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Altered C-tactile processing in human dynamic tactile allodynia

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ABSTRACT

Human unmyelinated (C) tactile afferents signal the pleasantness of gentle skin stroking on hairy (non-glabrous) skin. After neuronal injury, that same type of touch can elicit unpleasant sensations: tactile allodynia. The prevailing pathophysiological explanation is a spinal cord sensitization, triggered by nerve injury, which enables Aβ afferents to access pain pathways. However, a recent mouse knockout study demonstrates that C-tactile afferents are necessary for allodynia to develop, suggesting a role for not only Aβ but also C-tactile afferent signaling. To examine the contribution of C-tactile afferents to the allodynic condition in humans, we applied the heat/capsaicin model of tactile allodynia in 43 healthy subjects and in 2 sensory neuronopathy patients lacking Aβ afferents. Healthy subjects reported tactile-evoked pain, whereas the patients did not. Instead, patients reported their C-touch percept (faint sensation of pleasant touch) to be significantly weaker in the allodynic zone compared to untreated skin. Functional magnetic resonance imaging in 18 healthy subjects and in 1 scanned patient indicated that stroking in the allodynic and control zones evoked different responses in the primary cortical receiving area for thin fiber signaling, the posterior insular cortex. In addition, reduced activation in the medial prefrontal cortices, key areas for C-tactile hedonic processing, was identified. These findings suggest that dynamic tactile allodynia is associated with reduced C-tactile mediated hedonic touch processing. Nevertheless, because the patients did not develop allodynic pain, this seems dependent on Aβ signaling, at least under these experimental conditions.

1. Introduction

Dynamic tactile allodynia is a condition that results from neuronal disease or injury in which normally innocuous moving tactile stimuli produce unpleasantness or pain. People with tactile allodynia typically experience a burning, tender sensation during soft stroking of the affected skin [56]. Even a very light stimulus, such as a patient's garment brushing against the skin during movement, can evoke allodynia.

The prevailing hypothesis is that tactile allodynia is a consequence of nerve injury causing central sensitization, i.e., changes in tactile signaling in the spinal cord [9,68]. After central sensitization, low-threshold mechanoreceptors (LTMs) signal to nociceptive neurons in the dorsal horn and, from there, to cerebral pain processing areas [27,31,63,68]. The LTMs that signal allodynia, after central sensitization, are generally considered to be large myelinated (Aβ) afferents [10,41,64,67]. This view is mainly based on a large number of human selective nerve block experiments demonstrating that central sensitization type of tactile allodynia is abolished by compression or ischemic block of Aβ afferents [14,26,31,35,63].

Recent rodent studies suggest that a less explored class of unmyelinated LTMs, C-LTMs, also play a critical role in the pathophysiology of tactile allodynia. C-LTMs are highly sensitive mechanoreceptors with a conduction velocity of approximately 1 m s^{-1}, an intermediate rate of adaptation to a sustained indentation, a clear fatigue to repeated stimuli, and a strong response to slowly moving stimuli but poor sensitivity to quickly moving stimuli [4,20,28,33,72]. In experimental models of allodynic central sensitization, mice demonstrated reduced signs of mechanical hypersensitivity after knockout of C-LTM signaling [59]. Furthermore, electrophysiological recordings in rats have demonstrated a possible anatomical pathway for tactile allodynia where C-LTMs project to lamina I spinoparabrachial wide dynamic range (WDR) neurons [1].

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The human equivalent of C-LTMs are known as C-tactile afferents and are thought to contribute crucially to pleasantness perception during light stroking touch [38,45,47,51]. The spinal cord projection pathway for human C-tactile afferents is unknown, but it has been demonstrated that the posterior insula is the primary cortical receiving area for C-tactile signaling [5,19]. A particularly effective C-tactile afferent stimulus is slow, gentle brush stroking as demonstrated in human single afferent microneurography recordings [38]. Intriguingly, slow, gentle brush stroking is also a particularly effective stimulus for eliciting tactile allodynia in patients with neuropathic pain [58]. Furthermore, ongoing muscle pain induced by hypertonic saline muscle infusion may increase after slow brush stroking of the overlaying skin [48]. This type of allodynia survives compression block of myelinated cutaneous afferents, suggesting that it is selectively mediated by C-tactile afferents [48].

To study whether human C-tactile afferents contribute to dynamic tactile allodynia, we applied the heat/capsaicin model of central sensitization [53]. The subjects were examined by psychophysical techniques and functional magnetic resonance imaging (fMRI). By studying healthy subjects as well as 2 patients with rare selective denervation of large myelinated afferents, we were able to isolate the contribution of C-tactile afferent signaling to dynamic tactile allodynia.

