PAIN® 154 (2013) 1263–1273

PAIN®

www.elsevier.com/locate/pain

Neural coding of nociceptive stimuli—from rat spinal neurones to human perception

Shafaq Sikandar *, Irene Ronga, Gian Domenico Iannetti, Anthony H. Dickenson

Department of Neuroscience, Physiology and Pharmacology, University College London, United Kingdom

Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

ARTICLE INFO

Article history:
Received 31 July 2012
Received in revised form 21 March 2013
Accepted 29 March 2013

Keywords:
Dorsal horn neurone
Electroencephalography
Nociceptive stimulation
Psychophysics
Spatial summation
Translational research

ABSTRACT

Translational studies are key to furthering our understanding of nociceptive signalling and bridging the gaps between molecules and pathways to the patients. This requires use of appropriate preclinical models that accurately depict outcome measures used in humans. Whereas behavioural animal studies classically involve reports related to nociceptive thresholds of, for example, withdrawal, electrophysiological recordings of spinal neurones that receive convergent input from primary afferents permits investigation of suprathreshold events and exploration of the full-range coding of different stimuli. We explored the central processing of nociceptive inputs in a novel parallel investigation between rats and humans. Using radiant laser pulses, we first compared the electrophysiological responses of deep wide dynamic range and superficial nociceptive-specific neurones in the rat dorsal horn with human psychophysics and cortical responses. Secondly, we explored the effects of spatial summation using laser pulses of identical energy and different size. We observed 3 main findings. Firstly, both rodent and human data confirmed that neodymium–yttrium aluminium perovskite laser stimulation is a nociceptive-selective stimulus that never activates Aβ afferents. Secondly, graded laser stimulation elicited similarly graded electrophysiological and behavioural responses in both species. Thirdly, there was a significant degree of spatial summation of laser nociceptive input. The remarkable similarity in rodent and human coding indicates that responses of rat dorsal horn neurones can translate to human nociceptive processing. These findings suggest that recordings of spinal neuronal activity elicited by laser stimuli could be a valuable predictive measure of human pain perception.

1. Introduction

Pain is an important clinical problem that represents a major burden for society and calls for better understanding of nociceptive signalling in the peripheral and central nervous systems. Behavioural studies in animals shed light on the roles of molecular targets, but usually only inform on events related to nociceptive thresholds [12]. Sophisticated operant methods are useful for affective or cognitive measures of pain, but recording from spinal neurones that receive convergent input from primary afferents is one way to study and explore the full-range coding of different stimuli from low to high intensities. Translational approaches from animals to humans are key to progressing in pain research [27] because these electrophysiological measures allow investigation of neural events provoked by suprathreshold nociceptive stimuli that might underlie processes in patients reporting high pain levels.

This study used rats and human participants to characterize and correlate the behavioural and electrophysiological responses elicited by heat generated by infrared neodymium–yttrium aluminium perovskite (Nd:YAP) laser pulses. Laser heat is a natural stimulus and corresponds well with common painful experiences [4]. Cutaneous heat stimuli are transduced by transient receptor potential channels and transmitted by thin myelinated Aδ and unmyelinated C fibres [33]. Laser stimulation of the skin activates both high-threshold, fast conducting type II A-mechano-heat fibres (15 m/s) and low-threshold, slow conducting C nociceptors (0.5 to 1.5 m/s [4,15]). Because of these different conduction velocities, a single brief laser stimulus that coactivates Aδ and C skin nociceptors elicits a double sensation of first pricking pain (mediated by Aδ-fibres), followed by a second burning pain (mediated by C-fibres) [30].

Second-order spinal neurones receive the summated input from several primary afferents that innervate cutaneous structures and contribute to coding of stimulus location and intensity [31]. Electrophysiological recordings of deep dorsal horn wide dynamic range (WDR) neurones and nociceptive-specific (NS) lamina I neurones in both rats and primates show that these cells can encode the intensity of mechanical and thermal stimuli [18,20,23,46]. Spatial summation is also an important aspect of sensory coding and may be an important mechanism of pain amplification in some chronic pain conditions [44]. Indeed, psychophysical studies have

* Corresponding author. Address: Department of Neuroscience, Physiology and Pharmacology, University College London, London WC1E 6BT, United Kingdom. Tel.: +44 (0) 20 7679 3737.
E-mail address: shafaq.sikandar@ucl.ac.uk (S. Sikandar).

0304-3959/$36.00
http://dx.doi.org/10.1016/j.pain.2013.03.041

shown that intensity of painful sensations is increased when spatially larger stimuli are applied, either within or across dermatomes [13,19,36,43].

Here, we used identical Nd:YAP laser stimulation to compare several features of the response of deep WDR neurones and superficial NS neurones in the rat dorsal horn with human cortical responses (laser-evoked potentials [LEP]) and psychophysics. We first characterized the responses evoked in rats and humans by graded intensities of radiant heat. Subsequently, we investigated the effects of spatial summation of laser stimuli at the spinal cord level in rats and at the cortical level and on psychophysical measures in humans.

2. Materials and methods

2.1. Animals

All animal experiments used male Sprague-Dawley rats (250 g; Central Biological Services, University College, London, UK). This was approved by the United Kingdom Home Office according to guidelines set by personal and project licenses and the guidelines of the Committee for Research and Ethical Issues of the International Association for the Study of Pain.

2.2. Rodent electrophysiology

Electrophysiology experiments were performed as previously described [17]. In rats anaesthetised with isoflurane (1.5%; 66% N_2O and 33% O_2), extracellular recordings in L4-5 segments were made from WDR neurones in the deep dorsal horn (lamina V to VI, 500 to 1000 μm) and NS neurones in the superficial dorsal horn (lamina I, 0 to 250 μm) using parylene-coated tungsten electrodes (A-M Systems, Sequim, WA). A number of WDR neurones in the superficial dorsal horn were encountered, but their responses are not shown. The characteristics and responses of these neurones were the same as deep dorsal horn WDR cells. Superficial neurones were classified as NS if 8g von Frey (vF) stimulation evoked $\leqslant$100 spikes. Activity of neurones was visualised on an oscilloscope and discriminated on a spike amplitude and waveform basis.

Electrical, mechanical, and laser stimuli were applied in the peripheral receptive field on the hindpaw glabrous skin. Data were recorded and analysed by a CED 1401 interface coupled to Spike 2 software (Cambridge Electronic Design, Cambridge, UK).

2.2.1. Electrical stimulation

Evoked spikes to a train of 16 transcutaneous stimuli (2-ms-wide pulses, 0.5 Hz, 3 × C-fibre threshold) constructed a poststimulus histogram. Responses evoked by Aβ- (0 to 20 ms), Aδ- (20 to 90 ms), and C-fibres (90 to 350 ms) were separated and quantified on the basis of latency. Neuronal responses occurring after the C-fibre latency band of the neurone were classed as postdischarge (350 to 800 ms). C-fibre and postdischarge responses evoked by the first stimulus of the train multiplied by the total number of electrical stimuli (16) are referred to as input, a measure of activity evoked by the stimulus before any subsequent hyperexcitability. This is the nonpotentiated response. Wind-up is calculated as the total number of action potentials at C-fibre and postdischarge latencies produced by the train, minus the input. This increased neuronal responsiveness after repeated stimulation at the same intensity gives a measure of hyperexcitability of the neurone [18].

2.2.2. Mechanical stimulation

All von Frey filaments (8g, 26g, and 60g) and dynamic brush were applied for 10 seconds, and the total number of evoked spikes was recorded. Use of these natural stimuli does not allow us to determine which peripheral fibre types convey these stimuli, unlike the electrical stimulation described earlier.

2.2.3. Laser stimulation

Radiant stimuli were generated by an Nd:YAP laser (wavelength: 1.34 μm; duration: 4 ms; ElEn Group). The laser beam was transmitted via an optic fibre, and its diameter was set to 6 mm (28 mm^2) or 12 mm (113 mm^2) by focusing lenses. To characterize neuronal responses to graded intensities of laser stimulation, we delivered laser pulses of 1 J, 2 J, 3 J, and 4 J using a spot size with a diameter of 6 mm. To determine the effects of different spot sizes of stimulation while using identical energies, we delivered laser pulses with fluencies of 71 mJ/mm^2, 142 mJ/mm^2, and 214 mJ/mm^2 using a spot size with a diameter of 12 mm (ie, energies of 2 J, 4 J, and 6 J). In human subjects, we did not deliver laser pulses at the highest energy (6 J over a spot size with a diameter of 12 mm, ie, 214 mJ/mm^2), as the elicited sensation was unbearable in repeated measures. Surface skin temperature of the rat hindpaw was monitored using an infrared thermometer.

2.3. Human psychophysics and electrophysiology

Eight healthy volunteers gave their informed consent to take part in the study (3 male and 5 female subjects; age range: 21 to 37 years; mean age: 28.7 ± 5.7). The study conformed to the standards set by the Declaration of Helsinki and was approved by the local ethics committee. Each subject was first familiarized with the experimental setup and exposed to 5 to 10 test stimuli.

