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Pinprick-evoked brain potentials: a novel tool to assess central sensitization of nociceptive pathways in humans

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Iannetti GD, Baumgärtner U, Tracey I, Treede RD, Magerl W. Pinprick-evoked brain potentials: a novel tool to assess central sensitization of nociceptive pathways in humans. *J Neurophysiol* 110: 1107–1116, 2013. First published May 15, 2013; doi:10.1152/jn.00774.2012.—Although hyperalgesia to mechanical stimuli is a frequent sign in patients with inflammation or neuropathic pain, there is to date no objective electrophysiological measure for its evaluation in the clinical routine. Here we describe a technique for recording the electroencephalographic (EEG) responses elicited by mechanical stimulation with a flat-tip probe (diameter 0.25 mm, force 128 mN). Such probes activate Aδ nociceptors and are widely used to assess the presence of secondary hyperalgesia, a psychophysical correlate of sensitization in the nociceptive system. The corresponding pinprick-evoked potentials (PEPs) were recorded in 10 subjects during stimulation of the right and left hand dorsum before and after intradermal injection of capsaicin into the right hand and in 1 patient with a selective lesion of the right spinothalamic tract. PEPs in response to stimulation of normal skin were characterized by a vertex negative-positive (NP) complex, with N/P latencies and amplitudes of 111/245 ms and 3.5/11 μV, respectively. All subjects developed a robust capsaicin-induced increase in the pain elicited by pinprick stimulation of the secondary hyperalgesic area (+91.5%, $P < 0.005$). Such stimulation also resulted in a significant increase of the N-wave amplitude (+92.9%, $P < 0.005$), but not of the P wave (+6.6%, $P = 0.61$). In the patient, PEPs during stimulation of the hypoalgesic side were reduced. These results indicate that PEPs *1*) reflect cortical activities triggered by somatosensory input transmitted in Aδ primary sensory afferents and spinothalamic projection neurons, *2*) allow quantification of experimentally induced secondary mechanical hyperalgesia, and *3*) have the potential to become a diagnostic tool to substantiate mechanical hyperalgesia in patients with presumed central sensitization.

central sensitization; electrophysiology; human; neuropathic pain; pain

AFTER SKIN INJURY, an increased sensitivity specific to stimulation with punctate mechanical stimuli occurs in a large, uninjured area surrounding the injury site (Hardy et al. 1952; Lewis 1936). This phenomenon, termed secondary hyperalgesia, is the consequence of neuroplastic changes leading to a state of sensitization in central nociceptive pathways (central sensitization) (Baumann et al. 1991; Meyer and Treede 2004; Simone et al. 1991).

Two forms of mechanical hyperalgesia occur in the area of secondary hyperalgesia: hyperalgesia to light stroking with tactile stimuli (dynamic mechanical allodynia) and hyperalgesia to needlelike stimuli (punctate hyperalgesia). Although both stroking and punctate hyperalgesia are due to sensitization of nociceptive pathways, they have different psychophysical characteristics and are mediated by different primary afferents (Meyer and Treede 2004): stroking hyperalgesia is signaled by low-threshold mechanoreceptors (Torebjork et al. 1992), whereas punctate hyperalgesia is signaled by capsaicin-insensitive, type I A-fiber mechano-heat (I-AMH) nociceptors that project to mechanosensitive spinal interneurons sensitized by strong activation of C-fiber nociceptors, e.g., after capsaicin injection (Magerl et al. 2001; Simone et al. 1991; Ziegler et al. 1999).

Hyperalgesia to punctate stimuli is a sign in many frequent clinical conditions, like some forms of postherpetic neuralgia (Fields et al. 1998) and other neuropathic pain conditions (Jörum et al. 2003; Maier et al. 2010), restless legs syndrome (Stiasny-Kolster et al. 2004), or postoperative pain (Lavand'homme et al. 2008). Neuropathic pain is defined as "pain caused by a lesion or disease of the somatosensory system" (Jensen et al. 2011). Sensitization of nociceptive neurons in the spinal cord by primary nociceptive afferent input is thought to be the mechanism underlying punctate hyperalgesia during neuropathic pain. Accordingly, patients with neuropathic pain and hyperalgesia exhibit a shift of stimulus-response function and incidence of hyperalgesia to stroking and punctate stimuli nearly identical to normal subjects after capsaicin injection (Baumgärtner et al. 2002). Thus capsaicin-induced experimental hyperalgesia is a valid human surrogate model for the study of mechanical hyperalgesia and supports the view that sensitization of nociceptive pathways is an important feature of neuropathic pain states (Klein et al. 2005; Magerl et al. 1998).

Despite its high clinical relevance, there is at present no electrophysiological measure for the objective evaluation of mechanical hyperalgesia in the clinical routine. Currently, mechanical hyperalgesia is only measured psychophysically, either by mapping the area where it is present (LaMotte et al. 1991) or by calculating the leftward shift in stimulus-response functions (Baumgärtner et al. 2002). However, these approaches rely heavily on the reliability and trustworthiness of the subject and on the experience of the operator. Because of *1*) the high clinical relevance of mechanical hyperalgesia, and *2*) the subjective nature of the psychophysical approaches to assess it, the availability of an objective laboratory measure of this common positive symptom of neuropathic pain has been long-awaited and would be extremely beneficial both in basic research and in clinical practice.

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Here we describe a technique for activation of I-AMH units (i.e., the subgroup of A-fiber nociceptors sensitized in mechanical hyperalgesia) with small-diameter flat-tip mechanical stimulators (Greenspan and McGillis 1991; Magerl et al. 2001; Slugg et al. 2000; Ziegler et al. 1999) modified to obtain time-locked electroencephalographic (EEG) responses (pinprick-evoked potentials, PEPs). We show that PEPs are conducted in the spinothalamic pathways of the spinal cord and brain stem and are significantly enhanced when skin with capsaicin-induced hyperalgesia is stimulated. Thus PEPs may represent a useful tool for the objective assessment of experimentally induced mechanical hyperalgesia in normal volunteers and pathology-induced mechanical hyperalgesia in patients.

METHODS

Subjects

Ten healthy volunteers, aged 30 ± 2.6 yr (range 22–49 yr), and one patient participated in the study. The patient was a 29-year-old woman who developed neuropathic pain after surgical removal of a spinal neurinoma at right C_1–C_2 level. After surgery she exhibited thermal hypalgesia on the left side of the body below the level of the lesion. All healthy volunteers and the patient gave their informed consent, the study conformed to the standards set by the Declaration of Helsinki, and the local ethics committee approved the procedures.

Mechanical Stimulation of Type I-AMH Afferents

Noxious mechanical stimuli were generated by a cylindrical stainless steel wire with a flat tip (diameter 0.25 mm; Fig. 1). The wire was mounted on a plastic rod with a weight, which was free to move within a polished, handheld stainless steel tube. When applied perpendicularly to the skin, the weight of the rod rested exclusively on the wire tip, thus exerting a constant force of 128 mN. Such mechanical stimulators preferentially activate capsaicin-insensitive type I-AMH afferents, a notion supported by the observation that desensitization with chronic capsaicin application abolishes sensations and brain potentials evoked by laser stimuli activating type II-AMH units but only minimally affects the sensations evoked by the pinprick mechanical stimuli used in the present study (Magerl et al. 2001; Mouraux et al. 2010) and corroborated by animal electrophysiology of nociceptive primary afferents (Slugg et al. 2000).