2. Materials and methods

2.1. Participants

The ethical review boards at the University of Gothenburg and McGill University approved the procedures. The experiments were performed in accordance with the Declaration of Helsinki. Informed consent was obtained from 43 neurologically intact subjects (median age 24 years, range 20–46 years, 21 men) and 2 Aβ-denervated subjects (subject 1, age 60, female; subject 2, age 58, male). Psychophysical data was collected from all participants, and 22 of them, including subject 1, also participated in functional magnetic resonance imaging (fMRI). In pilot experiments, we tested neurologically intact subjects with ages up to 79 years. The heat/capsaicin model was effective in inducing flare and dynamic tactile allodynia in older subjects as well [70].

Subjects 1 and 2 were diagnosed with a rare sensory neuronopathy syndrome, leaving them without functional large-diameter myelinated somatosensory afferents [61]. Subject 1 became ill at age 31 and subject 2 at age 19 [15,17,18,23]. Clinical and electrophysiological examinations have been performed regularly, and their condition has remained stable over the years. By EEG and MEG, nonpainful electrical stimuli of the peripheral nerves fail to produce observable sensory potentials or cortical evoked potentials [7]. Motor nerve conduction velocities and EMG findings are normal. They report intact temperature and pain perceptions. Thermal detection thresholds that use method of limits are normal or slightly reduced [16,51]. As is typical for the neuronopathy syndrome, the sensory disturbances of both subjects did not indicate a patchy loss of light touch or movement/position sense nor a patchy loss of small fiber function [8].

Results of a sural nerve biopsy in subject 1 demonstrated complete loss of Aβ afferents with preservation of small-diameter myelinated afferents [23]. Subject 2 sought neurological care 12 years after his illness, so biopsy was not indicated.

Initial clinical observations made when subjects 1and 2 first sought care suggested a total loss of tactile perception. However, it was later demonstrated that in 2-alternative forced choice (2-afc) situations, they can detect stimuli that effectively activate C-tactile afferents [50,52].

2.2. Heat/capsaicin experimental model

The heat/capsaicin sensitization model is a safe paradigm for inducing primary and secondary hyperalgesia [53]. In the model, a mild burn injury is induced, after which capsaicin cream is applied to that same skin area. Primary hyperalgesia develops in the treated skin zone and secondary hyperalgesia in the surrounding skin. In the secondary hyperalgesia zone, light touch is perceived as unpleasant or painful, a consequence of altered sensory processing in the central nervous system [69]. We hereafter refer to the secondary hyperalgesia zone as the allodynic zone.

2.3. Heat/capsaicin sensitization

We used a Peltier thermode (3 × 3 cm, Medoc, TSA 2001, Thermosensory Analyzer, Rimat Yishai, Israel; or 2.5 × 5 cm, Somedic, MSA Thermal Stimulator, Hörby, Sweden) to deliver a 45 °C stimulus to the subject's skin for 5 min, after which capsaicin cream (Capsina, 0.075%, Hants, UK) was applied to the preheated skin area for 30 min. Thereafter, the subject rated ongoing pain on a visual analog scale (VAS; 0 = no pain, 10 = worst pain imaginable). The pain rating after removal of the cream was 2 (median, range 0–7, $n = 43$) in neurologically intact subjects, 5 in subject 1 and 1 in subject 2. All participants developed a visible flare (healthy subjects median 29.4 cm^2, range 9–57.4 cm^2; subject 1, 37.6 cm^2; subject 2, 25.3 cm^2). In a subgroup of participants, punctate hyperalgesia was mapped with a monofilament (calibrated indentation force 0.20 or 0.24 N), and was 9.7 cm^2 (median; range 1.1–32.0 cm^2, $n = 15$) in neurologically intact subjects and 31.0 cm^2 in subject 2 (not mapped in subject 1 as a result of time constraints). The heat/capsaicin model was applied on the dorsal aspect of the left forearm or on the ventral aspect of the left thigh (for accessibility in the scanner). A control zone was marked $\geqslant$7 cm from the primary hyperalgesia zone.

2.4. Tactile stimuli

With a cotton swab or a goat-hair brush (both 3 mm wide), effective stimulation of C-tactile afferents (stroking velocity 3 cm s^{-1}) was delivered manually in the allodynic and in the control zones (stroking distance 9 cm, application force approximately 0.3 N [38]). All stimuli were delivered manually by one of us (JL), who was trained to apply the strokes with constant force and velocity. Although the flare prevented blinding of the experimenter, it is unlikely that unintentional variations in stimulation force or velocity could have influenced the results because C-tactile afferents are insensitive to changes in indentation force in the range 0.2 to 0.4 N or velocity in the range 1 to 10 cm s^{-1} [38]. During tactile stimulation, the subjects were prevented from seeing the tested extremity.

2.5. Psychophysical testing

Stimuli were preceded by an auditory cue, and the subjects were instructed to specify which of the paired stimuli was the most unpleasant (2-afc). Subjects 1 and 2 both reported a distinct difference in stroking sensation between the two zones, but they did not perceive unpleasantness. When asked to describe it, they both independently used the words "weaker sensation" for stroking in the allodynic zone. Therefore, in 2-afc testing, subjects 1and 2 were instructed to specify which of the paired stimuli gave the weakest sensation. Finally, subjects completed the Short Form-McGill Pain Questionnaire (SF-MPQ) [44].

