Research Paper

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A comparative behavioural study of mechanical hypersensitivity in 2 pain models in rats and humans

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Abstract

The assessment of pain sensitivity in humans has been standardized using quantitative sensory testing, whereas in animals mostly paw withdrawal thresholds to diverse stimuli are measured. This study directly compares tests used in quantitative sensory testing (pinpricks, pressure algometer) with tests used in animal studies (electronic von Frey test: evF), which we applied to the dorsal hind limbs of humans after high frequency stimulation and rats after tibial nerve transection. Both experimental models induce profound mechanical hypersensitivity. At baseline, humans and rats showed a similar sensitivity to evF with 0.2 mm diameter tips, but significant differences for other test stimuli (all $P < 0.001$). When expressed as force divided by circumference, baseline thresholds for 0.8 mm probes were higher than for 0.2 mm in both species (both $P < 0.001$) suggesting spatial summation. At similar probe diameters, ramped stimuli showed higher baseline thresholds than stepped stimuli ($P < 0.01$) but similar sensitivity to change. For ramped stimuli sensitivity to change was higher with small probe tips than large blunt tips in both pain models ($P < 0.01$ in rat, $P < 0.05$ in humans). These data show that rat paw withdrawal threshold to punctate stimuli (0.2 mm diameter) can be used as surrogate parameters for human mechanical pain sensitivity, but probe size and shape should be standardized. Hypersensitivity to blunt pressure—the leading positive sensory sign after peripheral nerve injury in humans—is a novel finding in the tibial nerve transection model. By testing outside the primary zone of nerve damage (rat) or activation (humans), our methods likely involve effects of central sensitization in both species.

Keywords: Neuropathic pain, Quantitative sensory testing, Animal models, Translational research

1. Introduction

Neuropathic pain is characterized by the coexistence of positive sensory signs as hyperalgesia and allodynia and negative sensory signs as hypaesthesia in the same region.[42] Quantitative sensory testing (QST) is a standardized procedure to quantify both positive and negative sensory signs by measuring responses to calibrated stimuli[1] with an excellent test–retest and inter-observer reliability.[12] In preclinical research, the effects of medications on behavioural signs of neuropathic pain in animal models are measured,[15] but efforts at standardization are less advanced than in humans.[24] Although human pain ratings reflect pain perception, most animal studies test the sensitivity to mechanical, thermal, or chemical stimuli by using paw withdrawal thresholds (PWTs) as a surrogate for pain perception. In the present study, we aim to directly compare mechanical hypersensitivity in experimental pain models in rats and humans using identical test stimuli.

Mechanical hypersensitivity is a hallmark sign of secondary hyperalgesia in skin surrounding an injury.[40] Secondary hyperalgesia can also be induced by chemical or electrical stimulation,[16,23,38] which simulates injury-induced nociceptor activity without causing tissue damage. In the capsaicin model, mechanical hypersensitivity has been shown to be due to central sensitization of spinal nociceptive neurons to glutamatergic inputs.[6] Mechanical hypersensitivity is also a characteristic of neuropathic pain[8,13,31,40] that is reproduced in animal models of peripheral nerve injury.[15] In rodents, effects of standard analgesics on capsaicin-induced and nerve injury-induced mechanical hypersensitivity are similar.[17]

To translate sensory findings between human subjects and animals we used 2 experimental pain models leading to mechanical hypersensitivity: (1) electrical high frequency stimulation (HFS) in healthy human subjects,[20] which induces central sensitization through long-term potentiation (LTP) for about 1 day[20,37]; (2) tibial nerve transection (TNT) in rats as an animal model of neuropathic pain[27] resulting in mechanical hypersensitivity over a couple of weeks.

Tests to assess mechanical hypersensitivity differ widely between human and animal studies: the standard QST protocol for humans includes calibrated pinprick stimulators[5] to measure the mechanical pain threshold (MPT) of the skin and a pressure algometer to measure the pressure pain threshold (PPT) to blunt stimuli which is mostly due to compression of underlying muscle.[9] Animal studies commonly use von Frey type tests, either applied as ramped (electronic von Frey test, evF) or stepped stimuli (von Frey filaments, vFFs). von Frey filaments were initially introduced to measure the threshold for light touch[41]; to assess pain responses, force of vFF is increased by increasing the filament

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

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PAIN 157 (2016) 1248–1258

http://dx.doi.org/10.1097/j.pain.0000000000000515

diameter, which makes this a highly nonlinear parameter. Previous studies have shown systematic effects of stimulus application mode (ramped vs stepped), probe shape and size (punctate vs blunt), and skin type (glabrous vs hairy) on nociceptor activation, animal behaviour, and human pain.[3,14,18,43]

The aims of this study were to quantify the effects of stimulus parameters on baseline mechanical thresholds in rats and humans and to compare the sensitivity to change in experimental models of mechanical hypersensitivity across species. Thus, this study strives to be an example for translational pain research.

2. Material and methods

2.1. Ethical statement

All animal experiments conformed to the German Regulations (Animal Welfare Act of June 7, 2006 [BGBl. I S. 1313]. Project license AZ 35-9185.81/G-122/10 issued to R.-D.T. by the administrative district of Karlsruhe). Housing and handling of the animals as well as all experiments were conducted according to the Guidelines on Ethical Standards for Investigation of Experimental Pain in Animals.[45] This report follows the ARRIVE guidelines.[19]

The study on healthy volunteers was approved by the local Ethics Committee (FOR926 Teilprojekt 5) and conformed to the Declaration of Helsinki. Each subject was familiarized beforehand with the experimental procedures and gave written, informed consent. Subjects were not informed about the hypotheses of the study.

2.2. Animals and housing

We used 16 male rats (Wistar-Han rats weighing 188 ± 2 g [177-209 g] at arrival, corresponding to an estimated age of 47-51 days) divided into the following groups (n = 8 each): sham-operated animals and TNT-injured animals. This number was chosen according to our pilot experiments. The rats were randomly assigned to the sham or TNT group. Each rat was tested with all noxious stimuli: evF test with 3 different tips, pinprick stimulators, and vFFs. The experiments were performed as a baseline before surgery and weekly after the operation for 5 weeks. Rats were housed in groups of 4 per cage with access to standard rodent food and water ad libitum. Animals in one cage belonged to the same experimental group. The animals were kept under a standard light cycle (5:30 AM-6:30 PM) in a temperature- (22°C ± 2°C) and humidity-controlled (55% ± 5%) environment. The experiments were performed during day time (9:00 AM-1:00 PM).