The pinprick stimulator was held with the wire tip ~1 cm above the skin. The stimulation was performed by quickly moving the stimulator onto the skin at maximal speed (as in a needle prick) and then immediately removing it. To obtain precise information about when the stimulus was applied, an optical detector was mounted inside the tube just above the upper margin of the weight (Fig. 1, *B* and *C*). As soon as the weight started to move within the tube, the light beam was interrupted and an electrical signal (TTL) was generated. This device allowed us to synchronize the timing of the stimulation with the EEG recording and thus detect stimulus-locked EEG responses. The delay between the first contact with the skin and the TTL generation was 33 ± 1.7 ms (SE), as tested in 40 trials using a shortcut circuitry with digitization of the trigger responses.

To obtain precise information about the waveform of the mechanical stimulus, we recorded the force applied by the mechanical stimulus across time, using a fast force transducer with a sampling rate of 1 kHz (Siconn-pro Messtechnik, Geitmann Technik, Menden, Germany). Data were recorded with Dasylab software (Measurement Computing, Norton, MA). The stimulus was applied on the force transducer by the same experimenter who delivered the stimulation during the PEP recordings (U. Baumgärtner). To allow unrestricted depiction of the rise of force, the proportional-integral-derivative (PID) controller settings were adjusted for maximal slew rate.

Model of Mechanical Hyperalgesia

To induce a state of mechanical hyperalgesia, a 10 mM solution of capsaicin (40 μg capsaicin dissolved in 12.5 μl of 0.16% Tween 80 in normal saline; for details see Magerl et al. 2001) was injected intradermally into the center of the stimulated area of the right hand dorsum of the healthy volunteers. Subjects were asked to rate the magnitude of the capsaicin-induced pain for 5 min (every 10 s for the first minute and every 30 s thereafter) on a numerical rating scale ranging from 0 (no pain) to 100 (most intense pain imaginable). Even though the vast majority of I-AMH units are not sensitive to capsaicin (Ringkamp et al. 2001), the information they transmit is amplified via a heterosynaptic facilitation caused by the intense capsaicin-induced afferent volley in C-fiber nociceptors (Ziegler et al. 1999).

Experimental Protocol

In all subjects we recorded the brain potentials evoked by noxious mechanical stimuli (PEPs). In the healthy volunteers PEPs were recorded in two periods on the same day. Before the recording started, the area to be stimulated was defined by marking with a felt pen the edges of two circles (diameter 4 cm) centered on the right and on the left hand dorsum. The centers of these two areas, symmetrical on the two hands, were also marked. In each block, 40 mechanical stimuli were delivered to the dorsum of the right and of the left hand, in a balanced and randomized fashion (20 stimuli for each hand), with an interstimulus interval of ~10–15 s. Stimuli were delivered in a pseudorandom spatial pattern, ensuring that the same skin spot was not stimulated

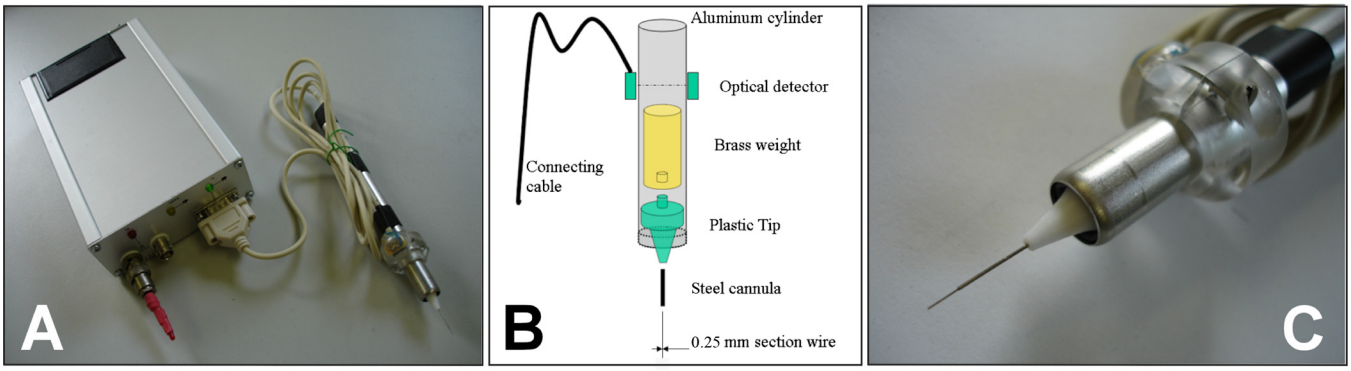

Fig. 1. Pinprick stimulation device. The mechanical stimulator consists of a very thin stainless steel probe with a flat tip (diameter 0.25 mm) that touches the skin (*A*). A brass weight freely moving within a polished stainless steel cylinder allows delivery of a constant force of 128 mN (*B*). To have accurate timing of the onset of the stimulation (a crucial requisite for reliable recording of stimulus-locked EEG responses), an optical detector was placed inside the cylinder. When the light of the detector was interrupted by the moving weight, an electrical signal (TTL) was generated by a trigger box connected to the stimulator (*C*).

again before 5 stimulus repetitions and thus producing an interstimulus interval of at least 1 min for the same skin site. Between 3 and 6 s after the stimulus, subjects were asked to provide a rating of the magnitude of the stimulus-induced pain on a numerical scale ranging from 0 (no pain) to 100 (most intense pain imaginable). Five minutes after the end of the first period, mechanical hyperalgesia was induced by capsaicin injection. Ten minutes after the capsaicin injection, the second period of stimulation and PEP recording was performed.

To investigate whether action potentials leading to PEPs are conducted by axons running in either the dorsal columns or the anterolateral tract of the spinal cord, we tested the function of both pathways specifically, by quantitative sensory testing and evoked potentials in the patient. Sensory testing was done semiquantitatively by scoring for proprioceptive, tactile, thermal, and nociceptive sensitivity. It consisted of passive movement of finger and toes for proprioception (maximum score of 4), touching with cotton wool and calibrated von Frey hairs for tactile sensitivity (maximum score of 8), stimulation with a tuning fork for vibration sensitivity (8/8 Rydel-Seyffer, maximum score of 4), brief or sustained contact with warm or cold water-filled glass tubes for thermal sensitivity (maximum score of 4 each), and pin prick, pulling of a single hair, sharp vs. blunt discrimination, and strong pressure to the tendons of the gastrocnemius and adductor pollicis muscles for nociception (maximum score of 2 each) (for a more detailed description of this simplified quantitative sensory testing see Spiegel et al. 2003). We assessed mechanoreceptive function by recording somatosensory evoked potentials (SEPs) elicited by the stimulation of the right and left median nerves and mediated by dorsal column pathways. Nociceptive function was assessed by recording laser-evoked potentials (LEPs) elicited by stimulation of the right and left hand dorsum and mediated by anterolateral spinothalamic pathways. PEPs were also recorded, as described above. LEP and SEP recordings were performed according to the recommendations described by Treede et al. (2003) and Cruccu et al. (2008a).

EEG Recording

Participants were seated in a comfortable reclining chair and asked to relax their muscles and keep their eyes open and gaze slightly downward. They were also instructed to focus on the stimulus. Brain electrical activity was recorded with silver disk electrodes from 31 channels in an equidistant modification (Easycap) of the International 10–20 electrode spacing system and digitized with a sampling rate of 1 kHz (Neurotop amplifier, Nihon Kohden). The EEG was recorded in sweeps with a prestimulus segment of 1 s and a poststimulus segment of 3 s. To monitor ocular movements or eyeblinks and subsequently correct contaminated trials, electrooculographic (EOG) signals were simultaneously recorded with surface electrodes. In the patient, we used a reduced six-channel recording (Fz, Cz, Pz vs. earlobe reference, T3, T4 vs. Fz reference, and vertical EOG).