2.6. fMRI

Twenty-one neurologically intact subjects were recruited for fMRI. Three subjects were excluded for technical reasons, leaving

18 subjects (all right-handed, age range 20–28 years, 10 men) for analysis. Subject 1 agreed to scanning. Subject 2 is claustrophobic and therefore declined scanning.

2.7. Design

Data were collected from 5 (median, range 3–5) consecutive fMRI runs (100 volume acquisitions) in each subject. Tactile stimuli, stroking over a 9 cm distance for 3 s, were delivered in the allodynic ($n = 8$ per run) and control ($n = 8$ per run) zones in a pseudorandomized order (interstimulus interval 15 s) by one of us (JL). Timing guidance was provided through a visual display generated by a Matlab (The MathWorks Inc, Natick, MA, USA) script. Subjects were instructed to focus on a fixation cross.

2.8. Data acquisition

Subject 1 and neurologically intact subjects were scanned in Montreal, Canada, and Gothenburg, Sweden, respectively, with 8-channel headcoil 3T magnetic resonance scanners (Montreal, Siemens TrioTim; Gothenburg, Philips Achieva). A T1-weighted protocol was used to acquire anatomical scans, and a blood oxygen level dependent (BOLD) sensitive protocol with a T2*-weighted gradient-echo, echo-planar imaging sequence was used for functional scans (Montreal: single-echo, TR 2.9 s, TE 30 ms, flip angle 90°, $2.9 \times 2.9 \times 2.9$ mm resolution; Gothenburg: double-echo [54], TR 3.1 s, TE 19 + 35 ms, flip angle 90°, $2.9 \times 2.9 \times 2.9$ mm resolution). Planes were oriented 30° from the anterior–posterior commissure line. These settings resulted in an adequate orbitofrontal cortex BOLD signal but the most superior part of the brain including primary somatosensory cortex (S1) was not covered. For image reconstruction, a short multi echo scan was acquired with TE 19, 36, 53, 70 and 87 ms after double-echo acquisition [54].

2.9. Preprocessing

Data were processed in SPM8 (Wellcome Department of Imaging Neuroscience, London, UK). Functional scans were motion corrected, unwarped to remove variance caused by the combination of movement and susceptibility, and spatially normalized to MNI (Montreal Neurological Institute) space (using the supplied EPI template, voxel size $2 \times 2 \times 2$ mm, trilinear interpolation and 6 mm FWHM Gaussian kernel spatially smoothing). The multi echo scan was then used to estimate the local T2* in each brain voxel [55]. A weighted summation of the preprocessed double echo images was performed with the normalized, estimated T2*map [55].

2.10. General linear model contrast analysis

Each condition was modeled by one predictor convolved with the standard SPM8 hemodynamic response function. Fixed effects analyses were performed in individual participants, and random effects analysis on a group level. Critical cluster sizes (k) corresponding to a family-wise error rate of 0.05 corrected for the whole brain volume were calculated by a Monte Carlo simulation procedure with 1000 iterations [60]. Individual level and group-level contrast were thresholded at $t = 2.34$ ($P = .01$; $k = 46$) and at $t = 3.65$ ($P = .001$; $k = 16$), respectively.

2.11. Multivoxel pattern analysis

Given ongoing nociceptor activation from the heat/capsaicin model during scanning, we expected the primary cortical target for C-afferents, the posterior insular cortex, to be continuously activated. Nonetheless, if C-tactile afferents are integral in tactile allodynia we would expect differences in the insular activation patterns in response to stimuli in the allodynic and control zones. To examine this, we applied multivoxel pattern analysis in a histologically defined region-of-interest (ROI) in the right (contralateral) posterior insular cortex [34]. This area is activated by C-tactile stimulation in humans [46]. After standard preprocessing (cf. above), further multivoxel pattern analysis (MVPA) specific preprocessing was performed with the Princeton MVPA Toolbox (http://www.pni.princeton.edu/mvpa); each voxel's response was normalized relative to the average of the time course within each scan. To account for hemodynamic delay, the condition labels were shifted by 2 volumes, after which linear trends were removed. Single trial estimates were formed by extracting the BOLD response corresponding to each of the stimuli. Multivoxel patterns differentiating the conditions were identified by locally multivariate brain mapping [6]. A linear support vector machine (SVM) classifier (in the LS-SVM implementation; with fixed regularization parameter C = 1) was used to model the conditions [62], and a leaveone–run-out cross-validation scheme was employed to robustly estimate individual voxel-wise SVM classification accuracies. Permutation testing was used to assess the significance of the classification accuracies [49]: the identical mapping procedure was iterated 999 times with different data label permutations to generate a probability distribution under the null hypothesis that there were no differences between the conditions. P values were computed as the proportion of permuted values that were at least as large as the true classification accuracy, and corrected for multiple comparisons by setting the false discovery rate to $q < 0.05$.