2.3. Tibial nerve transection

The rat neuropathic pain model was induced with TNT, the reference model of the Innovative Medicines Initiative Europain consortium. The surgery was performed on the basis of the original description by Lee et al.[27] Shortly, the rats were anesthetized with isoflurane. The right leg was shaved, disinfected, and the skin was incised. After separation of the muscle tissue, the tissue around the tibial nerve was carefully removed. The nerve was exposed, tightly ligated with 2 ligatures (4-0) at a distance of 3 mm and then transected. The tibial nerve innervates the plantar foot. The common sural and peroneal nerves were left intact; hence, the test site on the dorsal foot was adjacent to but outside the denervated skin zone. The wound was then closed with one deep tissue suture and several skin sutures (4-0 Vicryl, V494H; Ethicon, Somerville, NJ). The sham-operation was performed by exposing the nerve in exactly the same way until visible but without touching it. Half of the rats received TNT; the other half was sham-operated and served as control. The rats were randomized into the treatment groups by throwing a coin. Surgery was performed after the baseline of the different tests was measured. After surgery, the animals were left to heal for 1 week, and then the experiments were conducted weekly for 5 weeks. On the surgery day, the rats weighed 420 ± 7 g (389-490 g); the weight of the rats was monitored weekly.

2.4. Healthy volunteers

The study was conducted in 10 healthy male volunteers (mean age 26.7 years, 2 left-handed, 8 right-handed). Exclusion criteria included history of chronic pain and psychiatric disorders, injuries to the testing site, drug abuse, and analgesic medication during the last 5 days. All subjects were tested on the left and the right feet with all noxious stimuli: evF test with 3 different tips, pinprick stimulators, and pressure algometer. The experiments were performed as a baseline and at 2 points in time after HFS. High frequency stimulation and all experimental tests were performed by the same operator as the experimental tests on animals.

2.5. High frequency stimulation

Conditioning electrical pulse trains were applied by simultaneous stimulation through a homemade circular array (diameter: 3.5 cm) of 48 punctate electrodes with a diameter of 0.25 mm each. The electrodes were mounted in a plastic frame and attached to the skin with a rubber band. Superficial nociceptive Aδ- and C-fiber afferents are activated already at low stimulus intensities by this configuration of the electrode.[20] With the help of a constant current stimulator (model DS7A; Digitimer Ltd, Welwyn Garden City, United Kingdom), cathodal electrical stimuli were applied to the dorsum of the foot. A large surface electrode on the same calf was used as anode. The stimulation side was the medial dorsum of the foot opposite to handedness of the subject, the exact localization was determined with help of the extensor hallucis longus tendon and the second and third toes. The electrical detection threshold (EDT) was determined as the geometrical mean of 3 suprathreshold and 3 subthreshold values of single stimuli of 2 milliseconds duration. The conditioning stimulation consisted of HFS trains of 100 Hz for 1 second (pulse duration 2 millisecond, stimulus intensity 10 × EDT, model DG2A; Digitimer Ltd) repeated 5 times at a 10-second interval. This protocol has previously been shown to induce LTP at primary afferent synapses with rat spinal cord neuron and primary and secondary hyperalgesia in humans.[23] The test site on the dorsal foot was in the secondary hyperalgesia zone. The subjects were asked to rate the magnitude of pain to the conditioning stimulation on a numerical rating scale ranging from 0 (not painful) to 100 (most intense pain imaginable).

2.6. Test stimuli and test sites

This study aims at applying the same tests for mechanical sensitivity to humans and to rats to facilitate the translation between basic and clinical studies.

For both animals and humans, mechanical test stimuli consisted of an evF esthesiometer (SENSEBox; Somedic AB, Hörby, Sweden) with 2 different cylindrical, rigid tips with 0.2 mm and 0.8 mm as a diameter and a blunt rubber tip with a 5 mm diameter. Furthermore, we used a set of standardized pinpricks with a flat tip and a diameter of 0.25 mm exerting the force of 8, 16, 32, 64, 128, 256, and 512 mN (MRC Systems GmbH, Heidelberg, Germany). Additionally, in all animal experiments, a set of standardized vFFs exerting the force of 8, 16, 32, 64, 128, 256, and 512 mN was used (contact areas varied with filament strengths). In human experiments, we also used a pressure algometer with a contact area of 1 cm^2 and a built-in pressure display (Force dial; Wagner Instruments, Greenwich, CT).

Selection of test sites: human QST is commonly applied to hairy skin (dorsal surface of the hand or the foot, face or trunk), whereas sensory testing in rat TNT and other nerve injury models is mostly applied to glabrous skin (plantar surface of the hind paw). We chose the dorsal surface of the rat hind paw and compared it with the dorsal surface of the human feet because of the following reasons: the 2 skin types differ in thickness and innervation by mechanoreceptors and nociceptors.[3] As the front paw is not suitable for testing because it is not easily accessible, we chose the dorsal hind paw. Furthermore, data might be confounded by partial nerve degeneration in the plantar skin region, whereas our study focused on positive sensory signs. We tested the dorsal central area of the hind paw using a modification of the method described by Ren[35] consisting of a black cloth wrapped around the upper body of the rats. In our experience, this helped to calm the rats down, they showed no signs of discomfort and it made our measurements more stable and reliable.

Because of distinctive posture of the paw after TNT surgery, the injured side was visibly recognizable. Therefore, blinding to the side of injury was not possible in animal experiments. For a parallel design, we did not attempt to blind the testing in humans either. As the mechanical stimulators are obviously different, we could not blind this part of the experiments.

The baseline of mechanical sensitivity and changes in sensitivity were tested in all rats on the operated side and on the contralateral side. Baseline measurements were performed twice before the surgery at least 7 days after the arrival and after 2 days of habituation to the experimentation room. After surgery, tests were performed weekly over a time-course of 5 weeks to pursue if the mechanical hypersensitivity changes over time. After a minimum of 45-minute habituation in the test room, animals were tested individually. Each animal underwent the same sequence of experiments, starting with the evF test with the 0.2 mm tip, followed by a break of 30 minutes and the test with the 0.8 mm tip. On the following day, the experiments using pinpricks, the blunt rubber tips and vFFs were performed. The animal experiments were split to 2 consecutive experimental days intending to minimize the influence of previous test stimuli on the results of test stimuli later used in the same skin area. After calibration, pressure of increasing intensity (25 g/s) was applied with the evF esthesiometer perpendicularly to the dorsal central area of the hind paw until the rat withdrew it. The PWT was calculated as the arithmetic mean of 3 independent measurements. All tests were performed first on the paw contralateral to the surgery, followed by the injured paw. Pinpricks and vFFs were applied in increasing order perpendicularly to the test area until the rat withdrew its paw. Then, the next lower pinprick or filament was used until no withdrawal was seen. The PWT was calculated as the geometrical mean of 5 suprathreshold and 5 subthreshold values.