2.3.1. Stimuli

The same Nd:YAP laser stimuli used for the animal experiments were used in the human experiments (identical duration, energies, and spot sizes except for the stimuli of 214 mJ/mm^2, see previous section). The laser beam was directed onto the glabrous skin of the index and middle fingers of the right hand (a territory similar to the one stimulated in rats) and stimuli were delivered arrhythmically at intervals of 10 to 15 seconds.

2.3.2. Experimental design

Participants were seated with their right hand on a desk and with a screen-blocked view of the stimulated hand. Acoustic isolation was ensured using white noise throughout the experiment. In experiment 1, we delivered 20 laser stimuli for each of the 4 laser energies used (80 stimuli in total); in experiment 2, we delivered 20 laser stimuli for each of the 2 different spot sizes (40 laser stimuli in total). Within each experiment, the order of stimuli was pseudorandomized.

2.3.3. Behavioural measures

In both experiments, 3 different behavioural measures were collected simultaneously to the electroencephalogram (EEG). (1) When stimuli were perceived, participants described the quality of perception choosing 1 of the following 7 descriptors: light touch, touch, shock, tingling, warm, pricking, and burning [35]. (2) Participants were then asked to report the intensity of perception using a numerical rating scale (NRS) ranging from 0 (no detection) to 100 (maximum pain). An anchor at the middle of the scale (NRS = 50) marked the border between nonpainful and painful domains of sensation [35]. (3) Participants were instructed to release a pedal with their right foot as soon as they perceived the stimulus. Reaction times, defined as the time elapsed between the onset of the stimulus and the button press, were measured with a digital chronometer with 1-ms resolution.

2.3.4. Electrophysiology

The EEG was recorded in 3 subjects using 5 Ag–AgCl electrodes placed on the scalp at positions Fz, Pz, Cz, T3, and T4 (International

10–20 system), and in 5 subjects using 32 electrodes, to allow exploring the scalp distribution of the observed effect. In both cases the nose was used as reference. Ocular movements and eye blinks were recorded using 2 surface electrodes placed at the upper-left and lower-right sides of the right eye. Signals were amplified and digitized using a sampling rate of 1024 Hz (Micromed, Treviso, Italy). All EEG processing steps were carried out using Letswave [21] and Matlab (The MathWorks, Natick, MA). Continuous EEG recordings were segmented into 2-second-long epochs (−0.5 to +1.5 seconds relative to stimulus onset), band-pass filtered (1 to 30 Hz), and baseline-corrected (reference interval −0.5 to 0 seconds). Epochs with amplitude values exceeding ±100 μV (ie, epochs likely to be contaminated by an artefact) were rejected. Separate average event-related potential waveforms were computed for each participant and stimulus type. For each waveform, the amplitude of the N1, N2, and P2 peaks of the LEP were measured as follows. The N1 wave was measured at the temporal electrode contralateral to the stimulated side (T3), referenced to Fz [25]. It was defined as the negative deflection preceding the N2 wave, which appears as a positive deflection in this montage. The N2 and P2 waves were measured at the vertex (Cz), referenced to the nose. The N2 wave was defined as the most negative deflection after stimulus onset. The P2 wave was defined as the most positive deflection after stimulus onset.

2.4. Statistical analyses

2.4.1. Rodent electrophysiology

The correlation between the number of spikes evoked by laser stimulation and spikes evoked by electrical/mechanical stimuli was assessed using Pearson r. For data related to graded energy codings (number of spikes evoked and duration of firing in rats, NRS, and reaction times in humans), a 1-way analysis of variance (ANOVA) with Bonferroni post-hoc tests were used and data were presented as mean ± SEM response. Student unpaired *t* tests were used to compare the evoked responses of WDR neurones to electrical and mechanical stimulation against those of NS neurones, with data presented as mean ± SEM response. For data involving 2 spot sizes of laser stimulation, a 2-way ANOVA with Bonferroni post-hoc tests was used, with stimulus energy and stimulus size as main factors Data were presented as mean ± SEM response.

2.4.2. Human psychophysics

In experiment 1, we assessed the effect of stimulus energy on intensity of perception, number of stimuli perceived as painful, and reaction times using a 1-way ANOVA with stimulus energy as factor (4 levels: 1 J, 2 J, 3 J, 4 J). In experiment 2, we assessed the effect of stimulus fluence and stimulus size on intensity of perception and reaction times, using a 2-way ANOVA with stimulus fluence (2 levels: 71 and 142 mJ/mm^2) and stimulus size (2 levels: 28 and 113 mm^2) as factors.

2.4.3. Human electrophysiology

To statistically compare the LEP waveforms, we performed a point-by-point, repeated-measures ANOVA at electrode Cz. This approach allows disclosing the time course of each experimental effect and yields a waveform expressing, for each scalp electrode, its significance across time. In experiment 1 we used a 1-way ANOVA to explore the effect of the experimental factor of stimulus energy (4 levels: 1 J, 2 J, 3 J, 4 J). In experiment 2, we used a 2-way ANOVA to explore the effects of the experimental factors stimulus fluence (2 levels: 71 mJ/mm^2, 142 mJ/mm^2) and stimulus size (2 levels: 28 mm^2, 113 mm^2). To account for multiple comparisons, intervals were considered as significant only when lasting more than 50 ms. F and *P* values are given at the maximum peak of each significant interval.

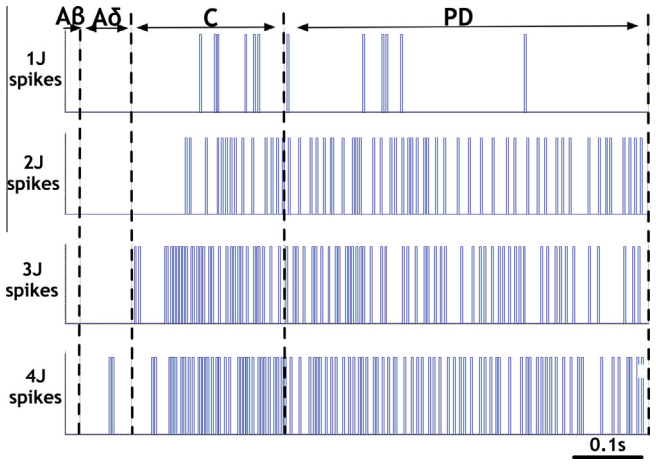

Fig. 1. Laser stimulation of the rat hindpaw is purely nociceptive. Example poststimulus time histograms show the activation and relative primary afferent input of a wide dynamic range neuronal response after radiant heat stimulation of the receptive field at different energies. Fibre types are separated according to latency of spikes; Aβ-fibres (0 to 20 ms), Aδ-fibres (20 to 90 ms), C-fibres (90 to 300 ms), and postdischarge (300 to 800 ms).

3. Results

3.1. Nd:YAP laser stimulation selectively activates Aδ- and C-fibres

Poststimulus time histograms quantify the relative primary afferent input to WDR neurones after peripheral stimulation (Fig. 1). This analysis clearly revealed that the laser stimulation applied to the rat hindpaw is selectively nociceptive, ie, it only activates Aδ- and C-fibres, but never Aβ-fibres. Respective contributions of primary nociceptive afferents varied as a function of stimulus intensity; at low laser energies only C-fibre afferents were activated, whereas at higher energies Aδ-fibres were also recruited.

There was no correlation between firing evoked by laser stimuli and firing evoked by Aβ-fibre input or innocuous mechanical stimulation (8*g* vF and brush; Figs. 2 and 3) in either neuronal class. Thus, laser-evoked activity in dorsal horn neurones is nociceptive-specific and not contributed by low-threshold Aβ-fibres. Further, for both populations of neurones, laser-evoked activity is significantly correlated with firing to noxious mechanical stimuli (Figs. 2 and 3).

In human subjects, the quality of laser-evoked sensations mostly involved descriptors unavoidably related to Aδ- and C-fibre activity, ie, warm, pricking, or burning (81% of the total number of perceived stimuli; Fig. 4C). Both reaction times and latencies of the cortical responses were never compatible with Aβ-fibre transmission (Figs. 4B and 4D). These findings confirm that heat stimulation produced by the Nd-YAP laser provides a selective nociceptive input to the central nervous system.

3.2. Graded laser stimulation produces graded responses in both rats and humans

Laser-evoked firing in both WDR (n = 56) and NS (n = 17) neurones clearly coded for stimulus intensity, with stronger laser stimuli eliciting neuronal firing of higher frequency ($P < .001$ for both WDR and NS neurones, 1-way ANOVA; Figs. 5A and 5B). Notably, NS neurones fired less than WDR neurones at the highest stimulus intensity (unpaired *t* test, $P < .05$) in all response time windows. Furthermore, the duration of WDR firing coded for stimulus intensity, whereas increasing stimulus intensities produced a ceiling effect on the duration of NS firing ($P < .001$ post-hoc test at 4 J, 2-way ANOVA; Fig. 5C and 5D).