2.12. Behavioral ratings

Ratings were made on a VAS with an magnetic resonance–compatible response unit, with anchor points unpleasant (0) and pleasant (10). VAS ranging from unpleasant to pleasant is reliable and sensitive in detecting subtle changes in affective touch perception in healthy subjects and in patients with tactile disturbances [11,12,22,43,57]. VAS data were not collected in subject 1 because she could not manipulate the response unit as a result of her lack of proprioception.

3. Results

3.1. Psychophysical measurements

3.1.1. Neurologically intact subjects perceived brush-evoked pain

Using the SF-MPQ, gentle stroking in the allodynic zone was described as "hot–burning" by 28 subjects, "tender" by 18 subjects, and "stabbing" by 18 subjects ($n = 30$, Fig. 1A). Stroking in the control zone was perceived as neutral or pleasant by all subjects. VAS ratings collected during fMRI confirmed that stroking in the allodynic zone was significantly less pleasant than stroking in the control zone ($P = .01$, Wilcoxon, median ratings allodynic zone 4.8 and control zone 5.8).

Nine neurologically intact subjects underwent 2-afc testing. These subjects perceived stroking to be most unpleasant in the allodynic zone for 13 of 16 stimulations (median, range 11–16, $P < .001$, binomial distribution).

3.1.2. Reduced C-touch sensation, but no brush-evoked pain, in Aβ-deafferented subjects

Stroking in the control zone evoked the C-touch sensation (faint sensation of pleasant touch) familiar to both subjects [50,51]. Stroking in the allodynic zone was neither unpleasant nor painful. However, both subjects spontaneously reported that the stroking

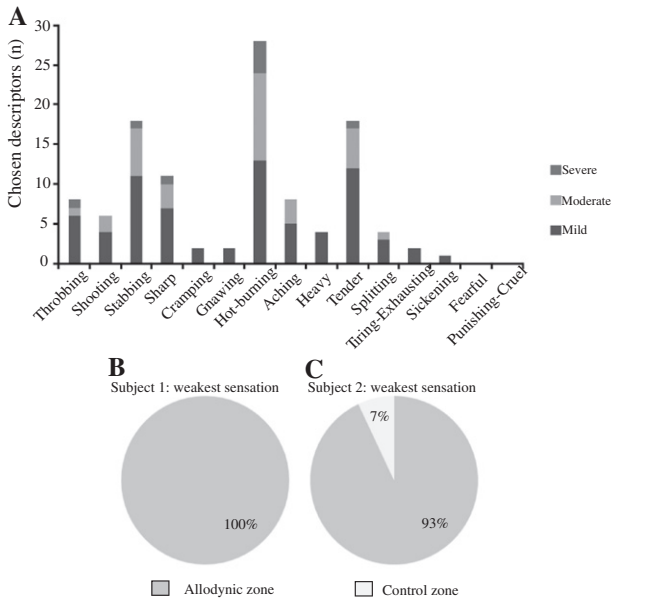

Fig. 1. Psychophysical testing. (A) Neurologically intact subjects ($n = 30$) completed the SF-MPQ of pain descriptors related to the stroking stimuli in the allodynic zone. (B and C) Paired stroking stimuli in the allodynic and in the control zone were delivered in a 2-afc testing session. The Aβ-denervated subjects 1 and 2 were instructed to specify which of the paired stimuli gave the weakest sensation.

sensation from the allodynic zone was different to the C-touch sensation. When asked to further describe how the sensation differed, they both, independent of each other, said "weaker sensation" for stimuli in the allodynic zone. None of the descriptors from the SF-MPQ were applicable. These subjective reports were quantified in 2-afc testings; subject 1 perceived stroking as being weakest in the allodynic zone for 10 of 10 stimulations (Fig. 1B, $P < .001$, binomial distribution), and subject 2 perceived stroking as being weakest in the allodynic zone for 15 of 16 stimulations (Fig. 1C, $P < .001$, binomial distribution). In control experiments, there were no differences in perceived intensity of sensation for stroking in 2 untreated zones (2-afc distribution; subject 1, 5 for each zone, $n = 10$, $P = .62$, binomial distribution; subject 2, 7 for one zone and 12 for the other, $n = 19$, $P = .18$, binomial distribution).

3.2. fMRI

3.2.1. Neurologically intact subjects demonstrated different activations for the two zones

Stroking in the allodynic zone evoked significantly stronger activation than stroking in the control zone in multiple areas including the inferior frontal gyrus, the anterior insular cortex, cerebellum, and the secondary somatosensory cortex (Table 1, Fig. 2A).

Stroking in the control zone evoked significantly stronger activation than stroking in the allodynic zone in large parts of the medial prefrontal cortex (mPFC), including medial orbitofrontal and frontal superior medial cortex, and extending into pregenual anterior cingulate cortex (Table 2, Fig. 2B).

