Observation of others’ painful heat stimulation involves responses in the spinal cord

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Observing others’ aversive experiences is central to knowing what is dangerous for ourselves. Hence, observation often elicits behavioral and physiological responses comparable to first-hand aversive experiences and engages overlapping brain activation. While brain activation to first-hand aversive experiences relies on connections to the spinal cord, it is unresolved whether merely observing aversive stimulation also involves responses in the spinal cord. Here, we show that observation of others receiving painful heat stimulation involves neural responses in the spinal cord, located in the same cervical segment as first-hand heat pain. However, while first-hand painful experiences are coded within dorsolateral regions of the spinal cord, observation of others’ painful heat stimulation involves medial regions. Dorsolateral areas that process first-hand pain exhibit negative responses when observing pain in others. Our results suggest a distinct processing between self and others’ pain in the spinal cord when integrating social information.

INTRODUCTION
Observing others’ painful experiences is central to knowing what is dangerous around us. Observing painful experiences elicits affective, cognitive, and physiological responses that are comparable to first-hand aversive experiences, including overlapping activity in neural brain networks. These brain networks include the anterior insula, the anterior cingulate cortex (ACC), and somatosensory cortices (SI and SII) (1–10). Processing of first-hand aversive experiences underlies functional connectivity with other parts of the central nervous system, the spinal cord in particular. Hence, first-hand aversive experiences involve ascending connections that relay sensory information from the spinal cord to the brain, as well as descending pathways from the brain that gate activity in the spinal cord (11, 12). The ACC, for example, modulates neurons at the dorsal horn of the spinal cord via polysynaptic inhibitory and excitatory inputs (13). Furthermore, midbrain pathways from the periaque- ductal gray (PAG) via the rostroventral medulla (RVM) modulate activity of the dorsal horn of the spinal cord by inhibitory GABAergic projections and endogenous opioids (12, 14). These descending connections are thereby central to integrating cognitive and affective processes (such as expectations, value, threats, and stress) at the level of the spinal cord, as evident from functional magnetic resonance imaging (fMRI) of the spinal cord in humans (15–17). Hence, descending inputs to the spinal cord have been shown to mediate cognitive and affective processes that result from first-hand sensory inputs (18, 19).

It is therefore tempting to assume that these descending cerebrospinal pathways are also involved in observational experiences. Indirect evidence comes from studies, showing that observing others’ aversive experiences can modulate corticospinal pathways in humans, indicated by enhanced corticospinal inhibition during transcranial magnetic stimulation (TMS) of the sensorimotor system (20, 21). In line with these results are experiments revealing that observation of aversive experiences can modulate peripheral readouts of spinal reflexes (22, 23). The idea that observational experiences modulate spinal cord activity is further suggested by electrophysiological recordings in monkeys, showing that inhibitory corticospinal projection neurons in M1 discharge when grasping is executed or when grasping is observed without sensory input (24).

Brain circuits that are involved in processing observation of others’ aversive experiences are functionally connected with the spinal cord, such as the ACC (4, 8, 25–29) and the midbrain PAG (30). It is therefore possible that a cerebrospinal pathway is involved in the processing of aversive experiences in general by enabling the integration of cognitive and affective experiences at the level of the spinal cord, even when first-hand sensory input is missing. While such a gate control mechanism has been established for first-hand aversive experiences (11), it is unclear whether a similar mechanism is at play when aversive events are merely observed.

The aim of the present study was to test the hypothesis that spinal responses reflect observation of others’ aversive experiences. To this end, we used high-resolution fMRI of the human cervical spinal cord based on the blood oxygenation level–dependent (BOLD) contrast (15, 17) in combination with an established protocol for observation of aversive experiences (31).

