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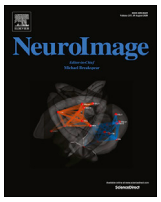

Keeping an eye on pain expression in primary somatosensory cortex

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ABSTRACT

Facial expressions of pain are composed of a subset of pain-indicative muscle movements. Amongst this subset, contracting the muscles surrounding the eyes (orbicularis oculi muscle) is the most frequent response and has been linked specifically to pain intensity, a fundamental aspect of the sensory dimension of pain. To further explore this link, the present study used functional magnetic resonance imaging (fMRI) to test the hypothesis that orbicularis oculi activation during pain reflects the magnitude of brain responses in areas being involved in processing the sensory dimension of pain.

Facial and brain (BOLD) responses to experimentally-induced heat pain applied to the left lower leg were assessed in twenty-two healthy participants after verbal suggestions were given to specifically increase perceived pain intensity and in control conditions involving no suggestion.

Increases in pain intensity produced the expected changes in facial responses characterized by a stronger contraction of the orbicularis oculi muscle. A regression model further demonstrated that stronger increases in orbicularis oculi activity reflected a larger increase in the BOLD response to the noxious stimulus in the leg area of the primary somatosensory cortex (S1) and a larger decrease in medial prefrontal activity consistent with previous finding suggesting disinhibition. Importantly, the positive coupling of orbicularis oculi with S1 activity was not accounted for by changes in other facial muscles.

These results are consistent with the notion that facial expressions of pain differentially encode the multidimensional pain experience and reflect, at least partly, the activity of the spino-thalamo-cortical pathway targeting the primary somatosensory cortex.

1. Introduction

Facial expressions of pain are a fundamental channel of pain communication and play an important role in social interactions, clinical decision-making and daily pain management. This particular form of non-verbal pain communication has been quantified using the Facial Action Coding system (FACS (Ekman and Friesen, 1987)) and is composed of a limited set of facial movements (Action Units, AU), including the "contraction of the eyebrows" (AU4), "contraction of the muscles surrounding the eyes" (AU6_7), "upper lip raise" (AU9_10), "closing of the eyes" (AU43) and "opening of the mouth" (AU26_27_28) (Kunz et al., 2019; Prkachin, 1992). This non-rigid, non-uniform set of pain-indicative facial movements is composed of various configurations that vary across individuals in the context of pain (different facial activity patterns) (Kunz and Lautenbacher, 2014; Kunz et al., 2019). Nevertheless, there is one pain-indicative facial movement that is displayed very consistently, namely the contraction of the muscles surrounding the eyes (orbicularis oculi muscle, AU6_7) (Kunz and Lautenbacher, 2014). Indeed, the orbicularis oculi contraction is the most frequent and most stable facial activity accompanying the experience of acute as well as chronic pain (Craig et al., 2001; Kunz et al., 2019). Furthermore, the orbicularis oculi contraction is unique in the sense that this facial movement seems to encode the sensory dimension of pain and more specifically the perception of pain intensity. In contrast, other pain-indicative facial movements are more closely associated with the affective dimension of pain (Kunz et al., 2012). Evidence for the close linkage between orbicularis oculi activation and the sensory dimension of pain stems from a previous study of our group (Kunz et al., 2012), where suggestions targeting pain intensity or pain unpleasantness were used to differentially modulate the sensory and the affective dimension of

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pain, respectively. We found that an increase in perceived pain intensity, following suggestions targeting pain intensity, was significantly associated with an increased contraction of the muscles surrounding the eyes (AU6_7), whereas other pain-indicative facial movements remained unchanged. In contrast, enhanced pain unpleasantness, following suggestions targeting pain affect, did not lead to a change in contraction of the muscles surrounding the eyes (AU6_7) but was accompanied by a more variable increase of other pain-indicative facial movements.