EEG Data Analysis

EEG data were imported and all analyses carried out with EEGLAB (**www.sccn.ucsd.edu/eeglab**), an open-source toolbox running under the MATLAB environment (Delorme and Makeig 2004) and Letswave (Mouraux and Iannetti 2008). EEG data were first downsampled to 256 Hz and band-pass filtered from 0.5 to 50 Hz. EEG epochs containing the somatosensory stimuli were subsequently extracted with a window analysis time of 2 s (from 500 ms before to 1,500 ms after the stimulus) and computed against average reference. For each epoch, a baseline correction for the data preceding the stimulus by 500 ms was performed. EEG epochs were visually inspected, and trials contaminated with artifacts due to gross movements were removed. Trials contaminated by artifacts due to eyeblinks were corrected with an independent component analysis (ICA) algorithm (Jung et al. 2000). In all data sets where this procedure was performed, individual eye movements could be seen in the independent component (IC) removed. The IC removed also had a large EOG channel contribution and a frontal scalp distribution.

EEG epochs were finally averaged across trials separately for each hand and period (for a total of 4 averages for each subject: left hand/precapsaicin, left hand/postcapsaicin, right hand/precapsaicin, right hand/postcapsaicin). For each subject we measured the peak latencies and the baseline-to-peak amplitudes of the main negative (N) and the main positive (P) waves of the vertex response (Cz against average reference). In 5 of 40 cases the N wave could not be unequivocally discriminated from EEG background noise, and in these cases the amplitude estimate of the N wave was replaced by average background noise level. The stimulus-locked responses were finally averaged across subjects to obtain group-level waveforms. To compare scalp distribution of PEPs, isopotential topographical maps were obtained by linear interpolation of the four nearest electrodes, using amplitudes from grand-averaged, reference-free PEP data of each condition.

Data Evaluation and Statistics

As the distributions of pain ratings and LEP amplitudes were found not to be normal, they were log-transformed to achieve secondary normal distribution and all statistics were calculated on these log values. To take into account the time-dependent effect of habituation, the psychophysical and electrophysiological responses obtained in *period 2* for both right and left hands were expressed, for each subject, as percent signal change compared with the corresponding responses obtained in *period 1*. The percent change differences of ratings and peak amplitudes were assessed with two-tailed, paired *t*-tests. The effect of habituation was estimated for each subject from the between-period percent change differences observed after left hand stimulation. To take into account these effects, the between-period percent change during right hand stimulation was compared with the between-period percent change during left (i.e., noninjected) hand stimulation. These differences were also tested by performing a two-way repeated-measures ANOVA, with "period" (2 levels: "precapsaicin" and "postcapsaicin") and "hand" (2 levels: "treated-right" and "untreated-left") as experimental factors, as well as the possible interaction between them, on the following parameters: pain ratings and latencies and amplitudes of N and P waves. All values are expressed as means ± SE.

RESULTS

Force Applied by Pinprick Stimulation

Figure 2 shows the plots of the force applied by the mechanical stimulation across time, using a fast force transducer. The measurements show that when the mechanical stimulus is applied on the force transducer, there is a clear overshoot in the first millisecond of the stimulation period and an undershoot at the stimulation offset. Note that the overshoot and subsequent ringing of the signal at onset and offset of the stimulus result from the undampened sensor-plus-amplifier circuitry. The stimulation force is constant, at ~128 mN, throughout the time window in which the stimulus is applied.

Psychophysics of Pinprick-Evoked and Capsaicin-Evoked Pain

The mechanical stimulation elicited a clear pinprick sensation in all subjects. Prior to capsaicin injection, average pain ratings on the 0–100 numerical rating scale were 7.8 ($\log_{10}$-mean: 0.892 ± 0.091) after left hand stimulation and 7.9 ($\log_{10}$-mean: 0.899 ± 0.085) after right hand stimulation. The ratings of the pain sensation elicited by the stimulation of the

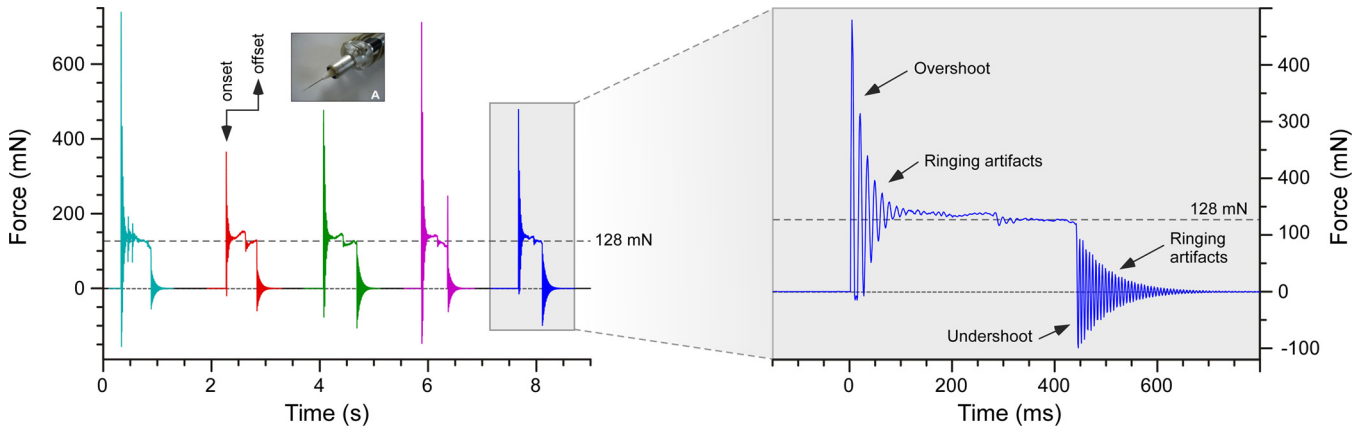

Fig. 2. Time profile of the force applied by the mechanical stimulus. The 128-mN mechanical stimulator (*inset A*) was used to apply the stimulus on an undampened fast force transducer with a sampling rate of 1 kHz. *Left*: application of 5 consecutive stimuli by the same experimenter. There is a pronounced overshoot in the first millisecond of the stimulation period and a similar undershoot at the stimulation offset. Note that the overshoot and subsequent ringing of the signal at onset and offset of the stimulus result from the high slew rate of undampened sensor-plus-amplifier circuitry. Note also that the resulting force is constant at ~128 mN throughout the time window in which the stimulus is applied. *Right*: enlargement of the 5th stimulus on *left*.

two hands were not significantly different ($P = 0.68$) and highly correlated ($r = 0.98$, $P < 0.001$).

Intradermal capsaicin injection into the dorsum of the right hand elicited a strong burning pain, which was maximal at the time of the injection (95.5 ± 8.6) and rapidly declined with a half-time of ~2 min (average pain rating at 2 min: 47.7 ± 5.8). Five minutes after the injection, the pain sensation had already disappeared in 5 of 10 subjects and, across all subjects, was not significantly different from zero anymore (5.8 ± 2.6). At this point, the skin surrounding the injection site exhibited enhanced pain sensitivity to mechanical stimuli in 9 of 10 subjects.