In this protocol, participants underwent first-hand aversive heat stimulation at the right ventral forearm and observed video clips of a demonstrator model that received the same heat stimulation in a randomized order (see Fig. 1A). In the video clips, the demonstrator displayed a facial expression of discomfort without movement of any other body parts. We further included two control conditions (see Fig. 2), one for observational experiences (video clip of a calm demonstrator) and another for sensory experiences (no first-hand experience). All four conditions were preceded by a colored square. Spinal cord fMRI responses were analyzed using a well-established toolbox (32).

RESULTS
Observational and first-hand experiences of heat pain are located at the same cervical level
Participants rated the first-hand experiences as painful (rated mean, >60 for each block; one-sample t test, all P < 0.001), whereas pain

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ratings for observational trials were not different from 0 (all \( P > 0.05 \); see table S3 and the Supplementary Materials). Participants furthermore expected first-hand pain by the presence of the associated colored cue (see table S4). Participants indicated no expectation for first-hand pain to the colored cue that preceded heat pain for the demonstrator (i.e., observational cue for the participant), because this cue was presented to the participants in separate trials without any aversive consequences (see table S4). Participants rated the intensity of the demonstrators’ heat stimulus comparable to their own painful heat stimulation (rated mean, 5.04; SD, 2.34 on scale from 0 to 10; >5 = higher intensity for the demonstrator; <5 = lower intensity for the demonstrator). All participants furthermore stated that they never perceived first-hand heat pain when observing the demonstrator receiving painful heat stimulation.

In a first analysis, we located our region of interest (ROI) for processing of first-hand painful stimulation within the spinal cord. To this end, we examined the BOLD responses elicited by first-hand heat stimulation and located the strongest responses in the lateral, dorsal section of the spinal cord ipsilateral to the stimulation side at the segmental level of C5, which corresponds to the dermatome C6 [one-tailed, one-sample \( t \) test, \( x,y,z \) (MNI) = 6,−46,−153; \( t_{29} = 3.19, P_{uncorr} = 0.0016 \); Fig. 1A] and resembles findings from previous studies (15–17).

Next, we tested for BOLD responses elicited by observed painful heat stimulation in others, within the defined ROI, a 4-mm-thick horizontal slice (12 mm–by–4 mm plane) that was centered at the same cervical height as the direct experience of heat pain. In accordance with our hypothesis, this analysis revealed a cluster of voxels reflecting BOLD responses related to observed painful heat stimulation [one-tailed, one-sample \( t \) test, \( x,y,z \) (MNI) = −1,−47,−155; \( t_{29} = 3.99, P_{corr} = 0.045, P_{uncorr} = 0.0015 \); Figs. 1B and 2A]. Specifically, BOLD responses in the spinal cord for observed painful heat stimulation were located more medial as compared to first-hand heat pain, i.e., activation occurred at the same spinal level (C6) as the direct experience of heat pain. Exploratory analyses for spinal cord responses at the level of C6 (within the ROI) during the anticipation phase (when only colored cues were displayed) yielded no voxel above the threshold of \( P_{uncorr} < 0.005 \) under any of the conditions (see also tables S5 and S6).

Responses in the spinal cord to observation of others’ painful heat stimulation are not related to the absence of sensory input