3.2.2. Aβ-deafferented subject 1demonstrated different activations for the two zones

In subject 1, stroking in the allodynic zone evoked significantly stronger activation than stroking in the control zone in the inferior frontal gyrus (Table 3, Fig. 2C). Stroking in the control zone evoked significantly stronger activation than stroking in the allodynic zone in several areas including large parts of the prefrontal cortex including frontal superior medial cortex and mid orbitofrontal cortex (Table 4, Fig. 2D).

Thus, both subject 1 and neurologically intact subjects had activations of mPFC that included both hemispheres for the contrast Control > Allodynia. For this contrast, there was an overlap of activations between subject 1 and neurologically intact subjects in bilateral frontal superior medial cortex (Fig. 3). Furthermore, subject 1 and neurologically intact subjects had activations in inferior frontal gyrus for the contrast Allodynia > Control. The activation in subject 1 was lateralized to the ipsilateral left hemisphere and in the neurologically intact group to the contralateral right hemisphere. However, when inspecting data for each individual, 11 of 18 subjects also had activations in the left inferior frontal gyrus.

Table 1
Activations for the contrast Allodynia > Control in neurologically intact subjects.[a]

Activated region	Peak X	Peak Y	Peak Z	*t* Score	Cluster size
Inferior frontal gyrus	50	46	−8	4.86	20
Anterior insular cortex	24	24	−10	4.73	32
Anterior insular cortex	−36	18	2	4.57	32
Anterior insular cortex	−26	26	−4	4.97	24
Anterior insular cortex	34	24	−2	4.65	17
Postcentral gyrus, S2	−64	−20	22	5.48	30
Inferior parietal cortex	42	−46	42	5.87	92
Inferior parietal cortex	56	−36	50	5.02	56
Cerebellum	30	−74	−30	9.54	256
Cerebellum	−18	−66	−26	6.81	228
Cerebellum	−42	−62	−32	6.44	417
Cerebellum	24	−70	−50	5.15	47
Cerebellum	6	−68	−32	5.97	24
Vermis cerebellum	2	−48	−14	6.35	201
Angular cortex	−42	−60	38	4.92	64
Midoccipital cortex	−26	−100	−4	6.14	38
Superior temporal pole	44	12	−16	4.61	23

S2, secondary somatosensory cortex. Full coverage of primary somatosensory cortex was not obtained.
[a] Contrast thresholded at $P < 0.001$, $k \geqslant 16$, cluster size corrected at $P < 0.05$.

3.2.3. Posterior insula multivoxel pattern differences between the two zones

There were significant differences in the BOLD response patterns to stroking in the allodynic and control zones in the posterior insular cortex in both subject 1 and neurologically intact subjects ($P < .05$, permutation testing, 999 iterations, Fig. 4A). The peak decoding accuracy in subject 1 was found at MNI coordinates [40–19 7], and nearby in neurologically intact subjects at [41–14 11] (Fig. 4B). Voxels differentiating the conditions ($P < .05$, permutation testing, 999 iterations) fell in the same portion of the posterior insular cortex in both the patient and healthy subjects (Fig. 4B).

4. Discussion

Using the heat/capsaicin experimental model, we were able to induce tactile allodynia in neurologically intact subjects but not in two subjects lacking Aβ afferents. Our findings confirm a previous report [64]. A novel observation is that the Aβ-denervated subjects reported *reduced* C-touch sensation for stroking in the allodynic zone. For both the Aβ-denervated and neurologically intact subjects, fMRI indicated reduced processing in mPFC as well as altered processing in the posterior insular cortex when comparing stroking in the allodynic and control zones. Because mPFC is a key area in C-tactile-mediated hedonic processing [25,32] and the posterior insular cortex is the primary receiving cortical area for C-tactile signaling [5,51], these findings suggest that the allodynic condition is associated with reduced hedonic touch processing after subcortical alteration of C-tactile signaling. Both subjects 1 and 2 developed a clearly reduced sensation of C-touch rather than