PEPs in Healthy Volunteers

In all subjects, pinprick stimulation elicited clear and reproducible PEPs, time-locked to the onset of the stimulus. PEPs recorded at the vertex (Cz) were characterized by a large biphasic negative-positive (NP) complex. Across all trials, the N and P waves had average peak latencies of 111 ± 7.8 ms and 245 ± 17.2 ms (when corrected for trigger delay), respectively. Average peak amplitudes of the N and P waves were 3.5 μV ($\log_{10}$-mean: 0.544 ± 0.323) and 11.1 μV ($\log_{10}$-mean: 1.045 ± 0.153), respectively (Figs. 3 and 4). Both N- and P-wave amplitudes were also highly correlated

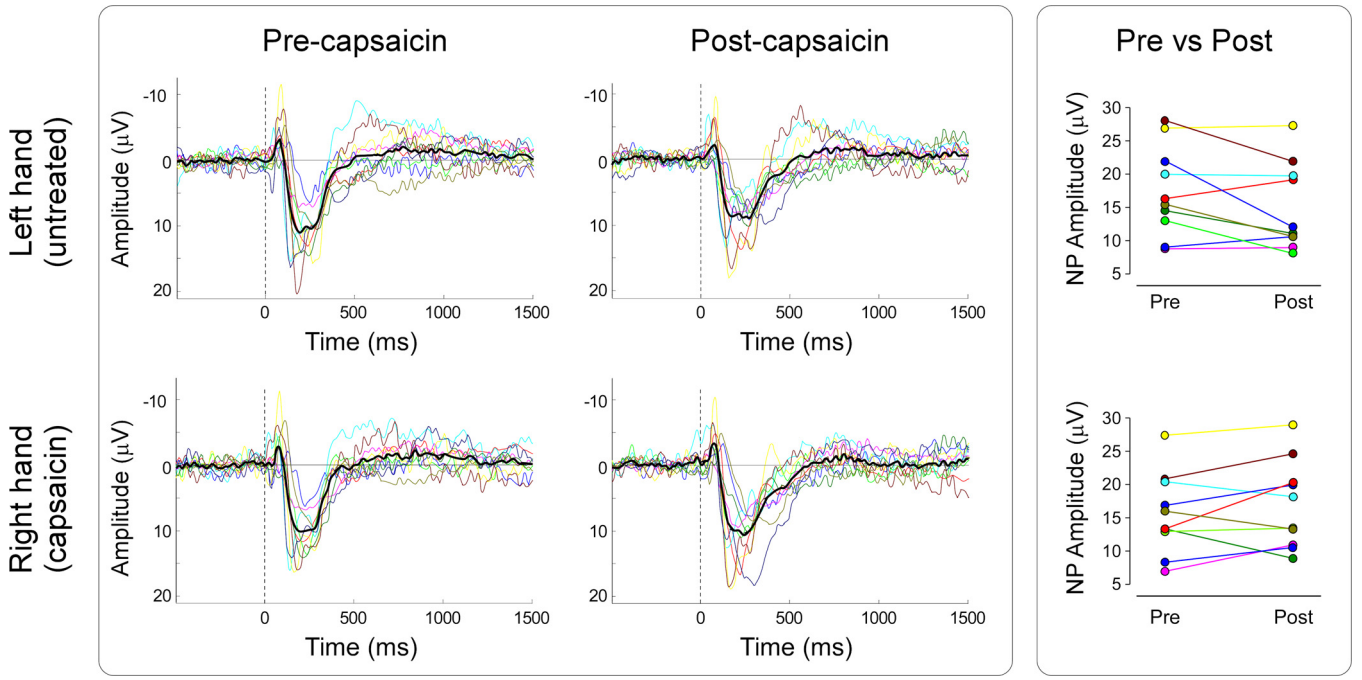

Fig. 3. Waveforms and amplitudes of pinprick-evoked potentials (PEPs). *Left*: average EEG responses to mechanical stimuli (PEPs). Mechanical stimuli were applied to the dorsum of the right and left hands before (Pre) and after (Post) intradermal injection of the vanilloid capsaicin on the dorsum of the right hand. Brain potentials, recorded from the vertex (Cz vs. average reference), are averaged time-locked to the onset of the mechanical stimulus (see METHODS for details). Thin colored waveforms represent single subjects, whereas the thick black waveform is the grand average across subjects. *Right*: single-subject amplitude of the main negative-positive complex at the vertex (N-P). Subjects are identified with the same colors as in the waveform plots. Note the increase in N-P amplitude after capsaicin injection into the dorsum of the right hand.

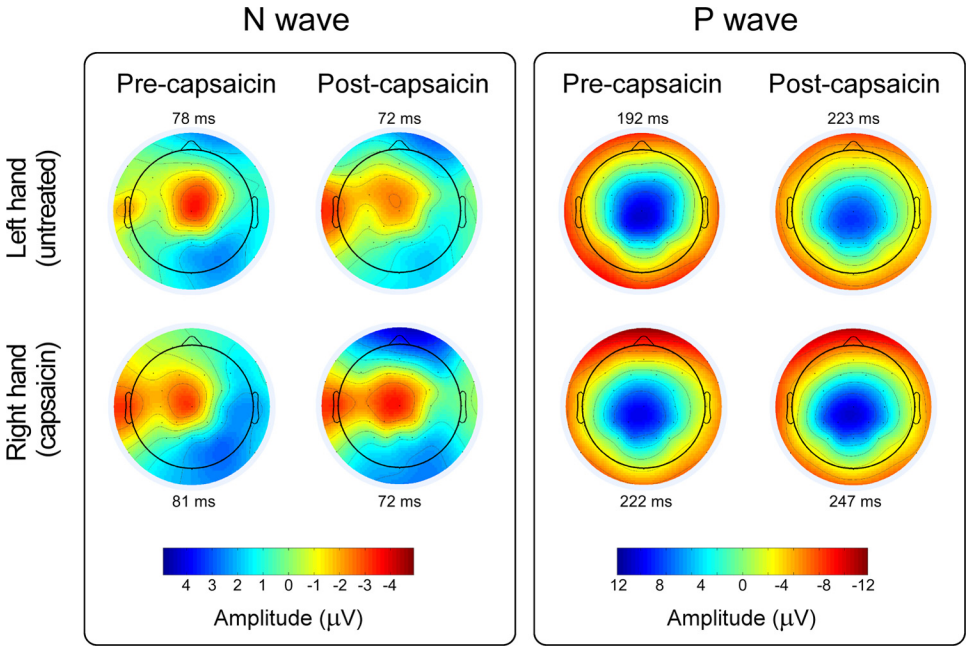

Fig. 4. Scalp topography. Scalp topographies of the grand-average PEPs after stimulation of the dorsum of the right and left hands before (*period 1*) and after (*period 2*) intradermal injection of the vanilloid capsaicin on the right hand dorsum. Both the main negative (N) and positive (P) peaks had maximal amplitude at the central electrodes. Note the negativity in the left temporal areas in the time window of the N peak. This activity is stronger when stimuli were applied contralaterally (on the right hand) but still prevalent on the left side also when stimuli were applied ipsilaterally (on the left hand) (see DISCUSSION).

between left and right hand stimulation ($r = 0.93$ and $r = 0.96$, respectively, $P < 0.001$ each).

The scalp distribution at peak latency of the N wave revealed a centrally distributed negative maximum, with an additional local negative maximum over the left temporal lead (electrode T7), after both right and left hand stimulation (Fig. 4). The group-level average time course of stimulus-evoked activity revealed an early negative wave only at the left temporal electrode (T7) regardless of the stimulated hand. The latency and amplitude of this early negative wave were 76 ms and 2.3 μV (right hand stimulation) and 74 ms and 1.0 μV (left hand stimulation) (Fig. 5). In contrast, the P wave had a central scalp distribution.