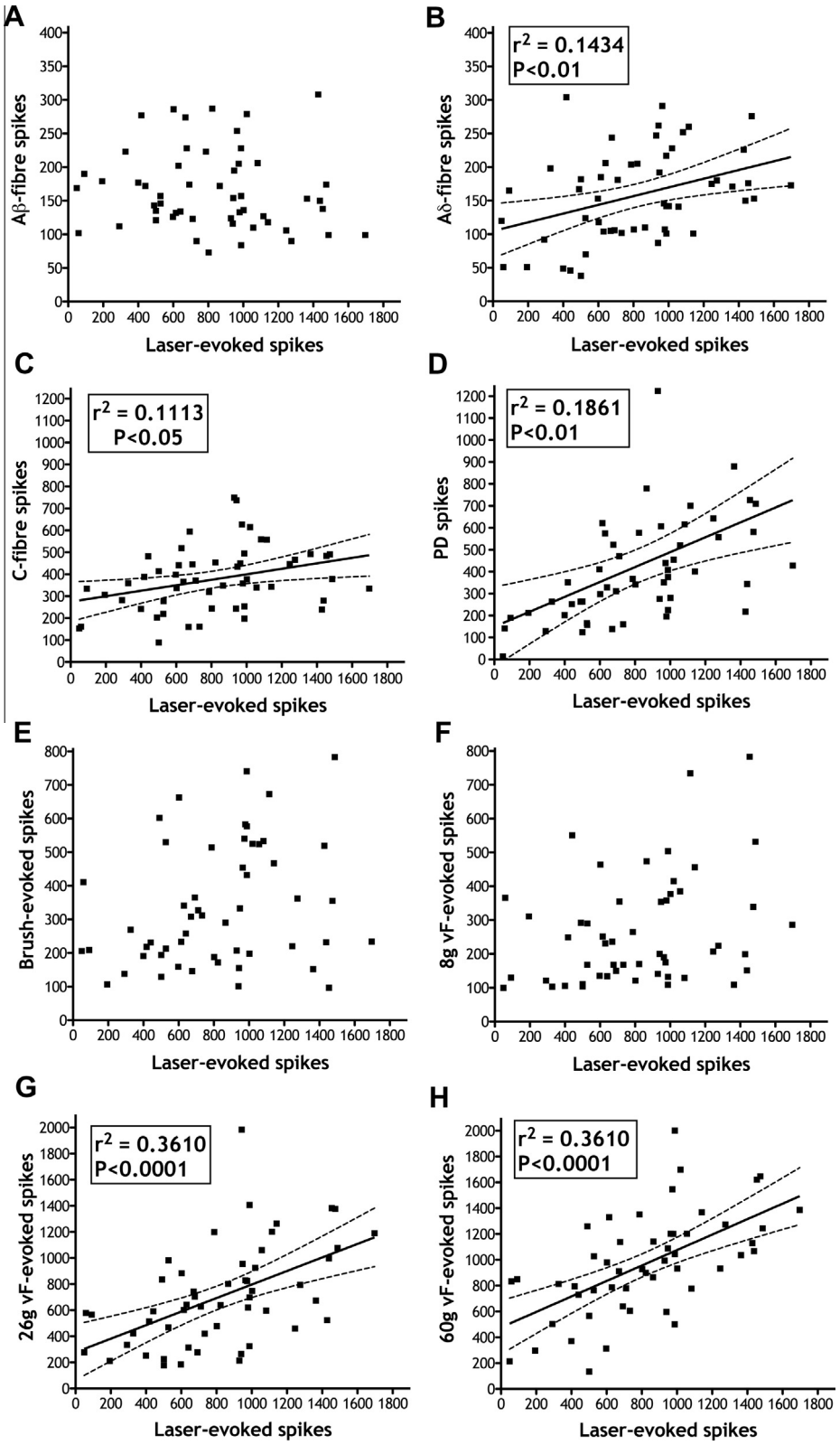

Fig. 2. Laser-evoked firing of deep wide dynamic range (WDR) neurones in the rat dorsal horn is significantly correlated with electrically evoked and mechanically evoked activity. WDR firing evoked by thermal stimulation (1- to 10-second cumulative spikes following 4 J laser energy) is significantly correlated with electrically evoked measures (all WDR neurones recorded n = 56; Pearson correlation). Specifically, Aδ-fibre input (B; $P < .01$), C-fibre input (C; $P < .05$) and postdischarge (D; $P < .01$), but not the Aβ-fibre input (A). Laser-evoked WDR firing is also significantly correlated with the evoked responses to noxious mechanical stimulation of 26*g* vF (G; $P < .001$) and 60*g* (H; $P < .001$), but not nonnoxious mechanical stimulation of dynamic brush (E) and 8*g* vF (F). The 95% confidence bands of the best-fit line are shown for significant correlations between evoked spikes by electrical/mechanical and laser stimulation.

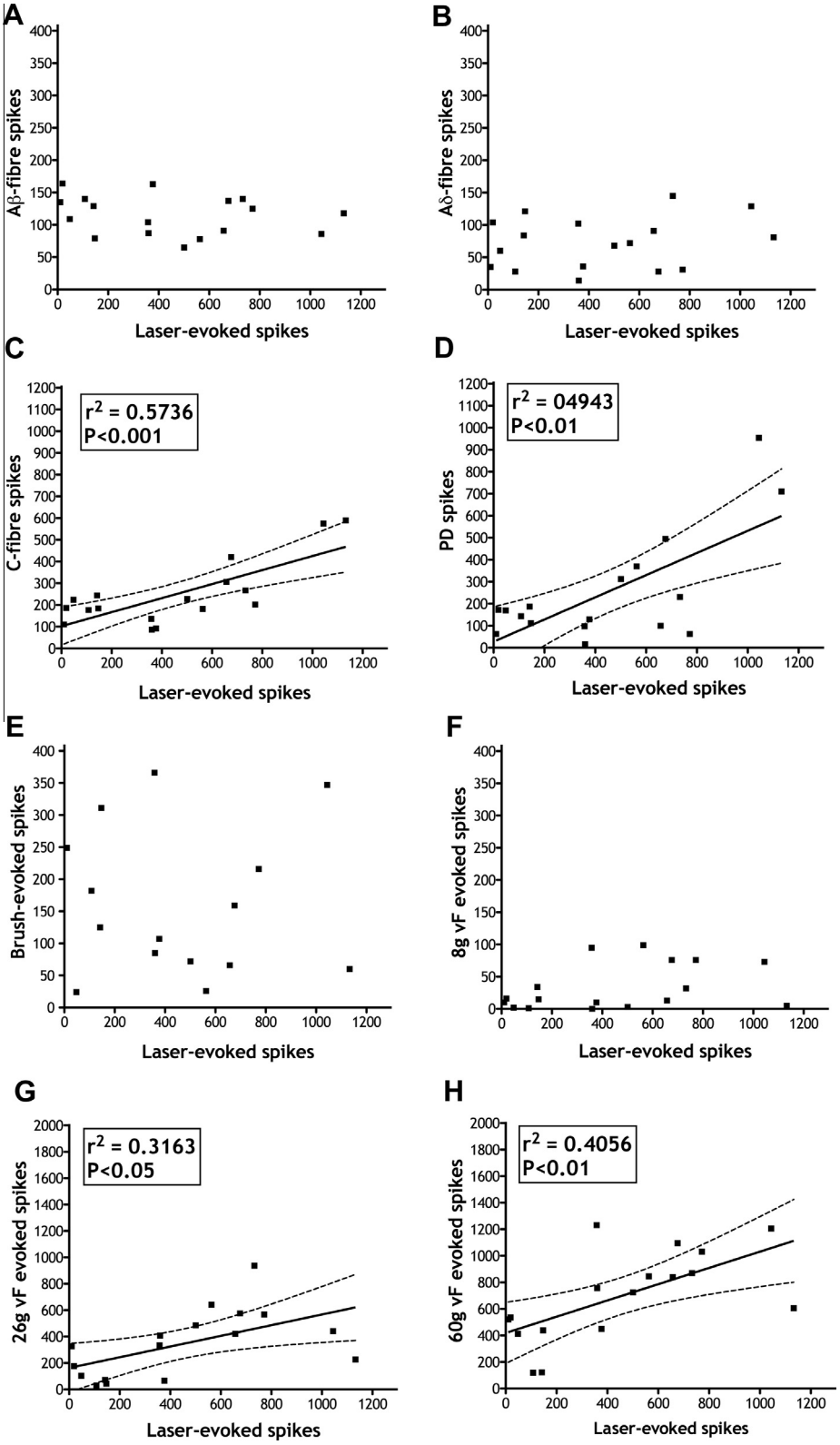

Fig. 3. Laser-evoked firing of superficial nociceptive-specific (NS) neurones in the rat dorsal horn is significantly correlated with electrically evoked and mechanically evoked activity. The thermally evoked firing of superficial NS neurones (1- to 10-second cumulative spikes following 4 J laser energy) is significantly correlated with electrically evoked measures (superficial NS neurones recorded n = 17; Pearson correlation). Specifically, C-fibre input (C; $P < .001$) and postdischarge (D; $P < .01$), but not the Aβ-fibre (A) or Aδ-fibre (B) input. Laser-evoked firing of NS neurones is also significantly correlated with the evoked responses to noxious mechanical stimulation of 26*g* vF (G; $P < .05$) and 60*g* vF (H; $P < .01$), but not nonnoxious mechanical stimulation of dynamic brush (E) and 8*g* vF (F). The 95% confidence bands of the best-fit line are shown for significant correlations between evoked spikes by electrical/mechanical and laser stimulation.

