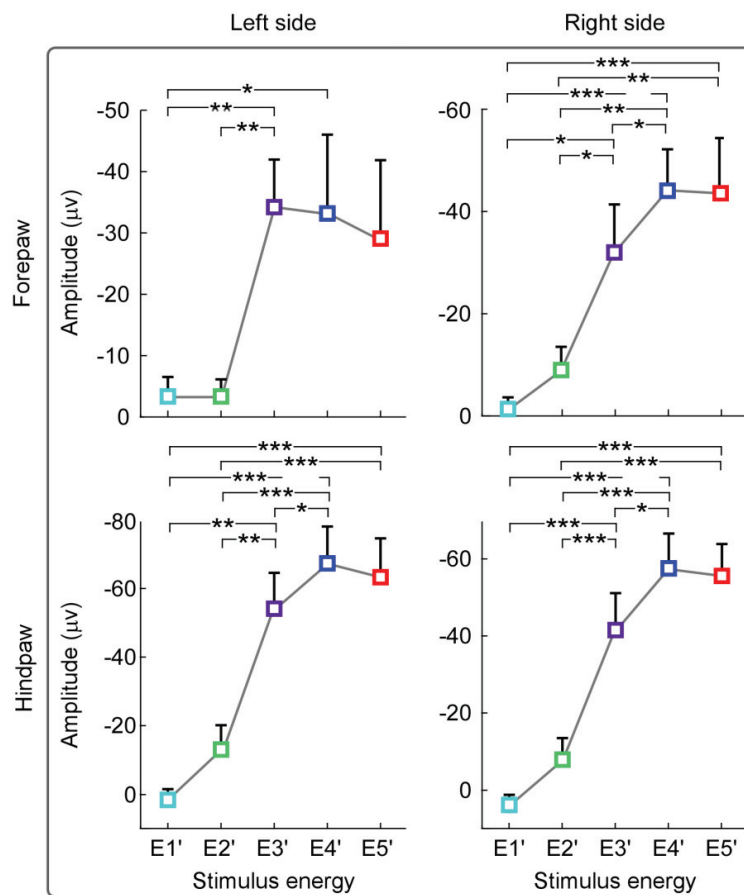


Figure 9. Dependence of rat C-N1 amplitudes on stimulus energy.

Group-level amplitudes of C-N1 waves elicited by stimulation of the left and right forepaws (*top panels*) and hindpaws (*bottom panels*) at different levels of stimulus energy (E1'-E5'). Error bars represent SEM across subjects. Note that, whereas human C-N1 amplitude is maximal at moderate level of stimulus energy (see Figure 6), rat C-N1 amplitude increases quasi-monotonically with stimulus energy, and only plateaus at E4'-E5'.

1080 **Tables**

1081

1082 **Table 1.** Latency and amplitude of LEP waves elicited by left and right hand stimulation.

	Left hand stimulation (mean ± SEM)		Right hand stimulation (mean ± SEM)	
	Latency (ms)	Amplitude (µV)	Latency (ms)	Amplitude (µV)
Aδ-N1	167 ± 3	-5.2 ± 0.3	173 ± 2	-4.3 ± 0.3
Aδ-N2	202 ± 2	-8.6 ± 0.4	206 ± 3	-7.8 ± 0.3
Aδ-P2	355 ± 4	6.1 ± 0.3	364 ± 4	5.7 ± 0.3
C-N1	736 ± 6	-3 ± 0.2	737 ± 6	-2.8 ± 0.2
C-N2	762 ± 5	-4.8 ± 0.2	765 ± 5	-4.9 ± 0.2
C-P2	886 ± 5	2.7 ± 0.2	890 ± 5	2.3 ± 0.2

1083 *Aδ-N2, Aδ-P2, C-N2, and C-P2 waves were measured at Cz-average.*
 1084 *Aδ-N1 and C-N1 waves were measured at Cc-Fz.*

1085

1086

1087 **Table 2.** Latency and amplitude correlations between LEP waves.