Habituation and Capsaicin-Induced Sensitization of Pinprick-Evoked Pain and PEPs

Two-way repeated-measures ANOVA on pain ratings revealed significant main effects of the factors "period" ($P < 0.01$) and "hand" ($P < 0.002$) and, crucially, a highly significant interaction between them ($F_{1,9} = 20.21$, $P < 0.002$; Table 1). Pain ratings to stimulation of the untreated (left) hand were significantly lower in the "post" than in the "pre" period (habituation effect), with an average reduction of -8.9% ($\log_{10}$-mean: -0.041 ± 0.015, $P < 0.05$), whereas pain ratings to stimulation of the treated (right) hand were significantly higher in the "post" than in the "pre" period (sensitization effect), with an average increase of $+74.4\%$, ($\log_{10}$-mean: 0.242 ± 0.059, $P < 0.005$; Fig. 5). When corrected for habituation (as assessed in the untreated hand), the capsaicin-induced sensitization caused an average increase of $+91.5\%$ ($\log_{10}$-mean: 0.282 ± 0.063, $P < 0.002$; Fig. 6, *right*).

Two-way repeated-measures ANOVA on the amplitude of the N wave of PEPs revealed no significant main effects of the factors "period" and "hand." However, the interaction between these factors was highly significant ($F_{1,9} = 14.94$, $P < 0.005$;

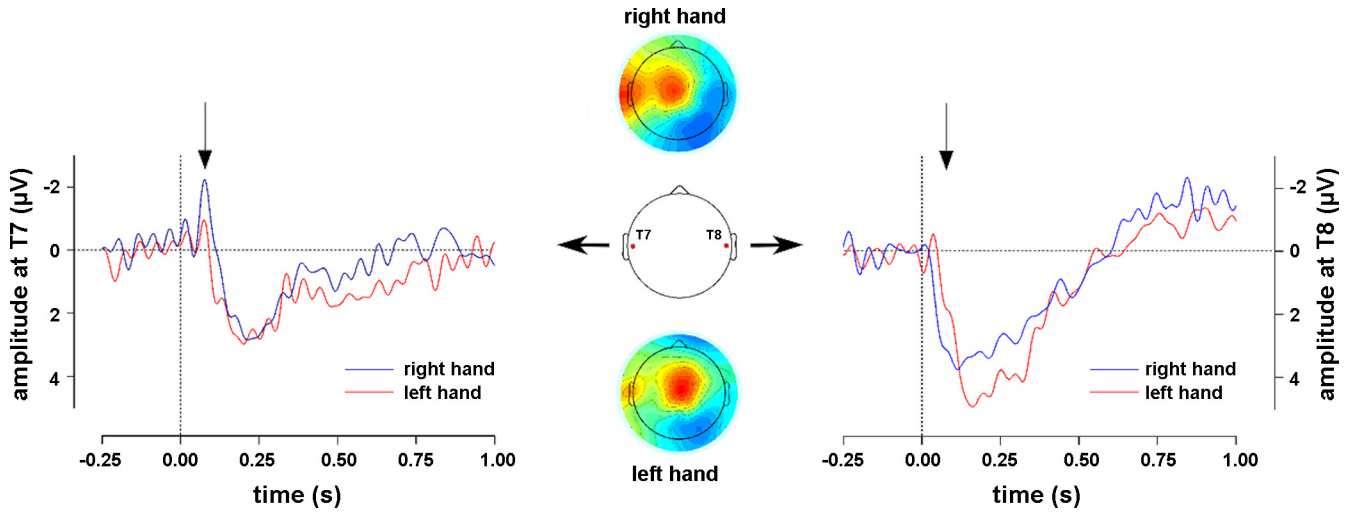

Fig. 5. Early-latency response. *Center*: scalp topography at the latency of the main negative peak. Note the additional, smaller maximum observed on the left temporal lead, independently of the side of stimulation. *Left* and *right*: response time course at left (T7) and right (T8) temporal electrodes. Arrows indicate the latency at which the scalp maps at *center* are plotted.

Table 1. *Summary of ANOVA results*

Pain ratings		
Main effect of "period"	$F = 11.37$	$P <$ **0.01****
Main effect of "hand"	$F = 20.84$	$P <$ **0.002*****
"Period" × "hand"	$F = 20.21$	$P <$ **0.002*****
N-wave amplitude		
Main effect of "period"	$F = 0.34$	$P = 0.57$
Main effect of "hand"	$F = 1.40$	$P = 0.27$
"Period" × "hand"	$F = 14.94$	$P <$ **0.005*****
P-wave amplitude		
Main effect of "period"	$F = 0.14$	$P = 0.72$
Main effect of "hand"	$F = 0.26$	$P = 0.62$
"Period" × "hand"	$F = 0.28$	$P = 0.61$
NP amplitude		
Main effect of "period"	$F = 0.43$	$P = 0.53$
Main effect of "hand"	$F = 0.01$	$P = 0.92$
"Period" × "hand"	$F = 3.87$	$P =$ **0.08**(*)
N-wave latency		
Main effect of "period"	$F = 1.16$	$P = 0.31$
Main effect of "hand"	$F = 2.72$	$P = 0.13$
"Period" × "hand"	$F = 0.39$	$P = 0.55$
P-wave latency		
Main effect of "period"	$F = 1.47$	$P = 0.26$
Main effect of "hand"	$F = 0.01$	$P = 0.91$
"Period" × "hand"	$F = 3.12$	$P = 0.11$

N, negative; P, positive. Significant P values are in bold: (*)$P < 0.1$, **$P < 0.01$, ***$P < 0.005$.

Table 1). While the N wave elicited by the stimulation of the untreated (left) hand tended to habituate with an average reduction of −32.8% ($\log_{10}$-mean: −0.179 ± 0.089, $P = 0.08$), the N wave elicited by the stimulation of the capsaicin-injected (right) hand tended to sensitize with an average increase of +27.6% ($\log_{10}$-mean: +0.106 ± 0.052, $P < 0.08$; Fig. 6, *left*). When corrected for habituation (as assessed in the untreated hand), the net capsaicin-induced sensitization caused an average increase of +92.9% ($\log_{10}$-mean: 0.285 ± 0.074, $P < 0.005$; Fig. 6, *right*).

In contrast, two-way repeated-measures ANOVA on the amplitude of the P wave of PEPs revealed neither significant main effects nor interaction between the factors (Table 1 and Fig. 6, *center*). Likewise, the increase after right hand stimulation corrected for habituation was only 6.6% ($\log_{10}$-mean: 0.028 ± 0.052, $P = 0.61$; Fig. 6, *right*). Similarly, the PEP peak-to-peak amplitude (NP complex) revealed only a trend for a capsaicin-induced increase of +23.1% ($\log_{10}$-mean: 0.090 ± 0.046, $P = 0.08$; see also Table 1 and Fig. 6, *center* and *right*).

No significant effects were found on the latencies of the N and P waves (Table 1). Finally, there was no significant correlation between the capsaicin-induced changes of pain perception (i.e., the hyperalgesia) and changes of the PEP amplitude (N wave: $r = -0.25$, P wave: $r = -0.28$, NP complex: $r = -0.28$; all $P > 0.30$).

Evidence that Anterolateral Quadrant of Spinal Cord Encompasses Ascending Pathway Responsible for PEPs

To investigate whether action potentials leading to PEPs are conducted by axons running in either the dorsal columns (mechanoreceptive; spinobulbar pathway) or the anterolateral tract (nociceptive; spinothalamic pathway) of the spinal cord, we tested the function of both pathways specifically, by quantitative sensory testing and evoked potentials (SEP and LEP), together with PEPs.