For the experiments on healthy human volunteers, mechanical and pressure pain sensitivity were measured before and immediately after HFS as well as after a break of 20 minutes. The area surrounding the conditioning electrode in a 1.5-cm broad strip, an area well within the previously reported secondary hyperalgesia zone,[7,25] was divided into 5 equal parts. Each test stimulus was assigned to one of these areas and to the equivalent area on the opposite foot. The experiments were conducted on both feet in the respective localizations before and immediately after the conditioning stimuli as well as after a 20-minute break. All subjects were tested with the evF test with the 3 tips described above as well as the pinprick stimulators and the pressure algometer. The test to start with was randomized with the help of a Latin square to prevent any influence of the time point of testing on the results, as HFS induces transient hyperalgesia. The standardized instructions were read out aloud to the subject before each baseline test and were repeated shortly before each following test. The exact wording was taken from the German QST manual published by the German Research Network on Neuropathic Pain (DFNS). The evF test was performed with the 0.2-mm and 0.8-mm tips to assess the MPT. After calibration, ramped stimuli (25 g/s) of increasing force were applied perpendicularly to the tested skin area until the subject indicated a pricking or stinging sensation additionally to the touch sensation. The MPT was calculated as the arithmetical mean of 3 independent nociceptive thresholds. Pinprick stimulators were applied perpendicularly to the test area until the subject felt a pricking or stinging sensation additionally to the touch sensation, the MPT to pinprick stimuli was calculated as the geometrical mean of 5 suprathreshold and 5 subthreshold values as proposed in the QST protocol.[36] To assess the PPT, a pressure algometer and the evF with the blunt tip were used. The pressure algometer was applied perpendicularly to the test area with ramped stimuli of slowly increasing intensity (0.5 kg/s) until the subject indicated a burning or drilling sensation additionally to the touching sensation. The evF test with a blunt tip was applied in the same manner (25 g/s). For both pressure algometer and blunt tip, the PPT was calculated as the arithmetic means of 3 independent measurements.

2.7. Drop-outs

There were no drop-outs due to unwell-being of the animals, which was defined as a weight loss of 20% or self-mutilation of the injured paw. We did not experience any drop-outs in the human group.

2.8. Statistical evaluation

Data were analyzed with Statistical Analysis System for Windows. The results are expressed as means ± SEM. To test for normality, we used the Shapiro–Wilk and the χ^2 tests. The Wilcoxon test for dependent data was used for all comparisons between the ipsilateral and contralateral sides in the same group. To assess the change over the experimental series, the Friedman analysis of variance was used. A *U* test for independent data was used for all comparisons between the human and the animal groups and across stimulus types. A P value < 0.05 was considered as significant.

We compared the results for MPT to pinprick stimulation with the reference database of healthy controls of the DFNS with the dorsal foot as reference site.[29] For calculation of the *z* values, we

normalized the subject data to gender and age group of the controls (z = [individual value − $mean_{database}$]/$SD_{database}$). Z values indicate hyposensitivity (z below 0) or hypersensitivity (z above 0) to the applied stimuli in comparison with age- and gender-matched controls. Proposed quality standards for between-laboratory variance for healthy subjects are mean Z: 0 ± 0.25 and mean SD: 1 ± 0.1.[29]

3. Results

3.1. Animal experiments

3.1.1. Weight monitoring

On the day of surgery, there was no difference in weight between animals in the TNT and the sham group (sham 421 ± 8 g, TNT 420 ± 12 g, $P = 0.789$). During the study, all rats gained weight regularly. However, at the end of the study, TNT animals had gained slightly less weight than sham-operated animals (sham 142 ± 9 g, TNT 117 ± 10 g, $P = 0.071$). Both groups looked healthy.

3.1.2. Effect of tibial nerve transection on paw withdrawal thresholds obtained with different test stimuli

The baseline measurements did neither reveal any difference between sham-operated animals and TNT animals nor between ipsilateral and contralateral paw. Regarding sham animals, there was no significant difference in PWTs between the sham-operated paw and the contralateral paw at all measuring points after surgery in all stimuli tested (data not shown). Accordingly, no difference was seen between sham animals and the contralateral paw of TNT animals (data not shown).

Tibial nerve transection animals exhibited significant reductions in all mechanical PWTs consistent over all measuring points assessed and a significant difference was seen compared with the baseline measurements (all $P < 0.01$, **Table 1**). Already 1 week after surgery, TNT animals showed a significant reduction in PWT compared with the contralateral side for all stimuli tested (all $P < 0.001$, **Fig. 1**). Ramped stimuli applied with the evF 0.2 mm and 0.8 mm tips showed a significantly higher reduction 2 weeks after the surgery compared with the first measurements after surgery (0.2 mm $P = 0.0264$, 0.8 mm $P = 0.0016$, Wilcoxon-test); for blunt stimuli there was a similar trend. On the other hand, pinpricks as step stimuli showed a higher reduction at the first point in time after surgery compared with 2 weeks after surgery ($P = 0.0076$, Wilcoxon-test), vFFs (also stepped stimuli) showed no statistically significant difference but a trend towards the same result as pinprick stimuli ($P = 0.0784$, Wilcoxon-test).

3.2. Human experiments

3.2.1. Induction of long-term potentiation and secondary hyperalgesia

Long-term potentiation of pain-perception was induced by applying five 1 second trains every 10 seconds (HFS) with the 10-fold EDT (0.348 ± 0.061 mA). High frequency stimulation evoked moderate to severe pain gradually increasing from the first (57 ± 7/100 NRS, range 20-90) to fifth train (77 ± 6/100 NRS, range 35-95, $P = 0.046$).

3.2.2. Effect of high frequency stimulation on mechanical pain thresholds obtained with different test stimuli

The baseline measurements did not reveal any difference between ipsilateral and contralateral sides for the tested stimuli except for the evF test with a 0.2-mm tip (**Fig. 2**). Immediately after HFS, all subjects showed a significant reduction of pain thresholds compared with the contralateral side for all stimuli tested, which became more prominent 20 minutes later. This reduction was consistent over the points in time assessed and a significant difference was seen when comparing both measurements after HFS with the baseline (**Table 2**). Calculating the effect size using the Cohen's d, HFS led to higher effect sizes at the second measuring point than immediately after HFS for all stimuli tested (**Table 2**).

To compare our results for MPT to pinprick stimulation with the reference database of healthy controls of the DFNS with the foot as reference site, we calculated the z score. For baseline measurements, the z score was 0.091 ± 0.694. The mean value is within the proposed quality standard (0 ± 0.25), whereas the SD is lower (proposed standard: 1 ± 0.1), which may be explained by the more homogenous subject population in this than in other studies.