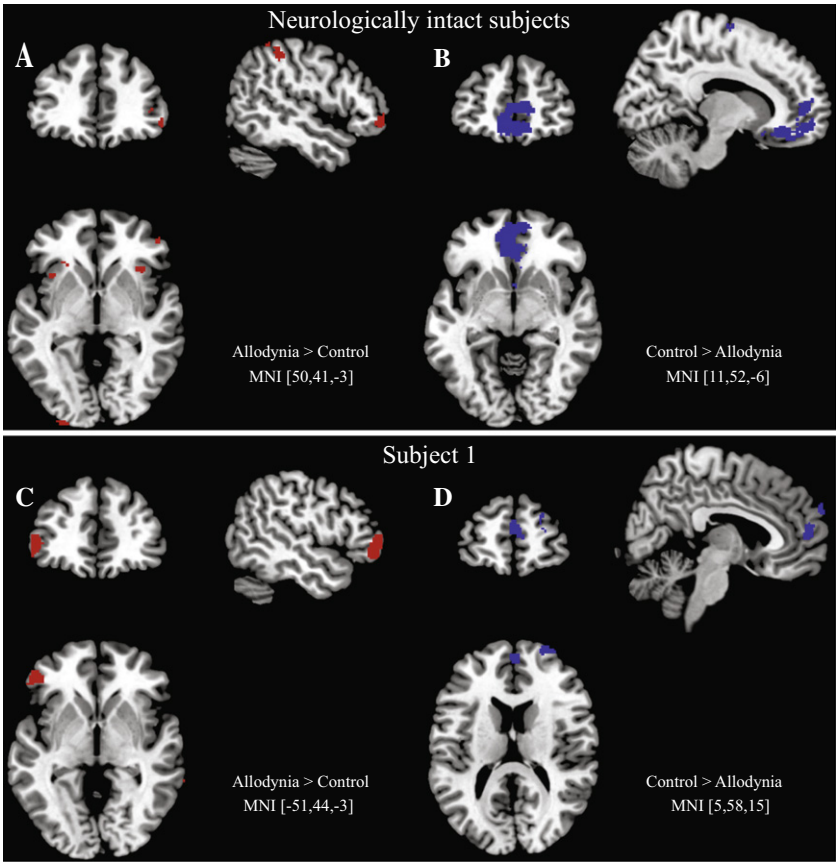

Fig. 2. fMRI of gentle brush stroking in the heat/capsaicin model. (A and B) Neurologically intact subjects. Activation maps thresholded at $P < .001$ uncorrected; $k \geqslant 16$ voxels, corrected at $P < .05$ (for complete activation lists, see Tables 1 and 2). (A) Stroking in the allodynic zone evoked significantly stronger activation than stroking in the control zone in multiple areas including inferior frontal gyrus and anterior insular cortex. (B) Stroking in the control zone evoked significantly stronger activation than stroking in the allodynic zone in large parts of the medial orbitofrontal cortices extending into prefrontal cortices. (C and D) Aβ-denervated subject 1. Activation maps thresholded at $P < .01$ uncorrected; $k \geqslant 46$ voxels, corrected at $P < .05$ (for complete activation lists, see Tables 3 and 4). (C) Stroking in the allodynic zone evoked significantly stronger activation than stroking in the control zone in the inferior frontal gyrus. (D) Stroking in the control zone evoked significantly stronger activation than stroking in the allodynic zone in the frontal superior medial cortex. All images are shown in neurological convention with the right side corresponding to the right hemisphere.

Table 2
Activations for the contrast Control > Allodynia in neurologically intact subjects.[a]

Activated region	Peak X	Peak Y	Peak Z	*t* Score	Cluster size
Medial orbitofrontal cortex	4	26	−12	11.00	1819
Postcentral gyrus, S2	−60	−6	16	5.29	23
Lingual	−8	−50	4	5.76	108
Midoccipital cortex	30	−82	22	5.54	51
Olfactory cortex	8	14	−16	5.52	107
Midtemporal pole	−46	12	−24	5.22	22
Inferior temporal cortex	−56	−2	−28	4.21	23
Supplementary motor area	−8	−12	66	6.06	36
Supplementary motor area	4	−8	68	5.81	169

S2, secondary somatosensory cortex. Full coverage of primary somatosensory cortex was not obtained.
[a] Contrast thresholded at $P < 0.001$, $k \geqslant 16$, cluster size corrected at $P < 0.05$.

Table 3
Activations for the contrast Allodynia > Control in Aβ-denervated subject.[a]

Activated region	Peak X	Peak Y	Peak Z	*t* Score	Cluster size
Inferior frontal gyrus	−52	44	6	3.16	94

[a] Contrast thresholded at $P < 0.01$, $k \geqslant 46$, cluster size corrected at $P < 0.05$.

allodynic pain. This suggests that the sensation of allodynic pain requires Aβ afferents, but, notably, it also indicates a role of C-tactile fibers in the allodynic condition through reduced hedonic processing.

4.1. No brush-evoked pain in Aβ-denervated subjects

In a previous study with subject 2, an intracutaneous injection of capsaicin did not elicit tactile allodynia [64]. In the present study, we detected robust 2-afc differences between the allodynic and control zones. These differences between the two zones were equally distinct in patients and in neurologically intact subjects. All participants were prevented from seeing the stimulated skin areas but subjects 1 and 2 were blinded to a greater extent because the C-tactile system only allows for a very crude spatial localization [50]. Hence, in the patients, any differences in perception from the two zones must be based on nonspatial cues.

After application of the heat/capsaicin model, subjects 1and 2 developed pain and flare, and in subject 2, punctate hyperalgesia was confirmed [64]. Punctate hyperalgesia is primarily mediated by central sensitization of input from capsaicin insensitive Aδ nociceptors [24,39,64].