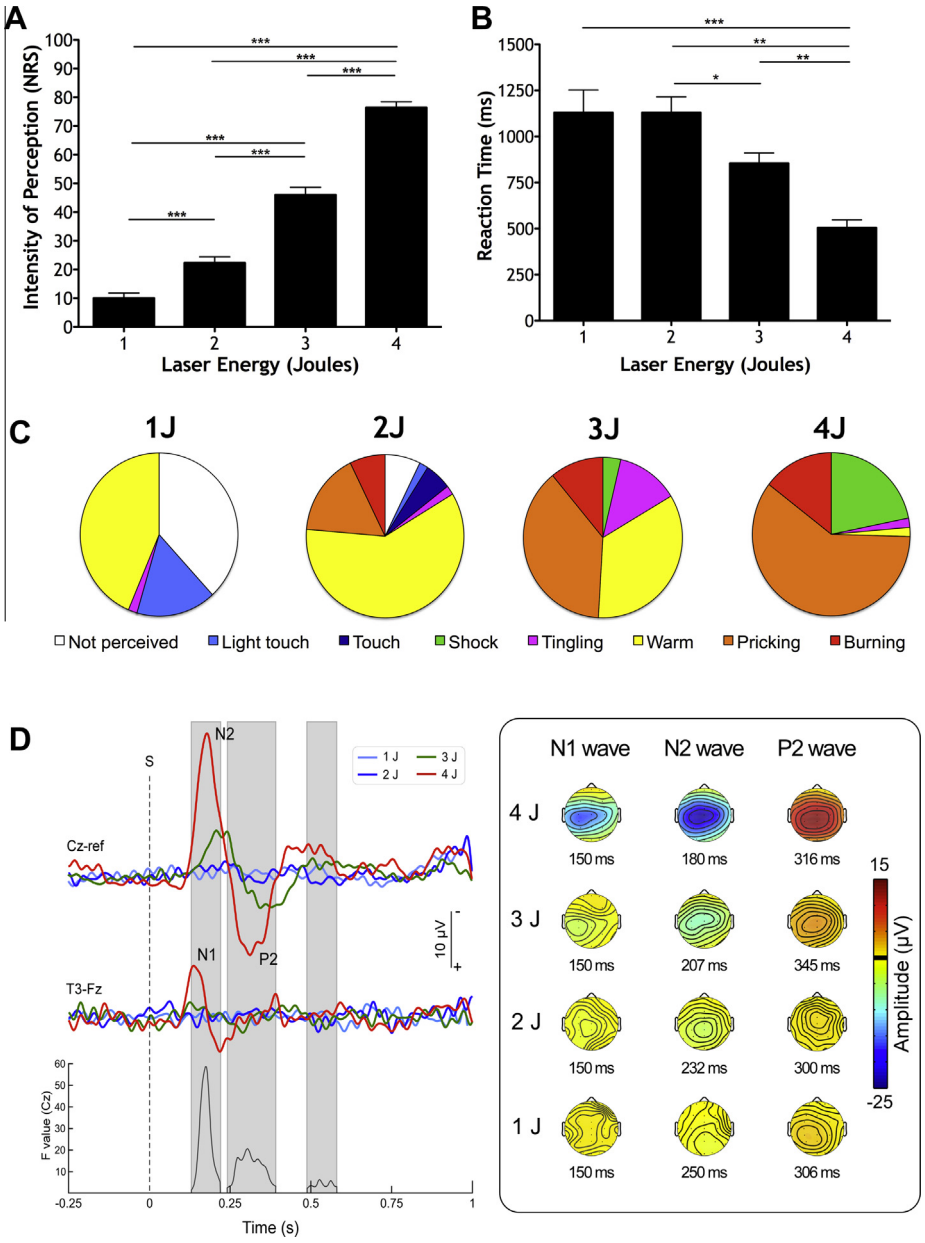

Fig. 4. Psychophysical and electrophysiological responses to graded laser stimulation of the human glabrous skin. Radiant heat neodymium–yttrium aluminium perovskite laser pulses were delivered on the volar surface of the index and middle fingers in 8 healthy participants, using 4 stimulus energies (1 to 4 J). (A) Relationship between stimulus energy and intensity of perception (***$P < .001$, post-hoc t tests). (B) Relationship between stimulus energy and reaction times (*$P < .05$, **$P < .01$, ***$P < .001$, post-hoc t tests). (C) Quality of perception: number of times each of the 8 descriptors was chosen, expressed as percentage of the total number of reports. (D) Group-level average laser-evoked potentials (LEPs) elicited by stimuli of different energies. Left panel shows the N1, N2, and P2 waves, at electrodes Cz (nose reference) and T3 (referenced to Fz). x-axis, time (seconds); y-axis, amplitude (µV). The vertical dashed line marks the stimulus onset. The time-course of the F-value expressing the significant effect of stimulus energy at Cz is shown below the LEP waveforms. Significant time intervals are highlighted in gray (consecutivity threshold = 50 ms). The right panel shows the scalpmaps at the latency of the N1, N2, and P2 peaks, for each energy of stimulation.

Interestingly, the duration of firing of WDR and NS neurones (eg, more than a minute following a single laser pulse at 4 J) matched the time profile of skin temperature following the same stimulus (Fig. 6). Stronger energies of laser stimulation produced higher peaks of skin temperature increases, and changes in surface skin temperature at all laser energies resolved to baseline temperature within 1 minute. The highest laser intensity produced skin temperature increases clearly above the Aδ heat nociceptor threshold (ie, 47°C [50]). The long duration of firing in dorsal horn neurones would be consequent to the gradual decline in skin temperature and likely to include responses evoked by warm skin temperatures.

We also observed that the convergent primary afferent input of NS neurones following electrical stimulation was smaller than those of WDR neurones (unpaired t-tests, Aβ-fibres [$P < .01$], Aδ-fibres [$P < .001$], and C-fibres [$P < .01$]; Fig. 7A). NS neurones also showed significantly smaller measures of cell excitability (postdischarge [$P < .05$], input [$P < .01$], and wind-up [$P < .001$]). Moreover, NS neurones fired significantly less than WDR neurones in response to both innocuous mechanical stimulation (dynamic brush [$P < .05$], 8g vF [$P < .001$]) and noxious mechanical stimulation (26g vF [$P < .001$], 60g vF [$P < .05$]; Fig. 7B).

The human behavioural responses to laser stimulation also were graded with stimulus energy. The number of non detected