3.3. Comparison of the thresholds to different mechanical stimuli in humans and rats

Although the baseline measurements of humans and animals were similar for the evF test with a 0.2-mm tip ($P = 0.7898$, U test), rats were more sensitive when testing with the 0.8-mm tip

Table 1

Change in rat paw withdrawal threshold over the repeated measurements compared with the baseline measurements.

Test	Side	Week after surgery						*P*
		Baseline	1	2	3	4	5	
evF 0.2 mm, mN	Ipsi	440 ± 19	211 ± 29	132 ± 24	144 ± 18	99 ± 8	115 ± 12	0.0001
	Contra	442 ± 13	484 ± 23	445 ± 31	470 ± 26	460 ± 14	489 ± 20	0.5702
evF 0.8 mm, mN	Ipsi	509 ± 20	268 ± 19	159 ± 13	208 ± 25	245 ± 27	253 ± 19	0.0002
	Contra	578 ± 16	536 ± 29	528 ± 27	572 ± 30	590 ± 33	601 ± 18	0.5190
evF blunt, mN	Ipsi	1574 ± 39	1060 ± 89	976 ± 49	1040 ± 68	1013 ± 38	1003 ± 53	0.0038
	Contra	1558 ± 27	1693 ± 47	1660 ± 57	1654 ± 90	1631 ± 37	1666 ± 27	0.4159
Pinprick, mN	Ipsi	136 ± 11	17 ± 5	30 ± 3	30 ± 7	24 ± 5	27 ± 3	0.0004
	Contra	134 ± 9	133 ± 16	132 ± 11	135 ± 21	140 ± 18	143 ± 21	0.5686
Von Frey filaments, mN	Ipsi	190 ± 20	33 ± 6	48 ± 9	61 ± 6	56 ± 6	54 ± 5	<0.0001
	Contra	198 ± 17	185 ± 18	180 ± 23	188 ± 31	176 ± 29	184 ± 20	0.9343

Rat paw withdrawal threshold was measured using the electronic von Frey test (evF) with a 0.2 mm, 0.8 mm, and a blunt tip as well as with pinpricks and von Frey filaments as a baseline before tibial nerve transection surgery and weekly for 5 weeks after surgery on the operated (ipsi) and on the contralateral, healthy leg (contra).
Data are presented as means ± SEM and *P* values calculated with the Friedman test for dependent observations.

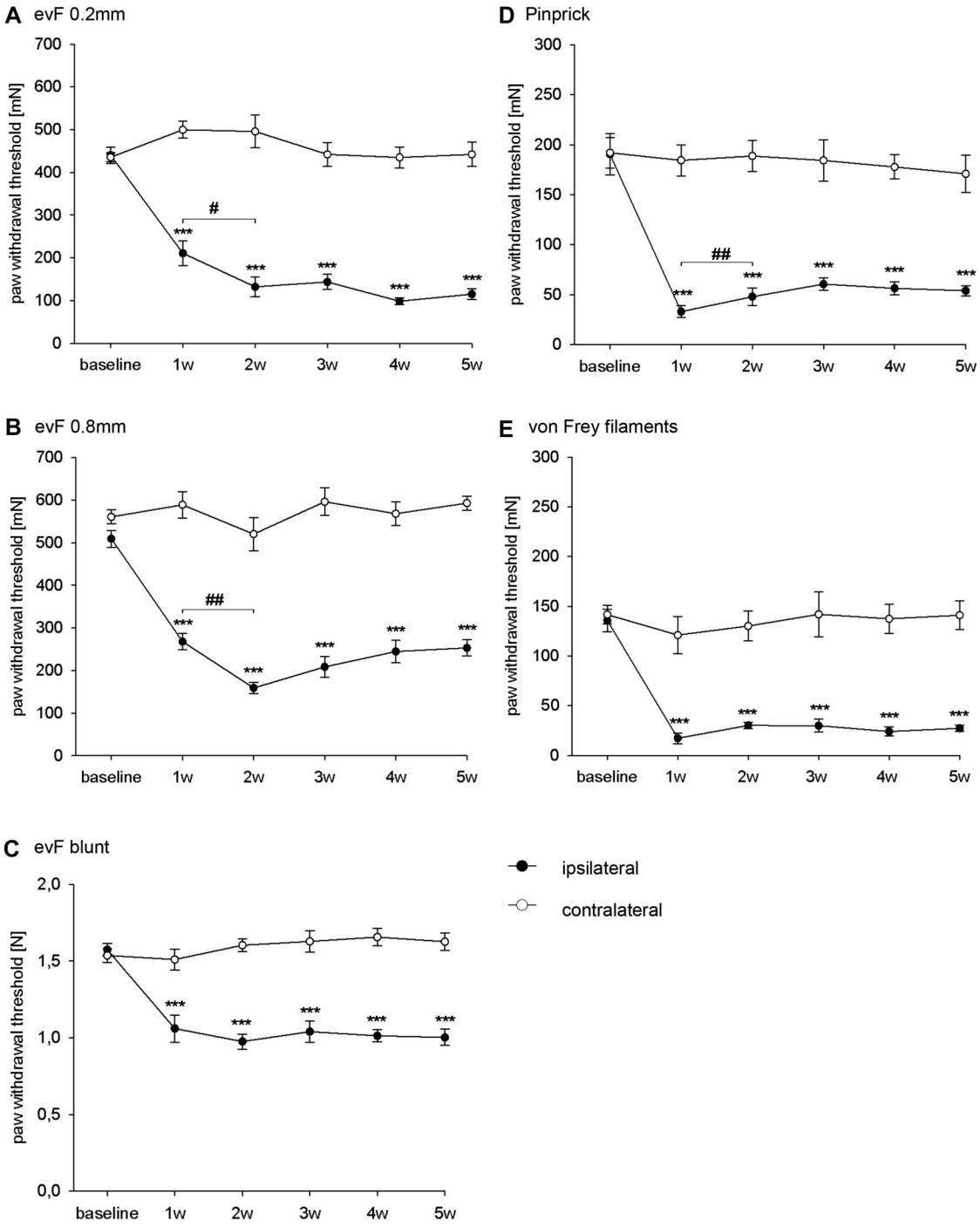

Figure 1. Paw withdrawal thresholds (PWTs) to ramped and stepped mechanical stimuli in a rat model of neuropathic pain. Paw withdrawal thresholds measured with the electronic von Frey test (evF, ramped stimuli) with a 0.2-mm tip (A), a 0.8-mm tip (B), and a blunt tip (C) as well as with standardized pinpricks (D) and von Frey filaments (E) as stepped stimuli. The PWT was measured in TNT animals on the ipsilateral (filled symbols) and contralateral (open symbols) sides before (baseline) and weekly after surgery (w = week after surgery). N = 8 animals. Data are depicted as mean ± SEM. ***$P < 0.001$, Wilcoxon test for dependent observations comparing ipsilateral and contralateral measurements. #$P < 0.05$, ##$P < 0.01$, Wilcoxon test for dependent observations comparing the ipsilateral measurements in week 2 with week 1.