Clinical examination. The clinical sensory examination of the patient's four extremities (done by simplified quantitative sensory testing according to Spiegel et al. 2003) revealed normal dorsal column function as assessed by stimulation of both hands and feet (64/64 = 100% for combined proprioception and mechanoreception). In contrast, both thermal sensitivity (1/16 = 6%) and nociceptive sensitivity (9/16 = 56%) were impaired on the left hand and foot, whereas on the right hand and foot both thermal and nociceptive sensitivity were normal (16/16 = 100%). This impairment of small-fiber function on the left side was significant both when tested against dorsal column function of the same limb ($P < 0.0001$, Fisher's exact test) and when tested against thermal or nociceptive scores of the contralateral (right) body side ($P < 0.01$, Fisher's exact test).

SEPs. SEPs also revealed that the function of lemniscal pathways was preserved. Both right and left median nerve

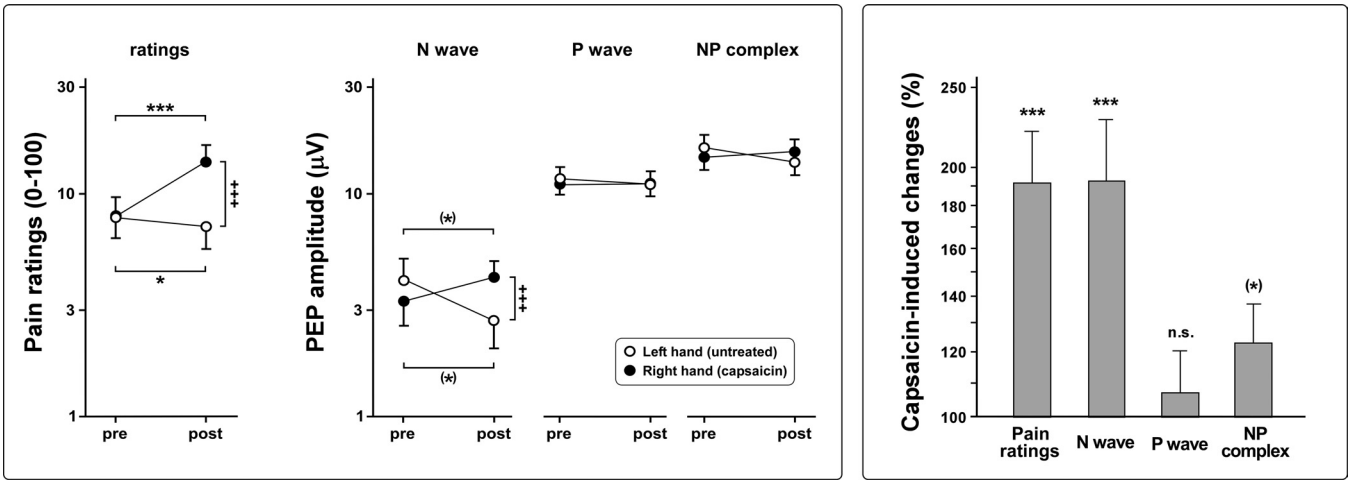

Fig. 6. Effect of capsaicin injection on psychophysics and PEP amplitude. *Left* and *center*: intradermal injection of capsaicin in the right hand elicited a site-specific significant increase of both pain ratings (*left*) and amplitudes of PEP responses (*center*). Note the habituation of both pain ratings and amplitudes of PEP responses in the control side (left hand). *Right*: % increases of pain and PEP amplitudes (right hand dorsum) corrected for habituation of the control side (left hand dorsum). A significant increase in pain responses (+91.5%) in the right hand dorsum is paralleled by a similar increase in the N-wave amplitude of the PEP (+92.9%) but not the P-wave amplitude of the PEP (+6.6%, $P = 0.61$). (*)$P < 0.10$, *$P < 0.05$, ***$P < 0.005$ vs. preinjection value; $^{+++}P < 0.005$ vs. untreated control hand (left). n.s., Not significant.

stimulation elicited an N20 wave of normal latency and amplitude (right stimulation: 17.5 ms, 2.0 μV; left stimulation: 17.8 ms, 2.2 μV) (Fig. 7, *top*).

Laser-evoked sensations and LEPs. The threshold for laser-induced pain in the unaffected (right) side was normal (270 mJ), but it was increased in the affected (left) side (420 mJ, abnormal in intraindividual, side-to-side comparison; for reference data, see Spiegel et al. 2000). During suprathreshold laser stimulation, all stimuli applied to the dorsum of the unaffected hand were detected (100%), and 72 of these 80 stimuli (90%) were perceived as painful. On the affected hand, however, only 60 of 80 stimuli were detected (75%), and only 23 of 80 (29%) were perceived as painful (χ^2, $P < 0.0001$ for incidence of detection and pain).

LEPs revealed that the function of the spinothalamic pathway was impaired ipsilaterally to the spinal lesion. Laser stimulation of the unaffected hand elicited an LEP of normal latency and amplitude (N2/P2 latencies: 164/216 ms; N2-P2 amplitude: 42.8 μV). In contrast, laser stimulation of the affected hand elicited an LEP of abnormally increased latency (N2/P2 latencies: 309/470 ms, i.e., both >3 SDs longer than normal; Spiegel et al. 2000) and decreased N2-P2 amplitude (reduced by ~60%: 17.1 μV, i.e., <3 SDs smaller than normal side; Spiegel et al. 2003) (Fig. 7, *middle*).

Pinprick-evoked pain and PEPs. Pinprick stimuli delivered to the unaffected (right) hand dorsum elicited an average pain rating of 45.8 ± 1.9. In contrast, pinprick stimuli delivered to the affected (left) hand dorsum elicited an average pain rating of 27.0 ± 1.7 (~40% lower, $P < 0.001$, *t*-test).

Pinprick stimulation of the unaffected hand elicited a PEP with peak latency and amplitude comparable to those of control subjects (N/P latencies: 131/225 ms; N-P amplitude: 31.7 μV), while pinprick stimulation of the affected hand elicited a PEP of comparable latency (N/P: 127/181 ms) but of reduced amplitude (N-P: 15.8 μV, ~50% lower) (Fig. 7, *bottom*).

DISCUSSION

Here we report three main results. First, when accurate information about the onset of the stimulation is available,