In order to further investigate the possibility of a specific facial encoding of pain sensation via contraction of the muscles surrounding the eyes, the present study examines the relation between orbicularis oculi activation and brain activity in pain related areas, especially areas that have been associated with the sensory dimension of pain, using functional magnetic resonance imaging (fMRI). There is a wide network of brain areas implicated in pain processing, including primary (S1) and secondary (S2) somatosensory cortices, middle cingulate cortex, insula, thalamus, and prefrontal areas (Apkarian et al., 2005; Duerden and Albanese, 2013). Although several of these regions may encode nociceptive intensity and perceived pain intensity (Coghill et al., 2001, 2003; Wager et al., 2013), suggestions to modulate the sensory dimension of pain have been previously shown to affect the response of S1 cortex to controlled noxious heat stimuli (Hofbauer et al., 2001).

Our main hypotheses propose that the contraction of the muscles surrounding the eyes (AU6_7) would increase following suggestions given for increased pain sensations, and that this increase would correlate with increased activity in target cortical areas of the spino-thalamocortical pathway, especially with S1.

2. Materials and methods

2.1. Participants

Twenty-three healthy volunteers (female: N = 12, male: N = 11) between the ages of 18 and 33 years (mean age 22.6 years; SD = 3.9) without history of chronic pain, neurological or psychiatric disorders, participated in this study. Given that our aim was to use verbal suggestions to increase the sensory dimension of pain and to investigate the impact of the manipulation particularly on facial responses, participants had to be suggestible as well as facially expressive in response to the experimental pain stimuli. They were selected out of a group of 60 participants recruited via advertisements posted on the campuses of the Université de Montréal and McGill University in a 1-h pre-selection session that took place 4–8 weeks prior to the present study. Detailed information on how suggestibility and facial expressiveness were assessed has been published previously (Kunz et al., 2012). Thirty-seven participants (out of the 60 recruited) did not meet the selection criteria and thus were excluded from the study (N = 14 due to the lack of suggestibility, N = 10 due to low facial expressiveness, and N = 13 due to both lack of suggestible and facially expressiveness). All participants provided written informed consent and received monetary compensation for their participation. Although the consent form did mention that facial expression would be monitored and recorded during the study, no emphasis was put on the importance of facial responses. The study protocol was approved by the ethics committee of the Centre de recherche de l'institut universitaire de gériatrie de Montréal.

2.2. Materials and procedure

Following the pre-selection, all 23 participants took part in a 1.5-h training session scheduled 2–4 weeks prior to the imaging study, during which participants were carefully familiarized with the experimental methods and suggestion procedures (see (Kunz et al., 2012) for details on the training session).

2.2.1. Pain stimulation

Pain was induced experimentally by a Peltier-based, computerized thermal stimulator with a 3 × 3 cm^2 contact probe (Medoc TSA-2001; Medoc Ltd, Ramat Yishai, Israel) attached to the left lower leg. Baseline temperature was always set to 38 °C. To ensure that temperature intensities were perceived as painful but tolerable in all participants (in order to prevent floor as well as ceiling effects), temperature intensities were tailored to the individual pain threshold. Following a familiarization trial, heat *pain thresholds* were determined using the method of adjustment in a mock MRI scanner immediately before the scanning session (the average of 5 trials was used as the threshold estimate). Participants were brought into the scanner room for the experiment immediately after.

Each subject underwent 6 × 8 min functional scans, where painful heat stimuli were applied. At the beginning of each run and before the first heat pain stimulus, participants received verbal suggestions, through MRI-compatible headphones, for increased pain intensity (2 runs of "↑pain intensity"), no suggestions (2 runs of "baseline") and suggestions for increased pain affect (2 runs of "↑pain affect"). The order of conditions was counterbalanced across participants (see also Fig. 1). Given that our main interest lies in the orbicularis oculi activation (contracting of the muscles surrounding the eyes; AU 6_7) and in whether this facial movement does indeed encode the sensory dimension of pain, this current report focuses on the "↑pain intensity" and baseline runs. During each of the six functional scans, ten heat stimuli with a temperature of +3 °C above the individual pain threshold were applied. The rate of temperature increase from baseline (38 °C) was adjusted individually to reach the target temperature in 2s, and remained at a plateau for 5s, before returning to baseline in 2s. A long and variable inter-stimulus interval (ISI) between 18s and 25s was used to prevent sensitization and to allow subjects to rate each stimulus. Additional precaution was taken to avoid local sensitization on the site of the stimulation by changing the placement of the thermode after each run.