($P = 0.0004$, *U* test) and the blunt tip ($P = 0.0004$, *U* test). With increasing diameter (0.2 mm, 0.8 mm and blunt tip) rats showed an increased PWT and humans showed an increased MPT ($P < 0.001$, Friedman test, **Fig. 3A**). However, when comparing the applied pressure the results were reversed, both humans and animals showed a lower threshold the larger the probe surface (**Fig. 3B**). When comparing force divided by perimeter, which is the adequate stimulus parameter for nociceptor activation,[18] thresholds were also lower for larger probe size, suggesting spatial summation (**Fig. 3C**).

Rats were less sensitive than humans when testing with pinprick stimuli but not with the small evF probe, although both probes have a similar diameter of 0.25 mm and 0.2 mm, respectively ($P = 0.0075$, *U* test, **Fig. 3D**). However, both humans and animals showed a smaller threshold to pinprick stimuli than to the ramped evF stimuli (humans $P = 0.002$, animals $P = 0.0078$,

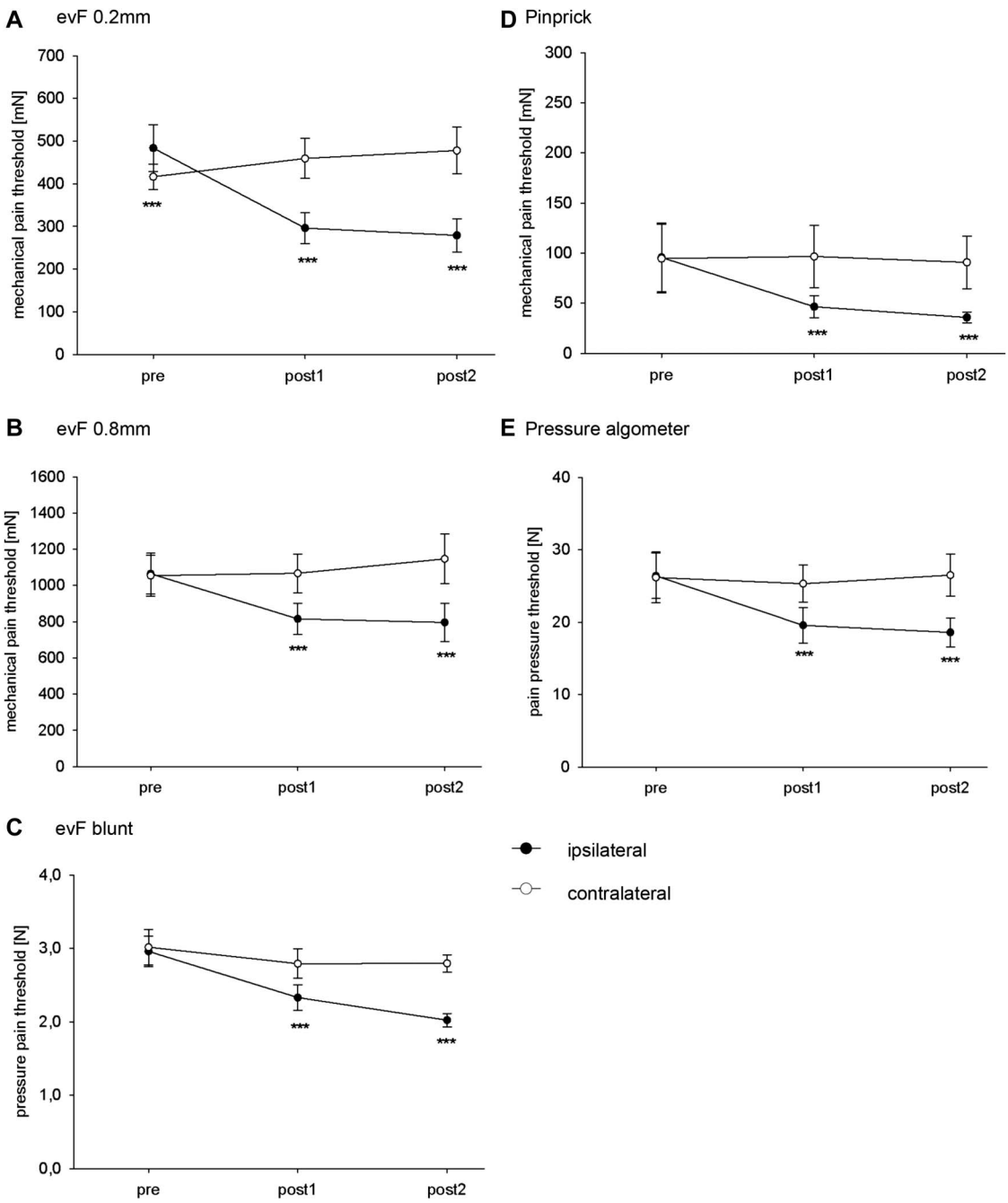

Figure 2. Mechanical pain thresholds to ramped and stepped mechanical stimuli in a human long-term potentiation model. Mechanical pain thresholds (MPTs) measured with the electronic von Frey test (evF) with a 0.2 mm tip (A), a 0.8 mm tip (B) as well as with pinpricks (D). Pressure pain thresholds (PPTs) measured with the evF test with a blunt tip (C) and the algometer (E). Mechanical pain threshold and PPT measured on the ipsilateral (filled symbols) and contralateral (open symbols) sides as a baseline (pre), immediately after high frequency stimulation (post 1) and after a 20-minute break (post 2). N = 10 subjects. Data are expressed as the mean ± SEM. ***$P < 0.001$, Wilcoxon test for dependent observations.

U test, **Fig. 3D**), suggesting the presence of reaction time artefact for the ramped stimuli.

3.4. Comparison of the effect of high frequency stimulation and tibial nerve transection on the thresholds to different stimuli

According to maximum effects of TNT and HFS, we chose the second week after surgery and the second time-point after HFS to compare the relative reduction in mechanical thresholds. Tibial nerve transection in rats had a much bigger effect than HFS in humans (**Fig. 4A**). High frequency stimulation led to a decrease in human MPT of 39% for the 0.2 mm, whereas TNT reduced the rat PWT by 70% ($P = 0.002$, *U* test). For the 0.8-mm tip, HFS reduced MPT by 23%, whereas TNT reduced the PWT by 71% ($P < 0.001$, *U* test). Similarly, human MPT was decreased by 21% for the blunt tip and rat PWT was decreased by 38% ($P = 0.003$, *U* test). However, in both rats and humans, the change in sensitivity to mechanical stimuli was higher for the 0.2 mm probe than for the blunt probe of the evF.