To further establish the specificity of spinal cord responses to observation of painful heat stimulation, we compared BOLD response estimates to other conditions that involve social observation, first-hand heat pain, and no sensory input. To this end, we extracted the estimates from the peak voxel representing the main effect of observed heat stimulation during both experimental and control conditions. The analyses revealed a main difference between responses across conditions [main effect analysis of variance (ANOVA), \( F_{2,3,68,0} = 3.344; P = 0.034; \eta_p^2 = 0.103 \)]. Follow-up paired comparisons revealed that responses toward observed painful heat stimulation were higher when compared to a control condition for social observation [video clip of a calm demonstrator, one-sided paired comparisons: \( t_{29} = 2.894, P_{uncorr} = 0.004, P_{corr} = 0.020, Cohen’s d = 0.528 \) (see Fig. 2B and table S2)]. Similarly, response estimates representing the observed painful heat stimulation were higher when compared to trials with no first-hand heat stimulation at the same voxel (one-sided paired comparisons, \( t_{29} = 2.573, P_{uncorr} = 0.008, P_{corr} = 0.032, Cohen’s d = 0.470 \); see Fig. 2D and see table S2), which suggests that missing sensory input did not drive the effect for observational experiences. BOLD responses at the voxel representing the main effect of observed painful heat stimulation were also higher when compared to first-hand painful heat stimulation at this voxel (one-sided paired comparisons, \( t_{29} = 2.416, P_{uncorr} = 0.011, P_{corr} = 0.033, Cohen’s d = 0.441 \); see Fig. 2C). We found no support for a differentiation between first-hand pain and control conditions when comparing responses at this voxel that reflects the main effect.
of observational heat pain (P_{uncorr} > 0.7). This result underlines our initial finding that observed painful heat stimulation and first-hand experiences of heat pain are located within different laminar parts of the spinal cord but involve processes at the same segmental level.

Prior experiments using TMS suggested that sensory, but not affective, aspects of observed experiences are related to corticospinal inhibition (20, 21). We found no conclusive statistical support for a negative association between responses toward observed painful heat stimulation and rated (inferred) intensity of heat pain for the demonstrator, when correcting for multiple comparisons (two-sided Pearson correlation with responses during observed heat pain: demonstrators’ intensity \( r = -0.407, P_{uncorr} = 0.039, P_{corr} = 0.156 \) and demonstrators’ intensity relative to own intensity \( r = -0.459, P_{uncorr} = 0.018, P_{corr} = 0.090 \). We found no support that responses to observed heat stimulation in the spinal cord were associated with the rated unpleasantness of the stimulation for the demonstrator or the unpleasantness of observing the stimulation for the demonstrator (all \( P_{corr} > 0.26 \); see table S3). Additional analyses did not support associations between responses in the spinal cord toward observed painful heat stimulation with empathy for the demonstrator or identification of the participants with the demonstrator or personality traits for anxious temperament or emotional empathy (all \( P_{corr} > 0.5 \); see the Supplementary Materials). Hence, our results cannot conclusively support that sensory evaluation of the observed painful heat stimulation is related (in a negative direction) to spinal involvement during observational experiences.

Responses in the spinal cord to observation of others’ heat stimulation are distinct from responses to first-hand painful experiences

Next, we aimed to further differentiate spinal cord responses to sensory, first-hand heat pain from observed painful heat stimulation. To this end, we extracted response estimates at the voxel in the dorsal/lateral horn to examine responses in this area, which represent first-hand pain processing (see Fig. 3A), during observed painful heat stimulation. The paired comparisons revealed higher responses toward first-hand pain than during observed painful heat stimulation in the dorsal/lateral horn (one-sided paired comparison, \( t_{29} = 3.384, P = 0.001 \); see Fig. 3B). Response estimates in the voxel that represents first-hand heat pain were negative during observation of others receiving heat stimulation (one-sided one-sample, \( t_{29} = -1.848, P = 0.037 \). To test whether several voxels in the segment of C6 exhibit negative responses during observed pain, we tested for negative BOLD responses with our ROI when participants observed heat stimulation. We found a dorsolateral cluster that exhibited negative responses when participants observed painful heat stimulation in others [one-tailed, one-sample \( t \) test, \( x,y,z \) (MNI) = 6,−45,−153; \( t_{29} = 3.94, P_{corr} = 0.049, P_{uncorr} = 0.00023 \); Fig. 3, D and E]. As already indicated by the analyses of the response estimates, this cluster was overlapping with the voxels reflecting the main effect of first-hand pain (see Fig. 3B).