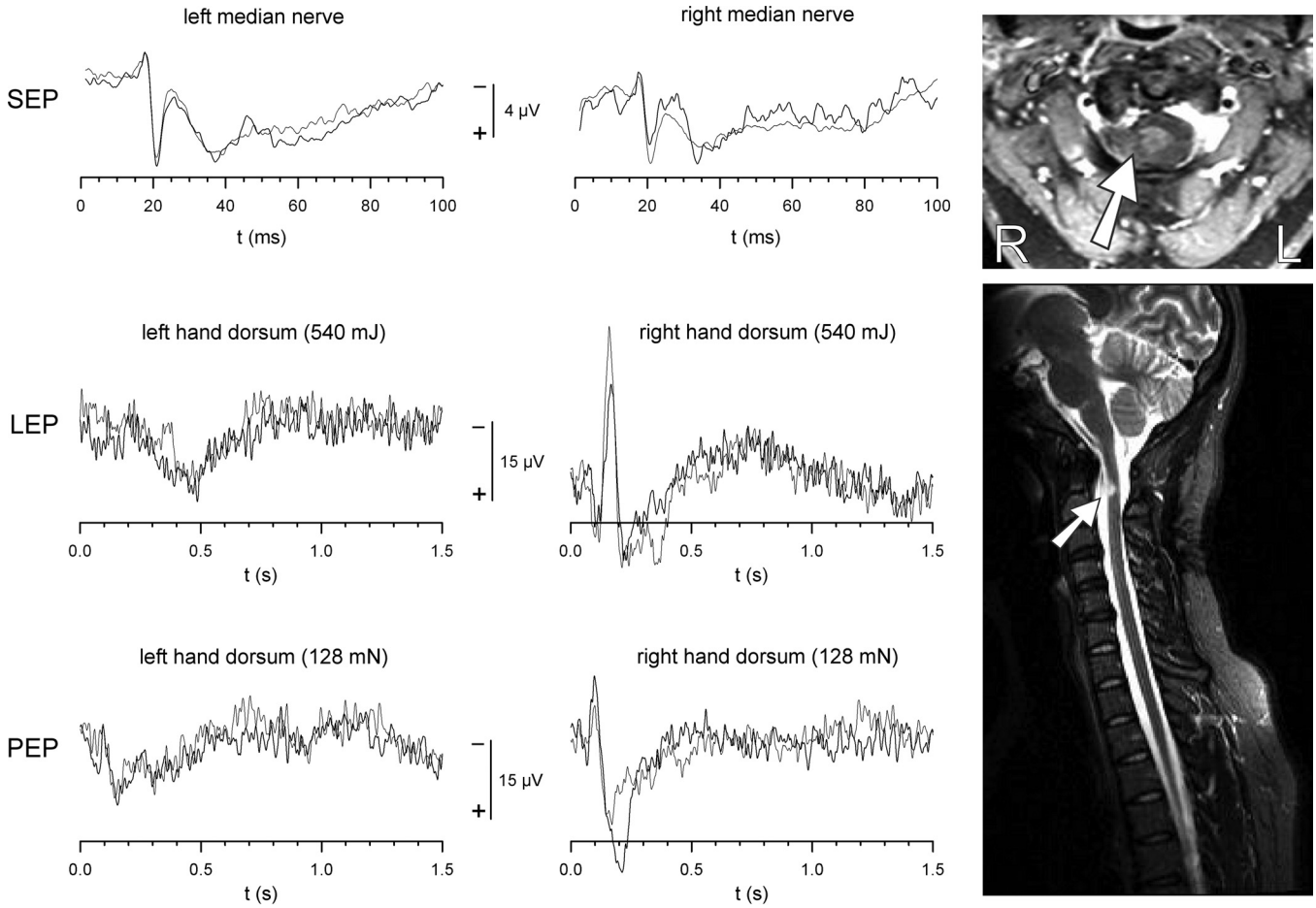

Fig. 7. Neurophysiological and MRI findings in a patient with thermal hypesthesia and pinprick hypalgesia following surgical removal of a spinal neurinoma at C_1–C_2 level, which was located right and anterior of the spinal cord, presumably compromising conduction in the nociceptive projection pathway (anterolateral tract). *Left*: neurophysiological findings: somatosensory evoked potentials (SEPs) elicited by the stimulation of different sets of primary sensory afferents [SEPs: Aβ low-threshold mechanoreceptors; laser-evoked potential (LEPs): heat-sensitive Aδ nociceptors; PEPs: mechanosensitive Aδ nociceptors]. All stimuli were delivered to the unaffected (right) and unaffected (left) hands. Transmission in Aβ pathways was preserved, as indicated by normal short-latency SEPs (*top*) following stimulation of both right and left median nerve. In contrast, transmission in Aδ pathways was impaired, as indicated by abnormal LEPs (*middle*) following stimulation of the left (affected) side. PEP results (*bottom*) paralleled LEP results, with a response of reduced amplitude when stimuli were delivered on the affected side, thus providing strong evidence that the increase in PEP amplitude primarily reflects cortical activities triggered by the somatosensory volley transmitted in Aδ primary sensory afferents and spinothalamic projection neurons. *Right*: MRI findings: axial (*top*) and sagittal (*bottom*) MRIs of the spinal cord collected 2 wk after the removal of the neurinoma. Note the anterolateral location of the lesion (arrows) at C_1–C_2 level.

mechanical stimulation of A-fiber skin nociceptors elicits robust time-locked EEG responses (PEPs). This demonstrates the existence of an EEG correlate of the activation of mechanical nociceptive myelinated fine afferents. Second, PEPs are selectively reduced in a patient with documented damage of nociceptive afferent pathways, thus indicating that these responses are triggered by somatosensory input traveling in the nociceptive pathways of the spinal cord and brain stem. Third, the magnitude of PEPs is significantly increased when mechanical stimuli are delivered to an area of experimentally induced secondary hyperalgesia.

Taken together, these findings indicate that PEPs reflect the state of the ascending mechanical nociceptive pathways and represent a useful tool to assess central sensitization in normal volunteers and, potentially, in patients with clinical hyperalgesia.

Afferent Somatosensory Input

Three classes of A-fiber skin nociceptors have been identified with teased-fiber recordings in primates: there is a heat-sensitive, but relatively high-threshold mechanonociceptive subtype (type II AMHs), and there are high-threshold or even heat-insensitive type I-AMHs and high-threshold mechanoreceptors (HTM; Szolcsanyi et al. 1988; Treede et al. 1998). Type I-AMHs have rather high thermal activation thresholds (>50°C) and relatively low mechanical activation thresholds (51 mN) (Treede et al. 1998) and are neither excited by acute nor blocked by chronic application of capsaicin, i.e., they are capsaicin insensitive (Ringkamp et al. 2001). Evidence from capsaicin desensitization and nerve block experiments indicates that pricking pain sensations elicited by punctate mechanical stimulators identical to those used in the present study are mediated by the activation of I-AMH units (Magerl et al. 2001). After peripheral lesions, these afferents exhibit increased firing rates to mechanical stimulation together with afterdischarges that may be a correlate of peripheral neuropathic pain (Andrew and Greenspan 1999). These A-fiber nociceptors project centrally through lamina I spinothalamic tract high-threshold mechanosensitive neurons, which specifically encode stimulus intensity and probe size (Andrew and Craig 2002).

Further evidence that PEPs are mediated by the activation of the spinothalamic tract is provided by recording PEPs in a patient with documented hypalgesia following surgical intervention in the spinal cord at right C_1–C_2 level. Indeed, PEP amplitude was significantly reduced when stimuli were delivered on the affected side (Fig. 7, *bottom*). In the same patient we also recorded short-latency SEPs, which selectively explore the function of Aβ afferent pathways (Cruccu et al. 2008b), and LEPs, which selectively explore the function of Aδ afferent pathways (Bromm and Treede 1991). SEP and LEP results showed a dissociated sensory loss, with a selective impairment of Aδ but not Aβ pathways (Fig. 7, *top* and *middle*). The results of PEP recording paralleled the LEP results, with a similar amplitude reduction when stimuli were delivered on the affected side, thus providing strong evidence that PEPs are mostly reflecting cortical activities triggered by the somatosensory volley transmitted in Aδ primary sensory afferents and spinothalamic projection neurons.

Pinprick-Evoked Brain Potentials

The mechanical stimulators described in the present report are designed to be hand held, and to be applied perpendicularly to the skin, in order to have the weight of the rod resting exclusively on the tip of the steel wire (Fig. 1, *left*) and thus apply a constant force (128 mN in the present experiment). By placing an optical detector inside the stimulator we were able to obtain exact temporal information about the onset of the retraction of the rod inside the cylinder (with a 33-ms delay), and thus synchronize the stimulation with the EEG recording.