When testing with pinpricks, HFS led to a reduction of MPT by 53%, whereas TNT reduced the PWT by 78% ($P = 0.045$, *U* test).

Table 2

Change in human mechanical and pressure pain threshold over the repeated measurements compared with the baseline measurements.

Test	Side	Pre-HFS	Post 1	Post 2	*P*	Post 1: *d*	Post 2: *d*
evF 0.2 mm, mN	Ipsi	484 ± 55	296 ± 37	279 ± 39	0.0005	1.18	1.28
	Contra	416 ± 29	459 ± 47	478 ± 55	0.4966	−0.42	−0.48
evF 0.8 mm, mN	Ipsi	1065 ± 113	815 ± 87	796 ± 106	0.0022	0.69	0.75
	Contra	1054 ± 114	1067 ± 107	1147 ± 137	0.7408	−0.03	−0.23
evF blunt, N	Ipsi	3 ± 0.2	2 ± 0.2	2 ± 0.1	0.0202	1.02	1.78
	Contra	3 ± 0.2	3 ± 0.2	3 ± 0.1	0.2725	0.24	0.37
Pinprick, mN	Ipsi	96 ± 34.0	47 ± 11	36 ± 5	0.0004	0.65	0.82
	Contra	95 ± 34	97 ± 31	91 ± 26	0.4966	−0.02	0.04
Algometer, N	Ipsi	26 ± 3	20 ± 2	19 ± 2	0.0004	0.78	0.96
	Contra	26 ± 4	25 ± 3	27 ± 3	0.4227	0.15	0.026

Human mechanical and pressure pain threshold was assessed with the electronic von Frey test (evF) using a 0.2 mm, 0.8 mm, and a blunt tip as well as with pinpricks and a pressure algometer. The tests were conducted as a baseline before high frequency stimulation (pre-HFS), immediately after stimulation (post 1), and after a 20-minute break (post 2) on the side of stimulation (ipsi) and on the contralateral side (contra).
Data are presented as means ± SEM and *P* values calculated with the Friedman test for dependent observations. Effect size (Cohen's *d*) is presented compared with the baseline values.

Concerning relative reduction of the human MPT or rat PWT, there was no difference between ramped stimuli of the evF with a 0.2-mm tip and stepped stimuli of the pinpricks (humans $P = 0.3750$, animals $P = 0.3125$, *U* test, **Fig. 4B**).

4. Discussion

This study compared 2 experimental models resulting in the development of hypersensitivity to mechanical stimuli over weeks in rats (TNT) and over hours in humans (HFS). We chose HFS because there are no human experimental models with equal duration as the rat model and because HFS leads to reproducible mechanical hypersensitivity in otherwise healthy subjects. In addition, we compare our findings with human clinical data from patients with peripheral nerve injury. This is the first study to directly compare results gained with the same test stimuli in humans and animals.

All tests were done at the foot/paw dorsum in normally innervated skin, ie, adjacent to but outside the denervated area in the rat TNT model and adjacent to but outside the conditioned skin area in the human HFS model. Thus, in both models it is likely that the observed changes in mechanical sensitivity were due to central sensitization.[4,30] There is only one example for which central sensitization has been conclusively shown,[2] in all other cases central sensitization is only inferred as a mechanism of "activity-dependent plasticity." The main findings were as follows:

(1) Humans and rats show a similar baseline for evF stimuli with 0.2-mm tips. Humans show a higher baseline threshold for 0.8-mm tips and blunt tips. Rats show a higher baseline for pinpricks.
(2) Contact surface plays a role for baseline (force increases with area) and for sensitivity to change (better for smaller probe).
(3) Ramp stimuli show a higher baseline than step stimuli but a similar sensitivity to change.
(4) Ramp stimuli need 2 weeks to establish stable reduction and step stimuli have maximal reduction already 1 week after surgery.

4.1. Changes in sensitivity to mechanical and pressure stimuli after tibial nerve transection

To investigate the relationship between neuropathic pain and its effects on sensitivity to different mechanical and pressure stimuli, we assessed the PWT using the evF with 2 rigid cylindrical tips of 0.2 mm and 0.8 mm diameter, pinpricks and vFFs. Tibial nerve transection -injured animals showed a significant reduction in PWT to mechanical stimulation with all 3 tips of the evF as well as with pinpricks and vFFs at all assessed measuring points. The reduction of PWT to ramped stimuli applied with the evF was lowest in the first week after surgery. The sensitivity to ramped stimuli showed a higher reduction in the second week after surgery suggesting that hypersensitivity to ramped stimuli might need more time to develop after traumatic nerve injury than hypersensitivity to stepped stimuli.

Using a blunt rubber tip for the evF, we demonstrated deep tissue mechanical hypersensitivity which is a novel finding in the TNT model. Our baseline thresholds were within the published range of other nerve injury models.[28,33,39] Hypersensitivity to deep tissue stimuli has already been shown for spinal nerve ligation,[32] and for nonneuropathic pain models.[10,11] As we applied all stimuli to the dorsum of the hind paw we cannot compare absolute values with previous studies that tested on the plantar surface.

For ethical reasons, no human nerve injury models exist, thus we compared our results with clinical data.[13] Approximately half of the patients with peripheral nerve injury showed increased sensitivity to blunt pressure, whereas only 12% to 34% were more sensitive to other mechanical, heat or cold stimuli; in total over 80% of the patients showed positive sensory signs after nerve injury. As hypersensitivity to blunt pressure is the most prominent positive sensory sign in humans after nerve injury, it is important to test this in animal models.

4.2. Changes in sensitivity to mechanical and pressure stimuli after high frequency stimulation

Electrical HFS is a method used to induce LTP in vitro and in vivo. In humans it leads to LTP-like pain amplification, including hyperalgesia to punctate and blunt mechanical stimuli as well as dynamic mechanical allodynia.[20,26] High frequency stimulation recruits nociceptive C-fibers by using a device consisting of 10 punctate electrodes with a 200 μm diameter.[16] This facilitates a high current density at low stimulus intensities with spatial summation in the receptive fields of the neurons and results in long-term changes in pain sensitivity to mechanical and electrical stimuli in the conditioned area lasting at least 3 hours.[20] Long-term potentiation on spinal cord level is the most likely mechanism of the long-term enhancement of pain perception observed at the conditioned skin site (homotopic hyperalgesia). Hyperalgesia to mechanical stimuli was also demonstrated in areas adjacent to the site of conditioning stimulation (heterotopic hyperalgesia).[23]

In this study, we measured mechanical hypersensitivity with pinpricks and the evF with 2 different tips of 0.2 mm and 0.8 mm