Two different regions were used for the heat/capsaicin testing, forearm and thigh. Human microneurography recordings have been made from both regions with similar physiological characteristics of myelinated and unmyelinated low-threshold mechanoafferents [21,65,66]. In contrast, there are no C-tactile afferents in glabrous skin and hence no C-tactile contribution to tactile allodynia here.

Table 4
Activations for the contrast Control > Allodynia in Aβ-denervated subject.[a]

Activated region	Peak X	Peak Y	Peak Z	*t* Score	Cluster size
Frontal superior medial cortex	0	56	12	2.77	131
Frontal superior medial cortex	4	66	28	2.68	48
Midorbitofrontal cortex	−32	40	−12	2.97	70
Frontal superior cortex	26	64	14	2.86	86
Inferior temporal cortex	−62	−14	−24	2.95	49
Cerebellum	−40	−42	−38	3.17	226

[a] Contrast thresholded at $P < 0.01$, $k \geqslant 46$, cluster size corrected at $P < 0.05$.

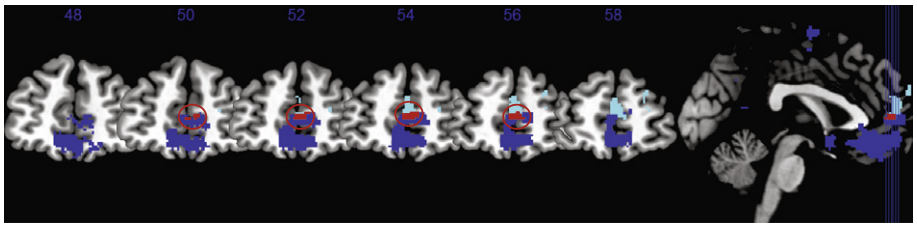

Fig. 3. Overlap of mPFC activations between subject 1 and neurologically intact subjects. Stroking in the control zone evoked significantly stronger activations than stroking in the allodynic zones in neurologically intact subjects (dark blue) and subject 1 (light blue). There was an overlap of activated voxels in bilateral frontal superior medial cortex (red circles). The stereotaxic levels (MNI space) are indicated above the frontal slices. Thresholding and conventions as in Fig. 2.

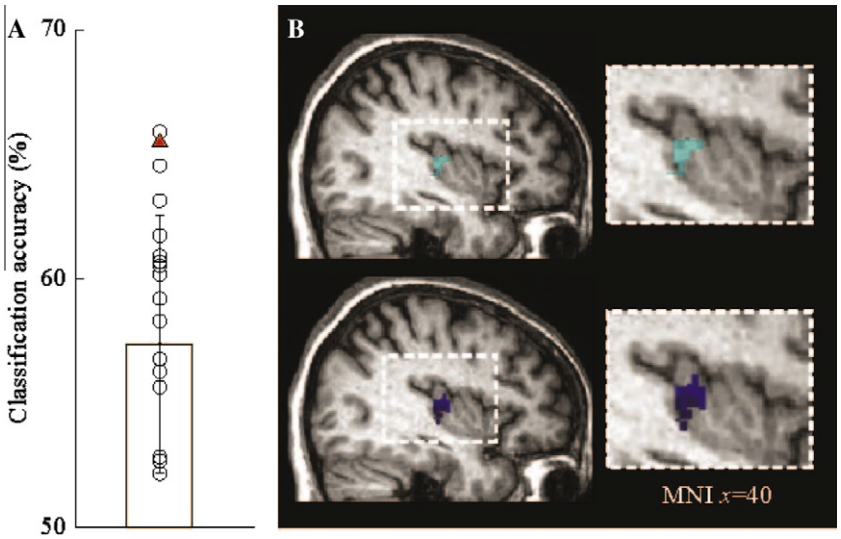

Fig. 4. Multivoxel pattern differences in posterior insula. (A) The posterior insula BOLD response patterns to tactile stimulation in the allodynic zone could be significantly differentiated from stimulation in the control zone in the subject 1 as well as in neurologically intact subjects ($P < .05$, permutation test, 999 iterations). The bar chart and error bars show the neurologically intact subject mean and standard deviation peak classification accuracies, respectively, and the individual accuracies are indicated by the triangle (subject 1) and circles (neurologically intact subjects). (B) Voxels with significant decoding ($P < .05$, permutation test, 999 iterations) were found in similar regions in subject 1 (top) and neurologically intact subjects (bottom).