		Latency correlations (R values)						Amplitude correlations (R values)					
		Aδ-N1	Aδ-N2	Aδ-P2	C-N1	C-N2	C-P2	Aδ-N1	Aδ-N2	Aδ-P2	C-N1	C-N2	C-P2
C-N1	Left	0.33	0.38*	0.18	---	0.70*	0.46*	0.35*	0.05	-0.10	---	0.18	-0.34*
	Right	0.26	0.26	0.08	---	0.61*	0.48*	0.46*	0.14	-0.17	---	0.44*	-0.22
C-N2	Left	0.20	0.15	0.11	0.70*	---	0.76*	0.13	0.56*	-0.43*	0.18	---	-0.49*
	Right	0.14	0.13	0.07	0.61*	---	0.76*	0.26	0.56*	-0.56*	0.44*	---	-0.69*
C-P2	Left	0.14	0.04	0.17	0.46*	0.76*	---	0.14	0.00	0.12	-0.34*	-0.49*	---
	Right	0.18	0.14	0.07	0.48*	0.76*	---	-0.12	-0.32*	0.42*	-0.22	-0.69*	---

1088 *: $P_{\text{dir}} < 0.05$; Significant correlations for both left and right hand stimulations are marked in bold.

1089

Table 3. Effect of laser energy (E1-E4) and stimulation site on LEP amplitudes elicited by left and right hand stimulation.

	Left hand stimulation (mean $\pm$ SEM)				Right hand stimulation (mean $\pm$ SEM)				Two-way ANOVA		
	E1	E2	E3	E4	E1	E2	E3	E4	'energy'	'site'	Interaction
Pain intensity (0-10)	3.4 $\pm$ 0.1	4.2 $\pm$ 0.1	5.7 $\pm$ 0.1	7.6 $\pm$ 0.1	3.3 $\pm$ 0.1	4.1 $\pm$ 0.1	5.5 $\pm$ 0.2	7.4 $\pm$ 0.2	F=726.5***	F=5.7*	F=0.5
A δ -N1	-0.3 $\pm$ 0.2	-0.9 $\pm$ 0.3	-5.3 $\pm$ 0.5	-11.2 $\pm$ 0.5	-0.1 $\pm$ 0.2	-1.2 $\pm$ 0.3	-4.0 $\pm$ 0.4	-9.5 $\pm$ 0.6	F=243.9***	F=7.3**	F=3.4*
A δ -N2	0.1 $\pm$ 0.3	-2.9 $\pm$ 0.6	-15.5 $\pm$ 1.3	-33.4 $\pm$ 1.4	-0.2 $\pm$ 0.4	-2.3 $\pm$ 0.5	-13.1 $\pm$ 1.1	-32.5 $\pm$ 1.4	F=370.8***	F=3.8	F=1.6
A δ -P2	0.8 $\pm$ 0.4	3.0 $\pm$ 0.7	13.3 $\pm$ 1.0	26.1 $\pm$ 1.2	-0.1 $\pm$ 0.5	2.8 $\pm$ 0.6	12.2 $\pm$ 1.0	25.9 $\pm$ 1.2	F=300.7***	F=1.9	F=0.3
C-N1	-2.3 $\pm$ 0.3	-3.1 $\pm$ 0.2	-3.3 $\pm$ 0.2	-2.5 $\pm$ 0.2	-2.4 $\pm$ 0.3	-2.7 $\pm$ 0.3	-3.0 $\pm$ 0.3	-2.4 $\pm$ 0.2	F=3.6*	F=2.0	F=0.4
C-N2	-6.4 $\pm$ 0.6	-9.3 $\pm$ 0.6	-8.5 $\pm$ 0.5	-6.2 $\pm$ 0.6	-6.4 $\pm$ 0.7	-9.3 $\pm$ 0.7	-8.5 $\pm$ 0.5	-7.7 $\pm$ 0.5	F=9.6***	F=1.1	F=1.3
C-P2	6.1 $\pm$ 0.7	7.3 $\pm$ 0.6	4.0 $\pm$ 0.5	2.0 $\pm$ 0.8	6.2 $\pm$ 0.6	7.3 $\pm$ 0.6	5.3 $\pm$ 0.5	1.8 $\pm$ 0.5	F=31.5***	F=0.6	F=1.0

*, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.

A δ -N2, A δ -P2, C-N2, and C-P2 amplitudes (μ V) were measured at Cz-average.

A δ -N1 and C-N1 amplitudes (μ V) were measured at Cc-Fz.