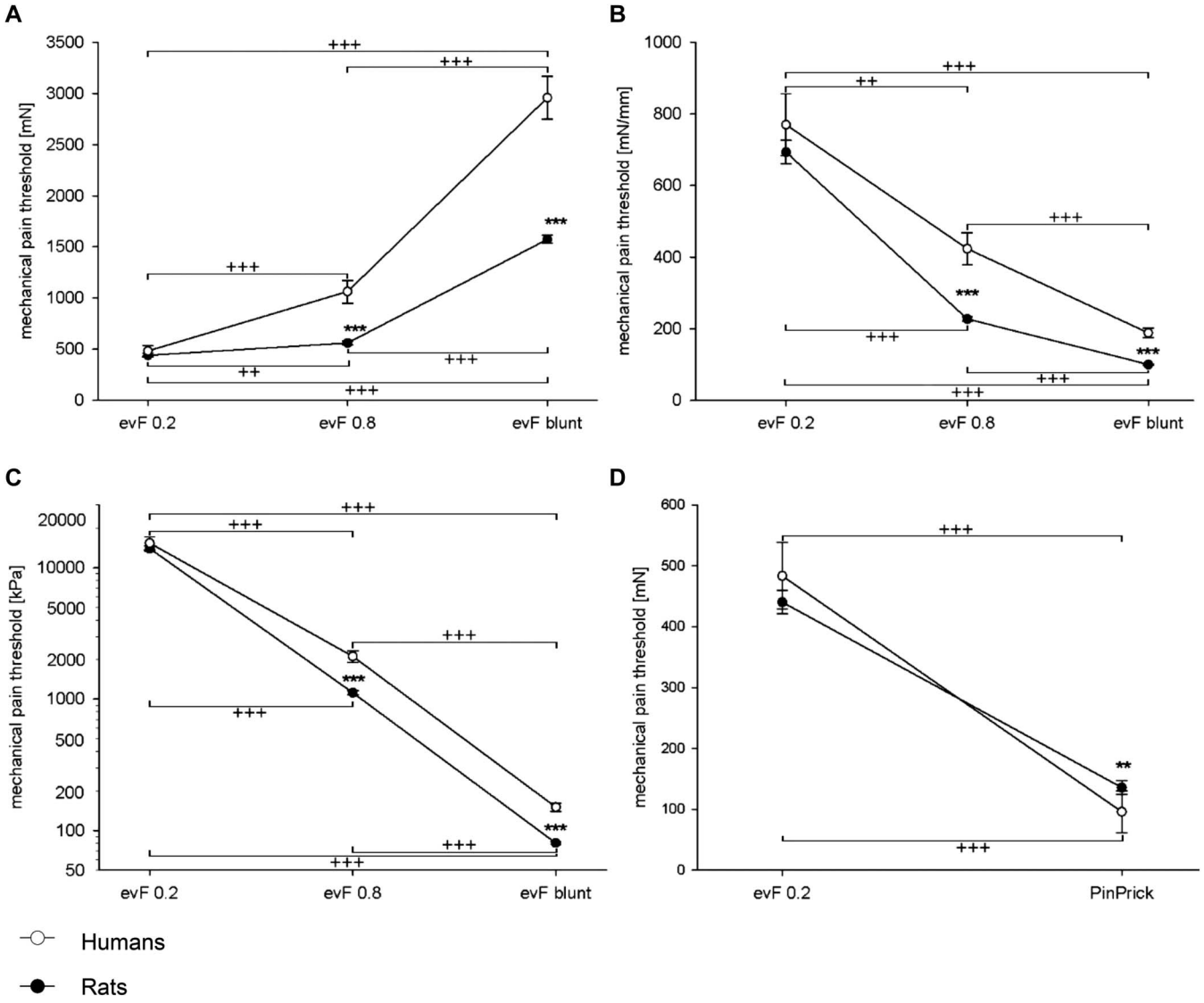

Figure 3. Influence of the diameter of the test probe (A-C) and the distinction between ramped and stepped stimuli (D) on the paw withdrawal threshold in rats and the mechanical pain threshold in humans. Human mechanical pain threshold (MPT, open symbols) and rat paw withdrawal threshold (PWT, filled symbols). The thresholds were measured on the side of high frequency stimulation or injury and represent the baseline values. (A) The influence of the diameter of the test probe on the force needed for MPT and PWT measured with the electronic von Frey test with a 0.2-mm tip (evF 0.2), a 0.8-mm tip (evF 0.8), and a blunt tip (evF blunt) is shown. (B) The influence of the diameter on the applied force divided by circumference is shown. (C) The influence on the applied pressure (force divided by area) is shown. (D) The comparison of stimuli with a similar probe size applied in a ramped (evF 0.2) or stepped (pinprick stimulators) manner is depicted. N = 10 subjects, n = 8 rats. Data are expressed as the mean ± SEM. Between-species comparisons: $^{**}P < 0.01$, $^{***}P < 0.001$, Within-species comparisons: $+++P < 0.001$, $++P < 0.01$, *U* tests for independent observations.

diameter. High frequency stimulation significantly reduced human MPT when stimulating with pinpricks and both rigid tips of the evF. Pressure pain threshold was assessed with a pressure algometer and a blunt tip for the evF esthesiometer. High frequency stimulation reduced PPT in both tests. Therefore, HFS enhanced the sensibility to both punctate and blunt mechanical stimuli in a heterotopic area.

We compared our data for pinprick stimulation with the reference database of healthy controls of the DFNS with the foot as reference site. Our baseline measurements conformed to the healthy control database with a mean *z* score close to 0. The lower standard deviation may be explained by a more homogenous subject population and by testing by a single observer in our study vs multiple centers in the reference database.

Also, we compared our data with the results of a study on patients with peripheral nerve injury.[13] Subjects after HFS showed less hypersensitivity to pinprick stimuli than patients with nerve injury (*z* score 1.51 vs 1.73 ± 0.61, data logarithmized).

There is no study using HFS on the dorsum of the foot; therefore, our absolute threshold values are not comparable to other studies using HFS. Most experiments use the increase in pain rating to pinprick stimuli[20–22,34] showing similar results to our study: whereas in our study HFS led to a 53% decrease in MPT, these studies measured a 48.8%[26] and 38%[23] decrease in MPT. As we used the pressure algometer on the dorsum of the foot and not over muscle tissue, PPT could not be compared with other studies.