4.2. Spinal mechanisms of tactile allodynia

Tactile allodynia is generally described in terms of central sensitization in which Aβ afferents, through altered spinal signaling, gain access to pain processing regions of the brain [10,41,64,67,68]. This theory is based on selective nerve block experiments in humans where the allodynic sensation is typically lost when Aβ afferents are blocked [14,26,35,63]. In addition, low-intensity electrical stimulation of Aβ afferents evokes pain if the receptive field of the stimulated afferent is in the allodynic zone [63]. The details of the anatomical and functional reorganization of the dorsal horn during central sensitization are controversial [9]. After capsaicin-induced C-fiber injury in rats, Aβ afferents have been demonstrated to sprout (from their normal terminations in lamina III–VI) and connect to lamina II, a region that normally receives only C-fiber input [42] (but see [3] for an alternative view). Another proposed mechanism is injury-induced unmasking (disinhibition) of polysynaptic low-threshold input to lamina I nociceptive output neurons [30]; such unmasking may be rapid enough to account for the acute onset of allodynia in the heat/capsaicin model.

Both subject 1 and neurologically intact subjects demonstrated differences in posterior insular processing when comparing stroking in the allodynic and control zones. This suggests that C-tactile afferent processing per se is altered during tactile allodynia.

Although C-LTMs activate nociceptive (WDR) lamina I projection pathways of the dorsal horn in rats, a C-LTM-specific pathway has yet not been observed [1]. It may, however, be that a lamina I WDR pathway to the posterior insular cortex terminates differently than a postulated pathway signaling the normal C-touch

sensation. In this scenario, noxious stimulation may suppress C-tactile signaling through the C-touch spinal pathway (resulting in the reduced perception of brush stimuli in the allodynic zone in Aβ-denervated subjects), whereas C-tactile signaling through the WDR pathway is enhanced. Hence, suppressed signaling in the C-touch pathway and increased signaling in the WDR pathway may contribute to the allodynic condition whereas the allodynic pain percept seems to be dependent on Aβ afferent signaling (at least under our experimental conditions). However, because fMRI does not allow distinction between bottom-up or top-down effects, we cannot exclude the notion that mechanisms for capsaicin-induced alteration of C-tactile signaling may be located at supraspinal levels.

An alternative possibility is that the patients have undergone compensatory plastic changes that prevent them from perceiving pain after C-tactile – lamina I WDR – posterior insular activation. However, we consider this less likely because subjects 1 and 2 have only slightly reduced or normal pain perception to nociceptive stimulation, suggesting they have largely intact pain systems [50].

It was recently suggested that the central terminations of A- and C- LTMs are somatotopically organized in a unifying pattern in lamina IIiv–IV of the mouse dorsal horn with projections through the dorsal column to the somatosensory cortices [36]. Whether this dorsal horn integration of A- and C- LTM signaling is affected in the allodynic condition has not been investigated.

In addition to altered C-tactile processing, tactile allodynia is also associated with reduced Aβ-fiber-mediated touch sensation [40]. After capsaicin injection, neurologically intact subjects reported numbness in an area surrounding the allodynic zone and reduced tactile detection within the allodynic zone [40]. A presynaptic inhibition of Aβ fibers by C-fiber input in the dorsal horn was suggested as a potential mechanism [29,40,71].

4.3. Cortical correlates of tactile allodynia

We examined subject 1 and 18 neurologically intact subjects using the same fMRI paradigm. We emphasize similarities in results for subject 1 and the neurologically intact subjects, and by doing so, we can draw conclusions on the C-tactile contribution to the observed phenomenon [5,13,51].

Recently, a hedonic network of brain areas beyond the posterior insula has been implicated in coding C-tactile affective touch [25]. This network includes mPFC and dorsoanterior cingulate cortex. For the contrast control minus allodynia, both subject 1 and neurologically intact subjects demonstrated reduced activation in frontal superior medial cortex (part of mPFC). Moreover, neurologically intact subjects demonstrated reduced activation in areas extending into the pregenual anterior cingulate cortex, which has also recently been suggested as being part of the C-tactile hedonic network [37].

A previous study of capsaicin-evoked pain in healthy subjects suggested that activation of the inferior frontal gyrus was specific for brush-evoked allodynia [27]. The findings in subject 1 demonstrated inferior frontal gyrus activation without her feeling pain, suggesting that it may reflect altered C-tactile processing rather than being related to the pain of allodynia. Neurologically intact subjects also demonstrated activations in several areas, including the anterior insular cortex, secondary somatosensory cortex, and cerebellum, that were not activated in subject 1. These additional activations are often associated with pain processing [2] and may reflect the critical contribution of Aβ signaling to dynamic tactile allodynia [14,26,31,35,63].

We conclude that the allodynic condition is associated with reduced C-tactile-mediated hedonic processing after subcortical alteration of C-tactile signaling. Furthermore, the lack of tactile-evoked pain in the Aβ-denervated subjects is consistent with the canonical view that Aβ afferents are necessary for perception of allodynic pain [10,14,26,31,35,41,63,64,67]. Nevertheless, supported by the observation that C-LTM knockout mice demonstrate decreased mechanical hypersensitivity to normally innocuous stimuli after experimental models of inflammation, nerve injury, and trauma [59], it seems possible that reduced C-tactile-mediated hedonic processing also plays an important role in the allodynic condition in humans.

Conflict of interest statement

The authors report no conflict of interest.

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