2.2.2. Suggestions

As we have done previously (Kunz et al., 2012), verbal suggestions for the modulation of pain were given without using hypnotic induction, given that this approach typically contains instructions to relax all muscles, thereby reducing potentially the likelihood of facial responses. Verbal suggestions for increased pain intensity (↑pain intensity) were given directly before the start of each of the 2 "↑pain intensity" scans (see also Fig. 1). Participants were instructed to keep their eyes closed during the suggestions to help them better focus on the suggestions. Importantly, these suggestions were explicitly formulated to prevent a generalization of the increase in pain intensity to pain unpleasantness. Detailed descriptions of these suggestions can be found as supplementary material (supplement 1). At the end of the suggestions participants were asked to open their eyes, look at the fixation cross presented at the centre of the screen, to attend to each stimulus, and to keep their head and body still. Following these instructions, the scan started and painful stimulations were administered.

2.2.3. Self-report ratings

After each stimulus (2-s delay), subjects provided ratings of the intensity and unpleasantness of the pain felt via a computerized VAS-scale that were converted linearly to values between 0 and 100. The VAS for sensory intensity of pain was labeled with verbal anchors from "no pain" (0) to "extremely strong pain" (100). Pain unpleasantness was labeled with "no pain" (0) to "extremely unpleasant pain" (100). All participants were explained the conceptual distinction between sensory intensity of pain and pain unpleasantness following the instructions of Price et al., 1983). VAS sensory and unpleasantness scales appeared successively and were displayed using E-Prime (Psychology Software Tools Inc.) and projected on a screen located at the head-end of the scanner and viewed by the subjects via a mirror attached above the head coil. The ratings were done by moving a computer-controlled cursor using the index and middle finger of the right hand and were recorded in E-Prime.

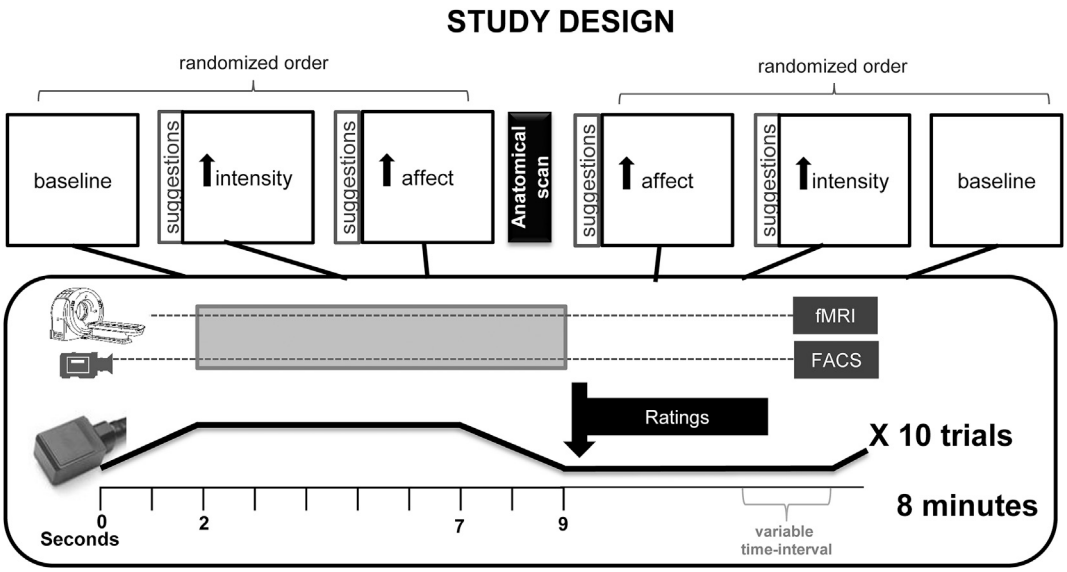

Fig. 1. Schematic illustration of the study design.