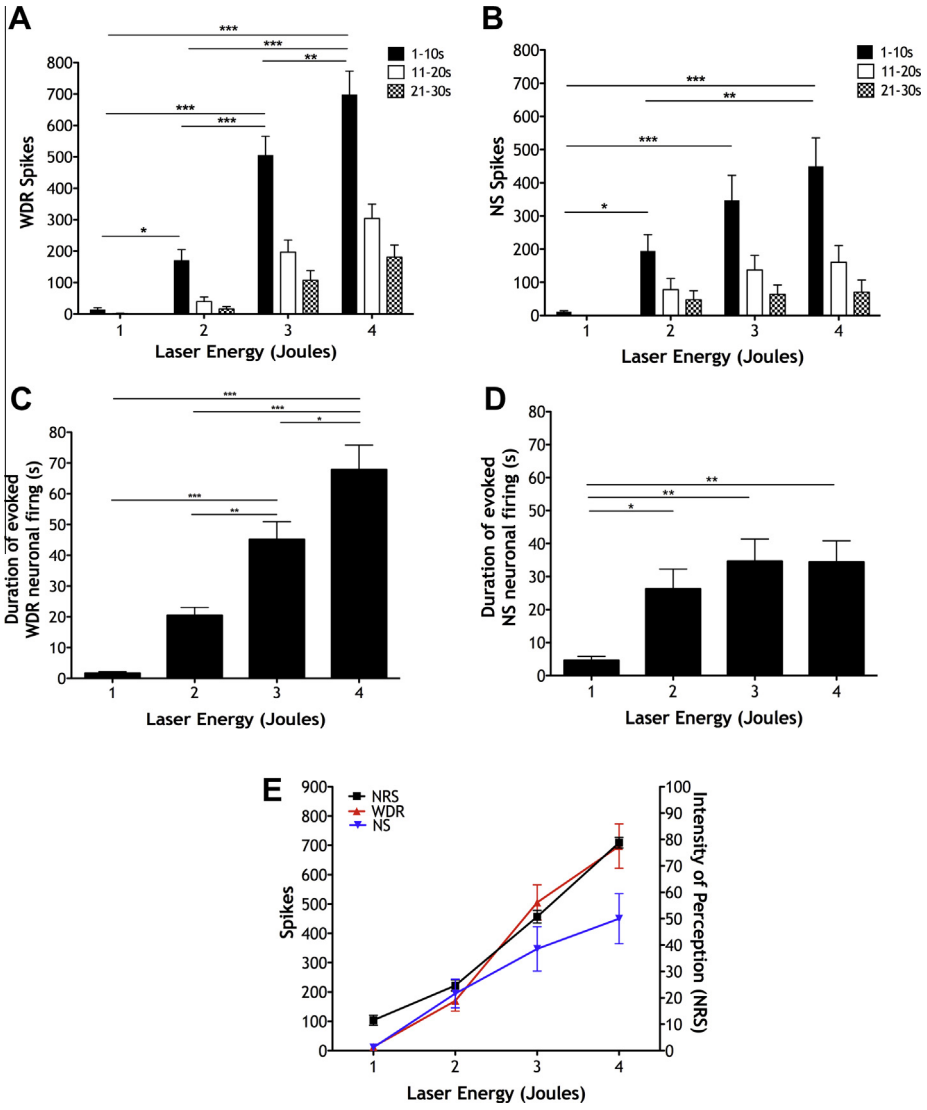

Fig. 5. Graded laser stimulation of the rat hindpaw produces graded firing of deep wide dynamic range (WDR) and superficial nociceptive-specific (NS) dorsal horn neurones that corresponds to human intensity reports. Evoked firing of WDR (A) and NS (B) neurones to increasing heat intensities, shown as the total number of spikes evoked in 3 response time windows (1 to 10 seconds, 1 to 20 seconds, and 21 to 30 seconds after laser stimulation; significance is shown only for response time window 1 to 10 seconds; $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$, post-hoc *t* tests). (C, D) Total duration of WDR and NS firing, respectively, following laser stimulation ($^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$, post-hoc *t* tests). (E) Comparison of the evoked firing of WDR and NS neurones in the rat dorsal horn (1- to 10-second cumulative spikes following 4 J laser energy) against the reported intensities of perception in human subjects with increasing laser stimulation intensities.

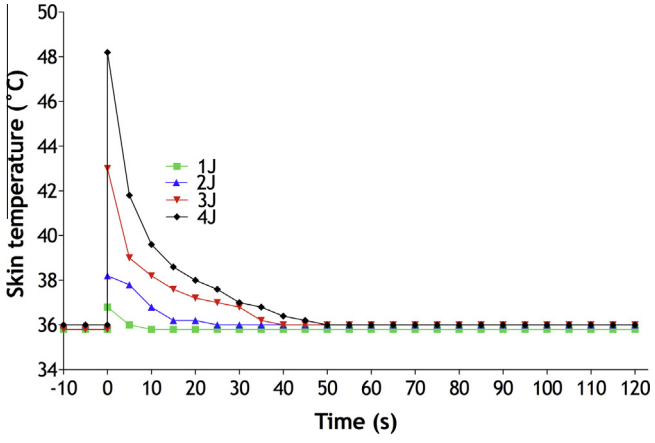

Fig. 6. Skin temperature profile following laser stimulation. Example skin temperature profile of a rat hindpaw illustrating the changes in surface skin temperature following laser stimulation at different energies.

stimuli decreased from 21 at 1 J to 4 at 2 J, whereas all stimuli were perceived at higher energies (Fig. 4C). The perceived stimulus intensity and the number of stimuli in the painful range (ie, perceived as pinprick or burning) increased significantly with stimulus energy (perceived intensity: $P < .0001$, 1-way ANOVA; Figs. 4A and 4C; 1 J: 0%; 2 J: 24% ± 0.15%; 3 J: 49% ± 0.27%; 4 J: 75% ± 0.35%). Interestingly, the energy-dependent increase of perceived intensity in humans was remarkably similar to the energy-dependent increase of neuronal firing in rats (Fig. 5E). When neuronal firing and human reports were normalised (data not shown), there was no significant difference between the WDR and NRS responses at any of the laser intensities. The NS and NRS responses only differed at a stimulus intensity of 2 J. RTs also shortened with increasing laser energy ($P < .0001$, 1-way ANOVA; Fig. 4B). RTs to laser stimuli at the 2 lowest energies were clearly in the C-fibre range (ie, >650 ms when stimulating the hand; [9]), and progressively shifted toward shorter latencies in the Aδ-fibre range when stimuli of 3 J and 4 J were applied. Lastly, the cortical response elicited by laser stimuli in humans was also graded with

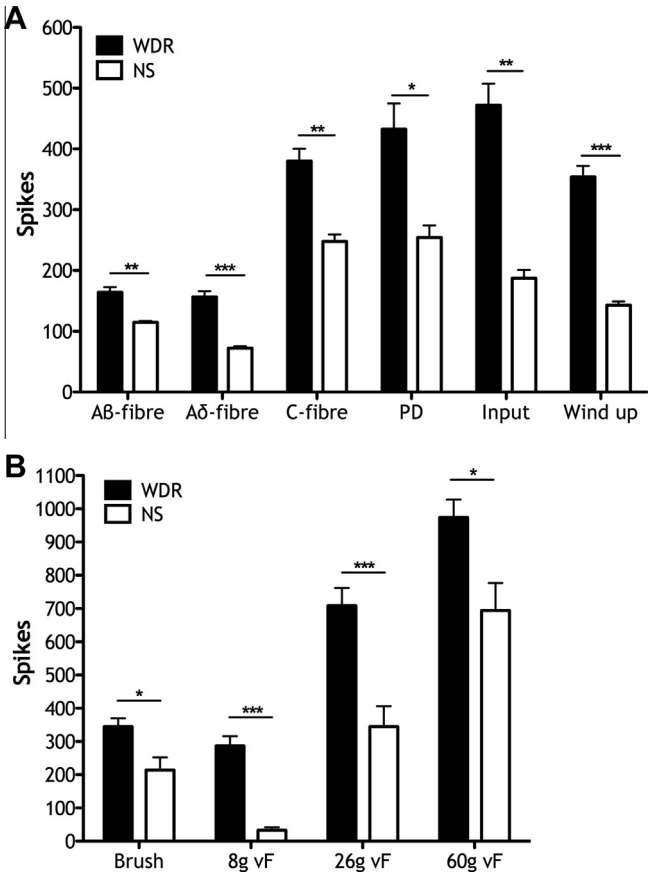

Fig. 7. Comparison of electrically and mechanically evoked responses in deep wide dynamic range (WDR) and superficial nociceptive-specific (NS) neurones in the rat dorsal horn. (A) Electrically evoked responses of WDR and NS neurones illustrating the convergent afferent input of recorded cells (Aβ-fibres, Aδ-fibres, and C-fibres) and measures of excitability (postdischarge, input, and windup). (B) Evoked responses of WDR and NS neurones to innocuous (brush, 8*g* vF) and noxious (26*g* vF, 60*g* vF) mechanical stimulation (*$P < .05$, **$P < .01$, ***$P < .001$).

stimulation intensity (Fig. 4D), with a clear LEP related to the activation of Aδ-fibres only elicited by stimuli of 3 J and 4 J. Stimulus energy was a significant source of variance within 3 different time intervals: 132 to 222 ms (corresponding to the N1 and N2 waves; $F = 58.54$, $P < .0001$, 1-way ANOVA), 244 to 390 ms (corresponding to the P2 wave; $F = 20.76$, $P < .0001$, 1-way ANOVA), and 490 to 579 ms (corresponding to the negative shoulder following the P2 wave; $F = 6.55$, $P < .01$, 1-way ANOVA).

3.3. Spatial summation of Nd:YAP laser input

To explore the spatial summation of nociceptive input, we applied laser stimuli of different fluences (3 in rats: 71, 142, and 214 mJ/mm^2; 2 in humans: 71 and 142 mJ/mm^2), each over a small (28 mm^2) and large (113 mm^2) spot size. The behavioural and electrophysiological responses evoked by laser stimuli of different size but equal intensity were remarkably different, indicating a clear effect of spatial summation in both species.