The EEG responses elicited by mechanical stimulation of nociceptive afferents had the typical characteristics of vertex potentials elicited by somatosensory stimuli (Figs. 3 and 5): their main constituent was a biphasic negative-positive wave, with a scalp distribution maximal at the vertex. A similar scalp distribution is observed when EEG responses are elicited by the activation of thermal nociceptive pathways with radiant heat (LEPs) or contact heat (contact heat-evoked potentials, CHEPs) (Greffrath et al. 2007; Treede et al. 1988) as well as salient and intense auditory, visual, and somatosensory (Aβ) stimuli (Mouraux and Iannetti 2009). The observation that *1*) similar vertex responses can be elicited by nonnociceptive sensory stimuli that are never perceived as painful, provided that they are salient (Mouraux and Iannetti 2009), and *2*) the well-known positive correlation between the intensity of perceived pain and the magnitude of LEPs can be disrupted in several experimental conditions, such as stimulus repetition at a short and constant interval (Iannetti et al. 2008; Treede et al. 2003), indicates that nociceptive event-related potentials, and, probably PEPs, are likely to reflect stimulus-triggered brain processes not directly related to pain intensity coding in the human cortex. However, this does not mean that their recording cannot be clinically useful to explore the function of the nociceptive pathways during mechanical hyperalgesia (as detailed in *Relevance for Clinical Practice*).

The latencies of the main negative and positive peaks of vertex PEPs were ~110 and 245 ms (Fig. 3) and thus lay in between Aβ-mediated and Aδ-mediated (laser induced) nociceptive latencies of vertex responses. This is conformable with available information on conduction velocity of I-AMH units from teased-fiber recordings in various species (including mouse, rat, cat, and monkey), indicating that they are substantially faster than heat-sensitive type II AMH units and exhibit maximal conduction velocities in the Aβ range (up to 70 m/s; Djouhri and Lawson 2004; Lawson 2002; Treede et al. 1998). In addition, the retraction of the weight inside the stimulator tube, and the consequent interruption of the light beam generating the TTL, most probably occurred before the first action potentials in I-AMH units, thus providing an underestimation of the actual latency of the evoked potential. For these reasons, a latency of 110 ms for the N wave of PEPs is entirely compatible with the activation of fast-conducting I-AMH nociceptors.

Effect of Secondary Hyperalgesia

The intradermal injection of capsaicin induced a robust secondary hyperalgesia in all subjects (Fig. 3). The neurophysiological basis of the secondary hyperalgesia observed in response to capsaicin injection is a state of sensitization in the central nervous system (Meyer and Treede 2004). Most likely this state of sensitization is consequent to plastic changes

happening at the level of the dorsal horn, consisting of a heterosynaptic modulation of a facilitating pathway (the C-fiber nociceptors stimulated by capsaicin injection) on a facilitated pathway (the I-AMH nociceptors stimulated by the mechanical probes) (Ziegler et al. 1999).

The increase of the perceived intensity to mechanical stimulation of I-AMH units was reflected in an increase of the PEP amplitude (Fig. 3). This finding indicates that, with the recording paradigm used in the present study, PEPs constitute a neurophysiological response that reflects the state of the I-AMH pathway in both experimental and clinical conditions and consequently can be used to assess sensitization of nociceptive pathways in healthy volunteers and patients. Interestingly, the amplitude increase during secondary hyperalgesia was observed mostly on the N wave of the biphasic NP vertex complex (Fig. 3), indicating that the neural sources generating the N wave are more reliably related with the state of the afferent pathway and the perceived stimulus intensity. This finding is in agreement with the observation that the N2 wave of LEPs correlates better than the P2 wave with the perceived stimulus intensity (Iannetti et al. 2005) and indicates that this rule holds also when the nociceptive input is amplified along the somatosensory afferent pathways. This dissociation between N and P amplitude is particularly interesting in relation to the evidence showing that the N2 and P2 waves of nociception-related evoked potentials are differentially modulated by cognitive tasks (Bentley et al. 2004; Legrain et al. 2002) and have different neural generators (Garcia-Larrea et al. 2003). The N2 wave is thought to be generated by the contribution of a bilateral source in operculoinsular areas and a source in the primary somatosensory cortex contralateral to the side of stimulation (Frot et al. 1999; Ohara et al. 2004; Tarkka and Treede 1993). Because of their similar scalp distribution and dependence on perceived intensity, it is likely that the N wave of PEPs and the N2 wave of LEPs share similar cortical generators and functional significance.

Relevance for Clinical Practice

So far the electrophysiological exploration of nociceptive pathways has been limited to the thermal spino-thalamo-cortical pathway, whose state can be indirectly but reliably assessed with LEPs (Bromm and Treede 1991; Mouraux and Iannetti 2009; Treede et al. 2003). LEPs have been widely used in studies investigating nociception in normal volunteers and patients and are recommended as the most useful tool to assess the deafferentation in spino-thalamo-cortical pathways and hence diagnose the neuropathic nature of clinical pain (Cruccu et al. 2004). However, although a value of LEPs as a predictive factor for the development of positive symptoms has been suggested (Garcia-Larrea et al. 2002), LEPs are mostly able to detect minus signs, i.e., amplitudes are not increased even when the subjective perception of the stimulus is enhanced in patients (Casey et al. 1996; Wu et al. 1999) or when laser stimuli are delivered to the area of secondary hyperalgesia (Valeriani et al. 2003), although some recent studies have suggested that shortening of CHEP latency (Madsen et al. 2012b) and increase of C fiber-related CHEPs (Madsen et al. 2012a) might reflect capsaicin-induced thermal hyperalgesia. However, thermal hyperalgesia is a much less frequent symptom than mechanical hyperalgesia in patients with neuropathic pain (Baumgartner et al. 2002), and, whatever the nature of the hyperalgesia (thermal or mechanical), an electrophysiological response able to objectify positive signs like allodynia and hyperalgesia in experimental central sensitization and neuropathic pain conditions has been lacking so far (Treede et al. 2003). Here we describe a technique that, by reliably assessing the state of mechanical spino-thalamo-cortical pathways (i.e., those pathways whose transmission is enhanced in central sensitization), reflects pain sensation, and it is hence able to provide a laboratory measure of plus signs in both experimental and clinical mechanical hyperalgesia. The lack of significant correlation between capsaicin-induced changes of pain perception and PEP amplitude at the single-subject level is not meaningful in this small cohort. To become meaningful, it would afford an $n > 100$ sample at this level of correlation (Maxwell 2000). For the reasons stated above, we believe that the recording of PEPs may be relevant in clinical practice and may also give useful information about the mechanisms of neuropathic pain syndromes. Coupled with the recording of LEPs, PEPs would provide an exhaustive exploration of both thermal and mechanical spino-thalamo-cortical pathways.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: G.D.I., U.B., R.-D.T., and W.M. conception and design of research; G.D.I., U.B., and W.M. performed experiments; G.D.I., U.B., and W.M. analyzed data; G.D.I., U.B., I.T., R.-D.T., and W.M. interpreted results of experiments; G.D.I., U.B., and W.M. prepared figures; G.D.I., U.B., and W.M. drafted manuscript; G.D.I., U.B., I.T., R.-D.T., and W.M. edited and revised manuscript; G.D.I., U.B., I.T., R.-D.T., and W.M. approved final version of manuscript.

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