This reverse pattern of activation found for sensory aspects of first-hand heat pain as opposed to observed painful heat stimulation was underlined by a negative association between rated pain during first-hand aversive experiences and response estimates during observed painful heat stimulation in the dorsal/lateral horn (two-sided Pearson \( r = -0.496, P = 0.014 \); see Fig. 3C). Hence, participants who experienced more pain during first-hand heat stimulation showed more negative responses in the dorsal/lateral horn when observing others’ pain. These results thereby underline again a distinction between processing of first-hand heat pain and observed heat stimulation within the spinal cord. The negative BOLD responses might suggest inhibitory processes (33) at the dorsolateral region of the spinal cord during observation of others’ pain.

DISCUSSION

Our results provide direct evidence that observational experiences involve central nervous responses outside of the human brain, within the spinal cord. They further suggest that observation of painful heat stimulation is already processed within spinal segments that are also involved in processing first-hand heat experiences. However, the spinal processes that underlie observational and first-hand experience most likely differ. These results are in line with findings
that observational and first-hand aversive experiences are implemented in overlapping regions in the brain (3, 4, 7, 25, 34–36), where underlying processes within these neural circuits compute domain general, as well as specific aspects of observed pain (8, 37–39). Our results underline the importance of processes that allow the distinction between self and others’ experiences when integrating social information (40, 41). We suggest that responses in the spinal cord allow us to differentiate implementation of aversive events by first-hand, nociceptive experience or as observed in others without actual sensory input.

Previous studies suggested involvement of the spinal cord in observational events; however, the precise implementation of social information was unclear. First of all, we demonstrate that observation of heat stimulation in others involves activity in the spinal cord, which is in line with activity during others’ pain within brain regions that are densely connected with the spinal cord [cerebellum and brainstem regions (7)]. We further demonstrate that spinal cord responses when observing painful heat stimulation involves positive responses within medial parts of the spinal cord and negative responses in dorsolateral regions that are related to processing first-hand painful experiences. Our findings are in line with inhibitory processes in corticospinal projections that underlie observational movement processing in monkeys (24). Similarly, studies in humans showed enhanced inhibition of corticospinal pathways when observing others’ aversive experiences (20, 21). It is possible that negative responses during observation reflect inhibitory processes of sensory signals. Studies in mice revealed inhibitory gating of the dorsal horn via projections from the PAG via the RVM and their modulation by endogenous opioids (14). Acute stress, which is known to release endogenous opioids, enhanced this inhibition. We could recently show that observation of others’ aversive experiences involves the PAG and release of endogenous opioids (30). One possibility is that observational heat stimulation recruits PAG-mediated opioid signaling that encodes the absence of sensory processing in the dorsal horn. This idea would be in line with spinal inhibition as a central mechanism for observational movements to prevent involuntary muscle activity toward all observed movements around us. Thereby, the spinal cord would have the important mechanistic function to integrate the absence of sensory input together with descending information from brain regions that process first-hand sensory experiences.

The medial spinal cord region that exhibited activity during observation of painful heat stimulation is associated with a connectivity pattern that is distinct from dorsolateral regions, both within the spinal cord itself and in connection to the brain (42, 43). While the dorsal regions were correlated with activity in the basal ganglia,
thalamus, and cerebellum, the medial regions showed coactivation with primary motor and somatosensory components (43). The activity that we found in the medial region might be related to neural responses in the central gray of the spinal cord, which has been found to receive afferents that are related to somatic and visceral pain processing (44).

Future studies are needed to unravel the topology of spinal responses when observing painful heat stimulation that is applied to different parts of the demonstrators’ body. In this study, we used one shared location for first-hand and observational stimuli. It will be interesting to see whether a converging location enables participants to make precise estimates of the others’ experiences and whether a distinct location for self and others’ stimulation maps onto different spinal segments. Moreover, our protocol might have fostered the distinction between self and others’ stimulation, because participants never experienced pain together with the demonstrator and participants were directly exposed to the cue that were followed by pain for the demonstrator (without first-hand experience of aversive consequences) and experienced first-hand painful stimulation that mirrored the observed stimulation. Hence, our results might be influenced by a protocol that enabled this clear distinction between self and others’ pain.