In rats, laser stimuli delivered over the larger surface evoked significantly more firing of both WDR and NS neurones, at all explored energies (main effect of stimulus size: $P < .001$ [NS] and $P < .0001$ [WDR]; main effect of stimulus energy: $P < .0001$ [NS] and $P < .0001$ [WDR]; no significant stimulus size × stimulus energy interaction for WDR and NS; 2-way ANOVA; Figs. 8A and 8B). Interestingly, while using the larger spot size, WDR firing was clearly graded with laser energy; with the smaller spot size, the WDR firing plateaued at the higher laser energy. In contrast, in NS neurones the intensity-dependent firing plateaued with the larger stimulus spot size.

In humans, laser stimuli delivered over the larger surface elicited significantly more intense sensations (main effect of stimulus size: $P < .0001$, 2-way ANOVA; Fig. 9A). Similar to what was observed in experiment 1, laser stimuli of higher fluence elicited significantly more intense sensations (main effect of stimulus fluence: $P < .0001$, 2-way ANOVA). There was also an interaction between stimulus size and stimulus fluence, indicating that the effect of stimulus size was significantly stronger at high fluence ($P < .01$; 2-way ANOVA).

Reaction times were shorter when laser stimuli were of higher fluence (main effect of stimulus fluence: $P < .0001$, 2-way ANOVA) and delivered over the larger surface (main effect of stimulus size: $P < .05$, 2-way ANOVA). Interestingly, there was a significant stimulus fluence × stimulus size interaction, indicating that the effect of stimulus size was only present when laser stimuli were of high fluence ($P < .05$, 2-way ANOVA; Fig. 9B).

LEP amplitudes were also affected by the spatial summation of the peripheral input (Fig. 5C). We observed significant main effects of both stimulus fluence and stimulus size. Stimuli delivered over the larger surface elicited significantly larger LEPs in the time interval 135 to 207 ms (main effect of stimulus size: $F = 57.23$, $P < .0001$; 2-way ANOVA), coinciding with the latency of both the N1 and the N2 waves. Similarly, stimuli of higher fluence elicited significantly larger LEPs in the time intervals 142 to 214 ms (corresponding to the N1 and N2 waves; $F = 32.54$, $P < .001$; 2-way ANOVA), 251 to 410 ms (corresponding to the P2 wave; $F = 28.55$, $P < .005$; 2-way ANOVA), and 482 to 532 ms (corresponding to the negative shoulder following the P2 wave; $F = 24.14$, $P < .005$;

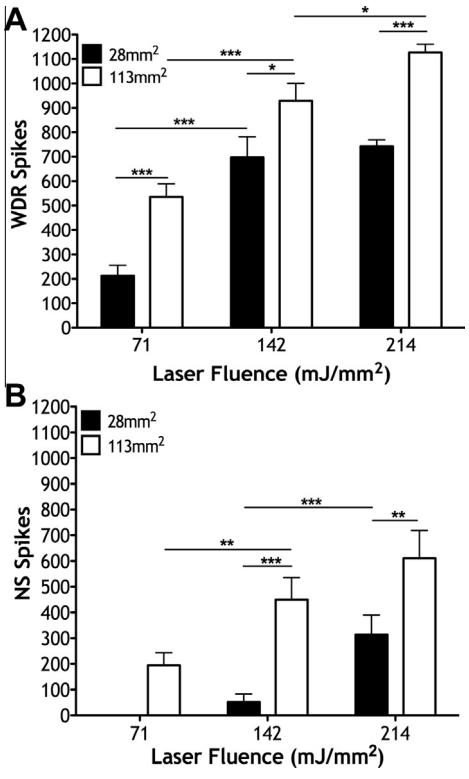

Fig. 8. Firing of deep wide dynamic range (WDR) neurones and superficial nociceptive-specific (NS) neurones in the rat dorsal horn can be spatially summated with laser stimulation. Evoked firing of wide dynamic range (A) and NS (B) neurones in the first time response window (1 to 10 seconds) following graded intensities of laser stimuli with a small (black bars) and large (white bars) spot size of stimulation (2-way ANOVA, *$P < .05$, **$P < .01$, ***$P < .001$).

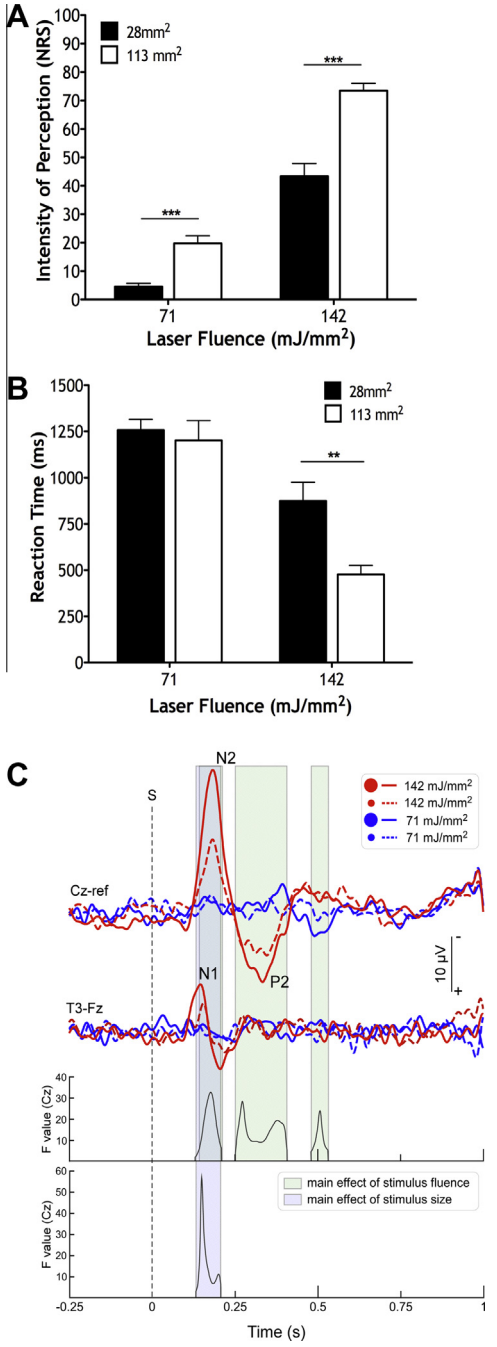

Fig. 9. Effect of spatial summation of nociceptive input on human psychophysical and electrophysiological responses. Nd:YAP laser pulses were delivered on the volar surface of the index and middle fingers in 8 healthy participants, using 2 stimulus fluences (71 and 142 mJ/mm^2) and 2 stimulus sizes (28 and 113 mm^2). (A) Relationship between stimulus fluence, stimulus size, and intensity of perception (***$P < .001$, post-hoc t tests). (B) Relationship between stimulus fluence, stimulus size, and reaction times (**$P < .01$, post hoc t test). (C) Group-level average LEPs elicited by stimuli of different fluence and spot size. N1, N2, and P2 waves are shown at electrodes Cz (nose reference) and Tc (referenced to Fz). x-axis, time (seconds); y-axis, amplitude (µV). The vertical dashed line marks the stimulus onset. The time-course of the F-values expressing the significant effect of stimulus fluence and size are shown below the LEP waveforms. Significant time intervals are highlighted in green (main effect of stimulus energy) and in light blue (main effect of stimulus size) (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

2-way ANOVA). These time intervals were similar to those observed in experiment 1. There was no significant stimulus size × stimulus fluence interaction on the amplitude of LEPs.

4. Discussion

In this study, we explored the central processing of nociceptive inputs in a novel parallel investigation using rats and humans. Using Nd:YAP laser pulses, we compared the responses of deep WDR neurones and superficial NS neurones in the rat dorsal horn with both behavioural and electrocortical responses in humans. We characterised the responses to graded laser stimulation and the effects of spatial summation.

We observed 3 main findings in both rodent and human measures. Firstly, laser stimulation over a range of energies never activated Aβ-fibres, thus showing that it represents a purely nociceptive stimulus. Secondly, graded laser stimuli elicited similarly graded electrophysiological and behavioural responses. Thirdly, there was a significant degree of spatial summation of the nociceptive laser input. The remarkable similarities in the rodent and human data suggest that responses of WDR and NS neurones in the rat dorsal horn can translate to human thermal sensory coding.

4.1. Selective activation of A and C nociceptors by Nd:YAP laser pulses

In the electrophysiological recordings from the rat dorsal horn, laser stimulation always activated C fibres, and at higher energies Aδ-fibres were also recruited. There was no correlation between laser-evoked firing and electrically evoked Aβ-fibre activity in dorsal horn neurones. LEPs evoked by the same laser pulses in humans showed no activity at Aβ-fibre latencies. Crucially, nociceptive laser stimulation never activated Aβ fibres.

The average human reaction times to 1 J and 2 J stimuli indicate that at these energies the afferent input is transmitted in C-fibres [7,51]. With the recruitment of Aδ fibres at higher laser energies, the reaction times of human subjects shortened, in parallel with an increase of reports of pricking sensations characteristic of Aδ-fibre activation [6].