4.3. Influence of stimulus parameters on mechanical (hyper-) sensitivity in rats and humans

The evF 0.2-mm tip and the pinpricks have a similar contact surface and can be used to directly compare ramped and

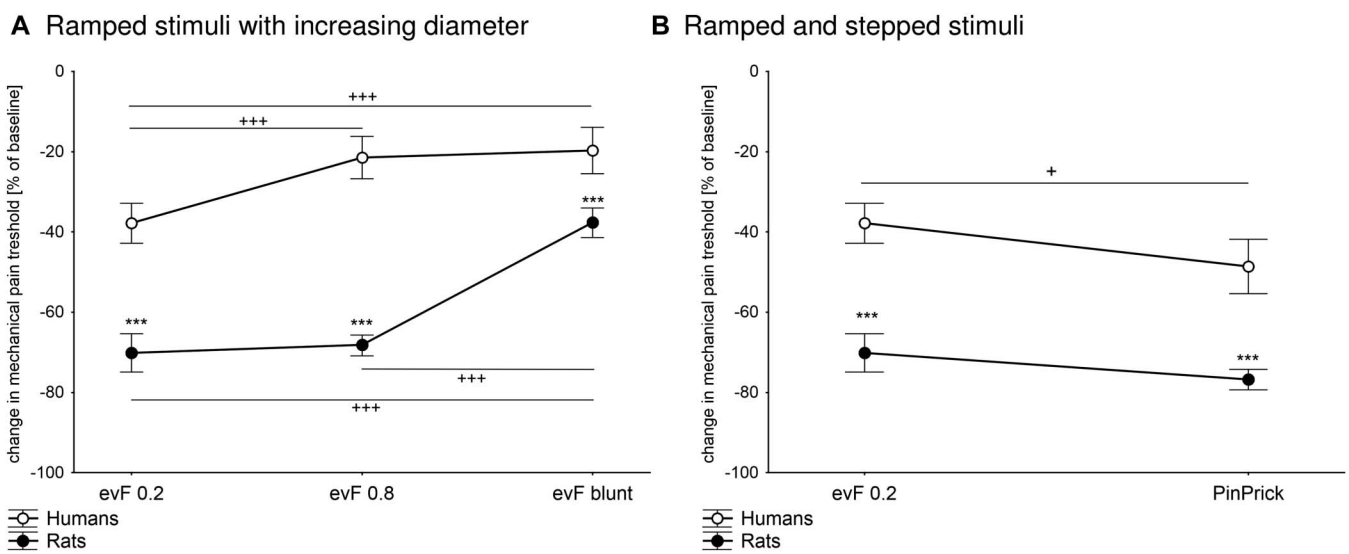

Figure 4. Influence of the diameter of the test probe on the sensitivity to change in paw withdrawal/mechanical pain threshold in the rat and human pain models and the distinction between ramped and stepped stimuli. Relative reduction in mechanical pain threshold in humans (MPT, open symbols) and paw withdrawal threshold in animals (PWT, filled symbols), respectively. Relative reduction was calculated by comparing the PWT on the ipsilateral side 2 weeks after surgery with the baseline for rats and by comparing the MPT on the ipsilateral side at the second measuring point 20 minutes after high frequency stimulation with the baseline for humans. (A) The influence of the diameter of the test probe on the relative reduction of MPT and PWT measured with the electronic von Frey test with a 0.2-mm tip (evF 0.2), a 0.8-mm tip (evF 0.8), and a blunt tip (evF blunt) is shown. (B) The comparison of stimuli with a similar probe size applied in a ramped (evF 0.2) or stepped (pinprick stimulators) manner is depicted. N = 10 subjects, n = 8 rats. Data are expressed as the mean ± SEM. Between-species comparisons: $^{*}P < 0.05$, $^{***}P < 0.001$. Within-species comparisons: $+P < 0.05$, $+++P < 0.001$. *U* tests for independent observations.

stepped stimuli. Ramped stimuli show a higher baseline threshold than stepped stimuli indicating a reaction time artefact for ramped stimuli, which was more pronounced in humans than in rats. This might be due to longer nerves in humans or to cortical processing needed to indicate the perception of sharpness opposed to the spinal processing occurring in the paw withdrawal. But both stimuli show a similar sensitivity to change, suggesting that ramped and stepped stimuli are equally useful to assess mechanical hypersensitivity.

The baseline threshold to mechanical stimuli depends on the contact surface of the test probe: the force needed to reach the threshold increases with increasing area. This seems to suggest that force per area (ie, pressure) may be the relevant stimulus parameter, but the pressure needed to reach the threshold decreased with increasing area in both species. Previous studies have shown that the response of nociceptors is primarily related to the tensile component of mechanical stimuli,[44] that compressive stimuli applied to skin that lies over soft tissue can result in substantial tensile loading around the indenting stimulus,[18] and that force divided by circumference might be the appropriate parameter for pain studies.[14] In our data, the force divided by circumference needed to reach the threshold decreased with increasing area, suggesting that larger probe diameters may cause more pain due to spatial summation. However, after both TNT and HFS, smaller probes showed a greater reduction of the measured threshold, suggesting that punctate probes offer a better sensitivity to change. Moreover, for ramped evF stimuli with a 0.2-mm tip humans and rats showed a similar baseline threshold making an interspecies comparison directly possible.

4.4. Summary and conclusions

This study showed that contact surface plays a role for baseline (force increases with area) and for sensitivity to change (better for smaller probe). Because humans and rats showed similar baseline sensitivity for the evF test using a 0.2-mm tip, but differences in either direction for other test stimuli, this test stimulus allows the most direct comparison between rat paw withdrawal and human mechanical pain sensitivity. This study shows that it is possible to use the same experimental test stimuli in humans and rats with the outcome of easily comparable data and validates that rat PWT is a reasonable surrogate parameter for human mechanical pain sensitivity. Probe size and shape have major effects in both species and should be standardized (eg, 0.2 mm cylindrical). We hope these results facilitate the translation between basic and clinical pain research and allow future studies directly comparing experimental animal models with patients suffering from neuropathic pain. Using these methods, we found hypersensitivity to blunt pressure in TNT, which is the most prominent positive sensory sign in humans after nerve injury, and a novel finding in this model.

Conflict of interest statement

The authors have no conflicts of interest to declare.

The research leading to these results is part of the Europain Collaboration, supported by the Innovative Medicines Initiative Joint Undertaking, under Grant agreement 115007, receiving financial contribution from the European Union's Seventh Framework Program (FP7/2007-2013) and EFPIA companies' in-kind contribution. D. Hrncic received financial support by the German Academic Exchange Service (DAAD). The work described in this manuscript is one of many outputs from this broad collaborative effort and has in no way direct commercial ties or obligations.

This article contains essential parts of the thesis of M.-C. Reitz to be submitted to the Medical Faculty Mannheim of the University of Heidelberg in partial fulfillment of the

requirements of the Master of Science degree in Translational Medical Research.

Article history:
Received 23 September 2015
Received in revised form 24 January 2016
Accepted 2 February 2016
Available online 8 February 2016

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