In sum, our results provide evidence for an involvement of the spinal cord in observation of painful heat stimulation. Coding of observational in distinction from first-hand experiences, however, differs at the implementational level and regarding their response function. The processes in the spinal cord thereby provide insights into how observational experiences are integrated distinctly from first-hand aversive experiences within the earliest level of the central nervous system. Our results provide a mechanistic perspective of how others’ experiences are integrated into neural processes of the observer.

**MATERIALS AND METHODS**

**Participants**

Thirty-five healthy, right-handed male participants (to exclude interactions between the gender of the model and the participants) were invited to participate in the study. Exclusion criteria were any history of neurological and psychiatric diseases, any chronic pain syndrome, current medication, and any MR contraindications. Furthermore, participants who had previously participated in any experiment including first-hand or observational heat experiences at our institute were excluded. Five participants had to be excluded from the sample [due to imaging artifacts (n = 3), technical problems (n = 1), and misunderstanding the instructions (n = 1)].

The final sample consisted of 30 participants (mean age, 23.9 ± 3.8 years). The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Medical Council of Hamburg. All participants gave informed written consent.

**Experimental design**

**First-hand heat stimulation**

First-hand heat stimuli were delivered using a Contact Heat-Evoked Potential Stimulator thermode (Medoc Advanced Medical Systems, Israel) with the 3 cm–by–3 cm thermode head tightly attached on the right forearm (located on dermatome C6) and having skin contact on the full active surface. The heat stimulus was individually adjusted from an initial temperature of 43°C, which was increased by 0.5°C until the participants did not want further increase in the temperature (mean temperature of the adjusted heat stimulus, 44.86°C ± 0.68°C). Participants rated the individually adjusted stimulus, on average, with 6.7 (±1.3) on a scale from 0 (no pain) to 10 (highest pain anticipated from the thermode). The thermode was always tightly attached to the participants’ right arm to prevent shifting. Furthermore, it was ensured that the entire thermode surface had full contact with the participants’ skin. The baseline temperature, when no heat pain was administered, was set to 37°C.

**Observational heat stimulation**

Observational heat stimulation consisted of a video clip, displaying a demonstrator model in front of a computer screen (displaying a colored rectangle), which was followed with heat stimulation to the demonstrator. The demonstrator was attached to the same thermode head, which was located at the right forearm (same position as for the participants). Upon heat stimulation, the demonstrator displayed facial expression of discomfort or pain, without movements of any other body parts. Similar video clips have been used in previous experiments that examined observational responses toward others’ aversive experiences (8, 30, 31). Participants rated the observed heat pain stimulus, on average, with 6.2 (±2.1) on a scale from 0 (not unpleasant for the demonstrator) to 10 (highly unpleasant for the demonstrator) and comparably painful to their individual first-hand experience with a rated 5.0 (±2.3) on a scale from 0 (less painful for the demonstrator) to 10 (more painful for the demonstrator), where 5 denoted an equally painful experience.

**Procedure**

The study consisted of a combined protocol for first-hand and observational nociceptive experiences [adapted from (31)]. Before MR scanning, participants came into the laboratory and gave their written consent after being informed about the study. Participants were informed that they would experience heat pain and see video clips (no further details were provided). Before the experiment, participants were positioned in the MR scanner, and preexperimental MR images to optimize the spinal cord data were performed (for further details, see the “fMRI data acquisition” section). This procedure lasted about 15 min and included a T1-weighted high-resolution image of the head and the neck and a T2-weighted image of the neck. An individual adjustment of first-hand heat stimulus preceded the experiment.