4.2. Spinal and cortical processing of thermal stimulation—parallel responses in rats and humans

We observed significant correlations between numbers of spikes elicited in WDR and NS neurones by noxious mechanical stimuli and 4 J laser stimuli. Second-order spinal neurones that fire to high-threshold heat stimuli are thus likely to produce comparable activity upon nociceptive mechanical stimulation. Peripheral mechanisms for transduction and coding of different somatosensory stimuli have been characterised peripherally [29], but knockout and microneurography studies show that primary afferents can code for more than 1 nociceptive stimulus modality [1,10,11]. Because sensory discrimination can involve overlapping of transduction for different stimulus modalities across different fibre types prior to central processing, central structures play an important role in discriminating between sensory modalities and intensities [14].

Indeed, second-order spinal cord neurones are the first central structures to receive somatic sensory input. Earlier work has illustrated that dorsal horn neurones participate in the encoding process of heat stimuli in primates [22,23,32]. We reproduced this intensity coding to laser stimuli in WDR and NS neurones in the rat dorsal horn and showed that the same stimulus parameters can evoke graded behavioural and electrophysiological responses in humans. WDR and NS neurones exhibited consistent responses within each population, suggesting that coding processes are common for projection and intrinsic neurones within these populations given that our recorded spinal neurones may be either. Although NS neurones show a positive relationship between stimulus inten-

sity and response frequency, they produced lower discharge frequencies to thermal and mechanical stimulation and cell excitability measures compared with WDR neurones (as expected [46]). This is likely a direct consequence of intrinsic properties and/or tonic GABAergic and glycinergic inhibition of the superficial dorsal horn [47]. Moreover, projections of lamina I neurones in the rat are dominated by medullary and parabrachial targets, and less so by direct spinothalamic projections that transmit sensory information to insular and somatosensory cortices for discriminative aspects of sensory processing [48]. It is thus conceivable that fine-tuned information regarding stimulus intensity is provided by WDR neuronal projections, whereas the activity of superficial NS neurones provides simple information about stimuli to induce appropriate autonomic and emotional responses. These factors underlying the difference in WDR and NS discharge frequencies may relate to an evolutionary pressure to conserve metabolically efficient neural codes [5].

Superficial NS neurones responded to low-threshold dynamic brush, but not low intensities of static von Frey stimulation. This may underlie our reports of touch to lower laser stimulation intensities, given that C-mechanoreceptors providing input to lamina I contribute to low-threshold activity [47]. C-tactile afferents are also involved in pleasant touch sensations [3,28]. Furthermore, dynamic regulation of touch and pain processing through inhibitory modulation of glycinergic neurones in the superficial dorsal horn may permit low-threshold inputs to penetrate to high-threshold lamina I cells [34,49].

The magnitude of LEP responses recorded in humans was also graded with both intensity of stimulation and subjective intensity of perception, as previously described [26]. Interestingly, there was a remarkable overlay between the number of evoked spikes in both WDR and NS neurones with the subjective intensity ratings of human subjects. This suggests that the ability of rat dorsal horn neurones to encode stimulus intensity through discharge frequency is likely related to perceptual outcome, presumably by transfer of graded nociceptive information to higher centres that is reflected by human intensity ratings (and LEPs at suprathreshold intensities) [8,39]. However, there was a striking difference in the respective durations of rat spinal cord activity and human perception. Indeed, whereas heat-evoked neuronal firing can last up to 1 minute, representing central excitability such as wind-up and corresponding postdischarge, the perception of a 4-ms-long laser pulse is very transient, lasting at most only a few seconds [41]. This continued low-frequency firing in WDR and NS neurones is unlikely to contribute to conscious sensory perception, but could facilitate late reflex responses in animals [45] or the nociceptive flexion reflex in humans [40].

Although identical laser stimulation parameters were used in both rats and humans, we must note that differences in thermophysical properties of the skin, including transparency and reflectance of the epidermal layers, do not necessarily implicate that the delivered stimulation was physiologically identical in the 2 species [2,24]. Furthermore, rodents were anaesthetized. Nevertheless, we have still shown that matching the parameters of laser stimulation produces highly correlated electrophysiological and perceptual responses of rats and human subjects.

4.3. Spatial summation of nociceptive heat input

Several studies have investigated spatial summation of innocuous or noxious heat stimulation in humans, showing that enlarging the stimulus area produces an increase in the intensity of the stimulus-evoked responses [13,19,36,43]. Here we provide the first parallel study of spatial summation in humans and rats using a range of heat intensities.

The mechanisms governing spatial summation are likely to happen at different levels of the central nervous system. Greater stimulation areas can activate peripheral zones of neighbouring receptive fields of spinal neurones [42], and through this recruitment of additional nociceptors and increasing stimulus intensities, it is possible to produce greater firing of spinal neurones [44]. We saw a clear effect of spatial summation of laser stimulation in both rats and humans using identical stimulation parameters. There was a significant increase in dorsal horn neuronal firing in rats and greater intensity ratings and LEP responses in humans, reinforcing the idea that spinal coding impinges on upstream perception.

The use of high-intensity stimulation (214 mJ/mm^2) while recording from central neurones in anaesthetised preparations was important to overcome limitations of behavioural studies using withdrawal thresholds or using near-maximum pain responses. As a result, we observe a plateau of evoked WDR activity at the highest intensity of stimulation over the smaller spot size, suggesting a maximum level of nociceptor recruitment within that spatial restraint. Similar reasoning may underlie the ceiling effect observed in the larger spot size in NS neurones, given that these are less excitable than WDR neurones.

4.4. Conclusions

We observed a strong concordance between the rodent spinal and human perceptual responses to laser stimulation, both for intensity coding and spatial summation. Thus, unlike threshold behavioural studies, the suprathreshold responses of dorsal horn neurones can relate to the high pain levels that patients report. Dorsal horn recordings in rats allows for such a quantitative analysis of neuronal coding to laser, or indeed any other form of stimulation at the full range of stimulus intensities, by permitting suprathreshold responses to be monitored. Indeed, these responses are critical in pain states but are not amenable to analysis by behavioural studies that can only determine withdrawal thresholds. Furthermore, characterisation of suprathreshold responses can also prove critical for the assessment of the efficacy of novel drugs, which may have different modulatory effects on responses to higher intensities of stimulation compared to threshold measures. These results are important for translational research. Imperatively, the concordance between the human and rat responses could be altered by changes in coding properties of neurones in pathological states [16,37,38]. Furthermore, spinal neurones can be modulated by a number of agents that alter pain ratings in patients [16]. However, the remarkable similarities in the rodent and human data indicate that responses of WDR and NS neurones in the rat dorsal horn can translate to coding of acute thermal sensations in humans. This correspondence of animal and human data suggests that recordings of central neuronal activity could be a valuable predictive measure of human thermal sensory perception.

Acknowledgements

There are no conflicts of interest to report. S.S. and A.H.D. are supported by IMI Europain and the Wellcome Trust London Pain Consortium. G.D.I. is University Research Fellow of The Royal Society and acknowledges the support of the El.En. group. I.R. is supported by Fondazione Caligara.

References

[1] Abrahamsen B, Zhao J, Asante CO, Cendan CM, Marsh S, Martinez-Barbera JP, Nassar MA, Dickenson AH, Wood JN. The cell and molecular basis of mechanical, cold, and inflammatory pain. Science 2008;321:702–5.
[2] Anderson RR, Parrish JA. The optics of human skin. J Invest Dermatol 1981;77:13–9.

[3] Andrew D. Quantitative characterization of low-threshold mechanoreceptor inputs to lamina I spinoparabrachial neurons in the rat. J Physiol 2010;588:117–24.
[4] Arendt-Nielsen L, Chen AC. Lasers and other thermal stimulators for activation of skin nociceptors in humans. Neurophysiol Clin 2003;33:259–68.
[5] Attwell D, Gibb A. Neuroenergetics and the kinetic design of excitatory synapses. Nat Rev Neurosci 2005;6:841–9.
[6] Beissner F, Brandau A, Henke C, Felden L, Baumgartner U, Treede RD, Oertel BG, Lotsch J. Quick discrimination of A(delta) and C fiber mediated pain based on three verbal descriptors. PLoS One 2010;5:e12944.
[7] Beydoun A, Morrow TJ, Casey KL. Pain-related laser-evoked potentials in awake monkeys: identification of components, behavioral correlates and drug effects. PAIN® 1997;72:319–24.
[8] Bromm B, Treede RD. Nerve fibre discharges, cerebral potentials and sensations induced by CO_2 laser stimulation. Hum Neurobiol 1984;3:33–40.
[9] Calvo M, Zhu N, Grist J, Ma Z, Loeb JA, Bennett DL. Following nerve injury neuregulin-1 drives microglial proliferation and neuropathic pain via the MEK/ERK pathway. Glia 2011;59:554–68.
[10] Campero M, Baumann TK, Bostock H, Ochoa JL. Human cutaneous C fibres activated by cooling, heating and menthol. J Physiol 2009;587:5633–52.
[11] Cavanaugh DJ, Lee H, Lo L, Shields SD, Zylka MJ, Basbaum AI, Anderson DJ. Distinct subsets of unmyelinated primary sensory fibers mediate behavioral responses to noxious thermal and mechanical stimuli. Proc Natl Acad Sci USA 2009;106:9075–80.
[12] Chapman CR, Casey KL, Dubner R, Foley KM, Gracely RH, Reading AE. Pain measurement: an overview. PAIN® 1985;22:1–31.
[13] Chen AC, Niddam DM, Crawford HJ, Oostenveld R, Arendt-Nielsen L. Spatial summation of pain processing in the human brain as assessed by cerebral event related potentials. Neurosci Lett 2002;328:190–4.
[14] Craig AD. Pain mechanisms: labeled lines versus convergence in central processing. Annu Rev Neurosci 2003;26:1–30.
[15] Devor M, Carmon A, Frostig R. Primary afferent and spinal sensory neurons that respond to brief pulses of intense infrared laser radiation: a preliminary survey in rats. Exp Neurol 1982;76:483–94.
[16] Dickenson AH, Donovan-Rodriguez T, Matthews E. Understanding central mechanisms of pain and pain modulation. In: Mao J, editor. Translational pain research, vol. 2. New York: Nova Science; 2006. p. 1–23.
[17] Dickenson AH, Sullivan AF. Electrophysiological studies on the effects of intrathecal morphine on nociceptive neurones in the rat dorsal horn. PAIN® 1986;24:211–22.
[18] Dickenson AH, Sullivan AF. Evidence for a role of the NMDA receptor in the frequency dependent potentiation of deep rat dorsal horn nociceptive neurones following C fibre stimulation. Neuropharmacology 1987;26:1235–8.
[19] Douglass DK, Carstens E, Watkins LR. Spatial summation in human thermal pain perception: comparison within and between dermatomes. PAIN® 1992;50:197–202.
[20] Doyle CA, Hunt SP. A role for spinal lamina I neurokinin-1-positive neurons in cold thermoreception in the rat. Neuroscience 1999;91:723–32.
[21] Drenth JP, Waxman SG. Mutations in sodium-channel gene SCN9A cause a spectrum of human genetic pain disorders. J Clin Invest 2007;117:3603–9.
[22] Dubner R, Kenshalo Jr DR, Maixner W, Bushnell MC, Oliveras JL. The correlation of monkey medullary dorsal horn neuronal activity and the perceived intensity of noxious heat stimuli. J Neurophysiol 1989;62:450–7.
[23] Ferrington DG, Sorkin LS, Willis Jr WD. Responses of spinothalamic tract cells in the superficial dorsal horn of the primate lumbar spinal cord. J Physiol 1987;388:681–703.
[24] Hardy JD. Body temperature regulation. In: Mountcastle VB, editor. Medical physiology. St Louis: Mosby; 1980. p. 14–8.
[25] Hu L, Mouraux A, Hu Y, Iannetti GD. A novel approach for enhancing the signal-to-noise ratio and detecting automatically event-related potentials (ERPs) in single trials. Neuroimage 2010;50:99–111.
[26] Iannetti GD, Zambreanu L, Wise RG, Buchanan TJ, Huggins JP, Smart TS, Vennart W, Tracey I. Pharmacological modulation of pain-related brain activity during normal and central sensitization states in humans. Proc Natl Acad Sci USA 2005;102:18195–200.
[27] IASP. Do animal models tell us about human pain? IASP Pain Clinical Updates July 2010; 18:5.
[28] Loken LS, Wessberg J, Morrison I, McGlone F, Olausson H. Coding of pleasant touch by unmyelinated afferents in humans. Nat Neurosci 2009;12:547–8.
[29] Lumpkin EA, Caterina MJ. Mechanisms of sensory transduction in the skin. Nature 2007;445:858–65.
[30] Magerl W, Ali Z, Ellrich J, Meyer RA, Treede RD. C- and A delta-fiber components of heat-evoked cerebral potentials in healthy human subjects. PAIN® 1999;82:127–37.
[31] Maixner W, Dubner R, Bushnell MC, Kenshalo Jr DR, Oliveras JL. Wide-dynamic-range dorsal horn neurons participate in the encoding process by which monkeys perceive the intensity of noxious heat stimuli. Brain Res 1986;374:385–8.
[32] Maixner W, Dubner R, Kenshalo Jr DR, Bushnell MC, Oliveras JL. Responses of monkey medullary dorsal horn neurons during the detection of noxious heat stimuli. J Neurophysiol 1989;62:437–49.
[33] Meyer RA, Campbell JN, Raja SR. Peripheral neural mechanisms of nociception. In: Wall P, Melzack R, editors. Textbook of pain. Edinburgh: Churchill Livingstone; 1994. p. 13–44.
[34] Miraucourt LS, Dallel R, Voisin DL. Glycine inhibitory dysfunction turns touch into pain through PKCgamma interneurons. PLoS One 2007;2:e1116.
[35] Mouraux A, Iannetti GD, Plaghki L. Low intensity intra-epidermal electrical stimulation can activate Adelta-nociceptors selectively. PAIN® 2010;150:199–207.
[36] Nielsen J, Arendt-Nielsen L. Spatial summation of heat induced pain within and between dermatomes. Somatosens Mot Res 1997;14:119–25.
[37] Palecek J, Dougherty PM, Kim SH, Paleckova V, Lekan H, Chung JM, Carlton SM, Willis WD. Responses of spinothalamic tract neurons to mechanical and thermal stimuli in an experimental model of peripheral neuropathy in primates. J Neurophysiol 1992;68:1951–66.
[38] Palecek J, Paleckova V, Dougherty PM, Carlton SM, Willis WD. Responses of spinothalamic tract cells to mechanical and thermal stimulation of skin in rats with experimental peripheral neuropathy. J Neurophysiol 1992;67:1562–73.
[39] Pertovaara A, Morrow TJ, Casey KL. Cutaneous pain and detection thresholds to short CO_2 laser pulses in humans: evidence on afferent mechanisms and the influence of varying stimulus conditions. PAIN® 1988;34:261–9.
[40] Plaghki L, Bragard D, Le Bars D, Willer JC, Godfraind JM. Facilitation of a nociceptive flexion reflex in man by nonnoxious radiant heat produced by a laser. J Neurophysiol 1998;79:2557–67.
[41] Ploner M, Gross J, Timmermann L, Schnitzler A. Cortical representation of first and second pain sensation in humans. Proc Natl Acad Sci USA 2002;99:12444–8.
[42] Price DD, Hayes RL, Ruda M, Dubner R. Spatial and temporal transformations of input to spinothalamic tract neurons and their relation to somatic sensations. J Neurophysiol 1978;41:933–47.
[43] Price DD, McHaffie JG, Larson MA. Spatial summation of heat-induced pain: influence of stimulus area and spatial separation of stimuli on perceived pain sensation intensity and unpleasantness. J Neurophysiol 1989;62:1270–9.
[44] Quevedo AS, Coghill RC. Filling-in, spatial summation, and radiation of pain: evidence for a neural population code in the nociceptive system. J Neurophysiol 2009;102:3544–53.
[45] Schouenborg J, Sjolund BH. Activity evoked by A- and C-afferent fibers in rat dorsal horn neurons and its relation to a flexion reflex. J Neurophysiol 1983;50:1108–21.
[46] Seagrove LC, Suzuki R, Dickenson AH. Electrophysiological characterisations of rat lamina I dorsal horn neurones and the involvement of excitatory amino acid receptors. PAIN® 2004;108:76–87.
[47] Takazawa T, MacDermott AB. Glycinergic and GABAergic tonic inhibition fine tune inhibitory control in regionally distinct subpopulations of dorsal horn neurons. J Physiol 2010;588:2571–87.
[48] Todd AJ. Anatomy of primary afferents and projection neurones in the rat spinal dorsal horn with particular emphasis on substance *P* and the neurokinin 1 receptor. Exp Physiol 2002;87:245–9.
[49] Torsney C, MacDermott AB. Disinhibition opens the gate to pathological pain signaling in superficial neurokinin 1 receptor-expressing neurons in rat spinal cord. J Neurosci 2006;26:1833–43.
[50] Treede RD, Meyer RA, Campbell JN. Myelinated mechanically insensitive afferents from monkey hairy skin: heat-response properties. J Neurophysiol 1998;80:1082–93.
[51] Treede RD, Meyer RA, Raja SN, Campbell JN. Evidence for two different heat transduction mechanisms in nociceptive primary afferents innervating monkey skin. J Physiol 1995;483:747–58.