In the experiment, each trial comprised an anticipation phase (5 s) that was followed by a first-hand, observational, or no heat stimulation. Trials for first-hand heat stimulation included a presentation of a colored rectangle (cue; duration, 9 s), which was followed 5 s after cue onset by heat stimulation (12 s). Accordingly, observational heat stimulation consisted of a display of the demonstrator model in front of a computer screen where a colored rectangle was displayed (observational cue) for 9 s, which was followed after 5 s with heat stimulation (12 s) to the demonstrator. Control conditions consisted of trials without heat stimulation, including either a presentation of a colored rectangle (duration, 9 s) without any stimulation or video clips displaying a calm demonstrator model in front of the computer screen that was displaying a colored rectangle (observational control cue) for 9 s. In addition, in separate trials, the colored squares that were presented on the demonstrators’ screen (observational cue and observational control cue) were presented directly on the screen of the participant. While observational learning protocol often used designs in which the observational trials and the direct presentation
of the observational cues to the participants are separated in blocks (31), we used an intermixed design to reduce the anticipation of first-hand pain to the observational cues in the participants. The color of each cue was unique to this cue for each participant (observational cues, yellow or blue; first-hand cues, brown or purple). The colors were counterbalanced within each condition across participants as in previous experiments examining first-hand and observational pain (8). All trials were separated by a variable intertrial interval (mean of 24 s). The order of the trial types was randomized, and the fMRI session consisted of 12 trials per condition in total.

Ratings
After three of each trial, a rating scale appeared on the screen, and participants had to rate the pain intensity of the preceding pain stimulus using a visual analog scale consisting of 100 rating steps from 0 to 99. The left anchor of the scale was labeled with "no pain" and the right anchor with "unbearable pain." Each pain rating after first-hand experiences was different from 0 (all P < 0.001; see table S3), whereas pain ratings for observational trials were not different from 0 within all rating (all P > 0.05; see table S3). Six participants were excluded from rating analyses, n = 3 due to missing data and n = 3 due to a technical failure of the rating procedure. Before and after the experiment, participants rated their fear/stress/tension toward each of the colored cues and how much they would expect a first-hand heat stimulation after each of these cues.

Postexperimental interview
After the experiment, we checked for contingency awareness (i.e., if they experienced first-hand heat pain stimulus, if they were aware which cue preceded the heat pain stimulus, and how many first-hand heat pain stimuli they experienced). We also asked whether they ever experienced heat pain during the observation of heat pain. To derive a measurement for the individually inferred stimulus intensity for the demonstrator, we asked participants how intense the heat pain stimulus was for the demonstrator model (absolute; 0 to 10) and how intense this stimulation was in comparison to their individual first-hand experience (0 to 10, when 5 is comparable to own experience). We further asked how unpleasant it was for the participant to observe the demonstrator receiving heat pain (0 to 10), how much empathy they experienced for the demonstrator (0 to 10), and how much they could identify themselves with the demonstrator (0 to 10). Three participants were excluded because of missing data.

fMRI data acquisition
MRI data acquisition was based on previous fMRI studies on first-hand pain processing within the human spinal cord (15, 17). Imaging data were acquired on a Siemens Magnetom Prisma Fit 3 Tesla System using a 64-channel head-neck coil. Individuals were positioned with the cervical spinal cord centered in the magnet. To minimize head and neck movements, we used a vendor-supplied immobilization pads to stabilize the position of the cervical vertebrae.

High-resolution (1 mm by 1 mm by 1 mm) T1-weighted anatomical images were acquired using a three-dimensional MPRAGE sequence (sagittal slice orientation; repetition time, 2.3 s; echo time, 3.45 ms; readout flip angle, 9°; inversion time, 1.1 s; field of view, 192 mm by 320 mm by 256 mm). The field of view ranged from the cingulate cortex to the second thoracic vertebrae. For this acquisition, all 64 coil elements were used, whereas, for the acquisition of functional images, we only used 12 neck elements. Functional images were acquired using a gradient-echo echo-planar imaging (EPI) sequence (repetition time, 860 ms; echo time, 30 ms; flip angle, 50°; field of view, 128 mm by 128 mm; matrix, 128 by 128; GRAPPA with a parallel acquisition technique factor of 2 and 48 reference lines). The target volume was centered on an area from the middle part of the fifth cervical vertebrae. We acquired 10 slices positioned approximately perpendicular to the spinal cord using a slice thickness of 5 mm to achieve an adequate signal-to-noise ratio despite a high in-plane resolution (1 mm by 1 mm), as in a previous study (15). Saturation pulses posterior and anterior to the target region, i.e., in the phase-encoding direction, were added to avoid ghosting and minimize inflow artifacts related to pulsatile blood flow in major vessels.

To minimize signal intensity variations along the z direction, a compensatory slice-specific gradient momentum was used (15, 45). This “z-shim” was determined on the basis of a reference scan acquisition with 15 equidistant gradient steps applied to all slices and subsequently selecting the gradient setting yielding the maximum signal intensity within the spinal cord for each slice.

The first five volumes of the fMRI acquisition were discarded to eliminate T1 saturation effects. To allow for retrospective physiological noise correction (46), which is critical in spinal fMRI, heart rate and respiration rate were measured using a clinical monitoring unit (Expression, Invivo, Gainesville, USA). Data were recorded together with the scanner pulses using Spike2 software (Cambridge Electronic Design Limited, Cambridge, UK) to ensure timing accuracy.

fMRI data preprocessing
fMRI data preprocessing was carried out using the Spinal Cord Toolbox (https://sourceforge.net/projects/spinalcordtoolbox/), an established toolbox for spinal cord fMRI data (32). The processing of the spinal cord structural MR scans started with an automatic segmentation (DeepSeg) and labeling of the cervical elements. If necessary, manual identification of C3 was used (n = 3). This was followed by a registration of the individual scans to an established unbiased multimodal MRI template of the spinal cord [Polytechnique, Aix-Marseille University and Montreal Neurological Institute (PAM)50; (47)] to normalize all images into a common space. Registration first included an affine alignment of the segmented images (slice-wise center of mass using mean of squares metric), followed by nonrigid symmetric registration of the images (B-spline approximation using cubic centimeter metric) across five iterations and a shrink factor of 2.

The preprocessing of the spinal cord fMRI data started with motion correction of the time series using spline interpolation and a mask around the spinal cords’ centerline (65-mm-diameter cylinder). Next, the mean image across all scans within one individual was segmented (using a deep learning algorithm or, if necessary, a support vector machine in n = 2). To optimize registration between EPI and PAM50 template, the EPI mean image was first registered to a PAM50 T2 template that was warped into individual native space. This registration was performed using an affine alignment of the segmented images (for n = 10, the default parameters were used; otherwise, these were optimized as regularized translations using mean of squares metric, smoothing factor of 2 mm, polynomial degree of regularization of 2, 20 iterations, gradient step of 0.2, initial translation alignment based on center of mass of images, and final interpolation using spline). Next, the mean functional image was registered to a PAM50 T2 template (common space) using warp fields that resulted from registration of the individual structural volume to the
comparisons were adjusted for multiple comparisons (Bonferroni-Holm method, five comparisons). All these comparisons tested directed hypotheses and were adopted as one-sided paired t tests.

We calculated two-sided Pearson correlation to test for association between the inferred stimulus intensity (absolute and, in comparison, to individual experiences) and spinal cord activity. Previous research (20) found a negative relationship between TMS-evoked responses and inferred stimulus intensity, yet we cannot assume that BOLD responses would show a similar direction and therefore tested two-tailed. We further tested for association with the unpleasantness for the participant to watch the demonstrator, empathy for the demonstrator, and identification with the demonstrator. These correlational analyses were corrected for multiple comparisons (five comparisons) using the Bonferroni-Holm method.

**SUPPLEMENTARY MATERIALS**

Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/7/14/eabe8444/DC1

View/request a protocol for this paper from Bio-protocol.

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