2.2.4. Facial responses

During all functional scans, the face of the subject was videotaped using a small MRI-compatible camera (MRC Systems, Heidelberg, Germany) mounted onto the head coil. The camera was carefully positioned to capture the face of the subject reflected through a mirror attached above the head coil, without blocking the visual field of the subject. The onset of each thermal stimulus was marked automatically on the video recording using a signal sent from the stimulator to the sound card. We quantified facial responses using the Facial Action Coding System (FACS (Ekman and Friesen, 1987)), a fine-grained anatomically based system that is considered the gold standard when decoding facial expressions, including the facial expression of pain. A certified FACS coder blind to the experimental tasks ("↑pain intensity" vs. control baseline) identified the frequency and the intensity (5-point scale) of all facial movements (FACS differentiates between 44 Action Units). A software designed for the analysis of observational data (Observer Video-Pro; Noldus Information Technology) was used to segment the videos and to input the FACS codes into a time-related database. Time segments of 7 s beginning with the stimulus reaching the target temperature (5s plateau + 2s offset) were selected for scoring.

For further analyses, we computed a product term for "AU 6_7" (orbicularis oculi muscle contraction) and for "All other AUs" (i.e. all AUs excluding AU 6_7) by multiplying the frequency and intensity values of the corresponding Action Unit in the baseline and the ↑pain intensity runs in each participant. These product terms were then square-root transformed to yield unskewed distributions (Karmann et al., 2016; Kunz et al., 2011, 2012) and used as regressors for the correlation with changes in the suggestion-induced pain-related BOLD responses.

2.2.5. fMRI image acquisition & analyses

Imaging data were acquired at the "Unité de Neuroimagerie Fonctionnelle" of the "Centre de recherche de l'Institut de gériatrie de Montréal" using a 3T Siemens Magneton TIM Trio magnetic resonance imaging (MRI) system with a 12-channel head coil. Participants were placed in a comfortable position and their head stabilized with foam pads and headphones. Earplugs were also given to reduce the noise from the scanner. The 6 functional runs were separated by one high-resolution anatomical scan of 9 min after the third run. A total of 100 whole-brain volumes were acquired during each functional scan using blood oxygen level-dependent (BOLD) contrast (Ogawa et al., 1990). Each functional volume comprised 40 interleaved axial slices of 3.40 mm thickness parallel to the AC-PC line (in-plane resolution 3.44 × 3.44 mm). Volumes were acquired using a gradient echo, echo-planar (EPI) T2*-weighted sequence (TR = 3000 ms, TE = 30 ms; flip angle = 90°; matrix = 64 × 64; FOV = 220 × 220 mm^2; bandwidth = 2440 Hz/Px). Structural images were acquired using a high-resolution, T1-weighted MP-RAGE sequence (TR = 2300 ms, TE = 2.91 ms; flip angle = 9°; FOV = 256 mm; matrix = 256 × 240; 1 × 1 × 1.2 mm voxels; bandwidth = 240 Hz/Px; 160 slices per whole-brain volume).

2.2.5.1. Image preprocessing. Image analysis was performed with SPM8 (Statistical Parametric Mapping, Version 8; Wellcome Department of Imaging Neuroscience, London, UK), executed in Matlab 7 (Mathworks, Sherborn, Massachusetts). Functional images were first pre-processed with slice-time correction, and motion corrected by realigning all images to the first image using six-parameter rigid body transformation and re-slicing with fourth degree B-spline interpolation. The motion correction parameters were carefully examined for each individual run and led to the complete exclusion of one participant who showed several instantaneous movements exceeding half of the voxel size and reaching a maximum of about 6 mm. Thus, 22 subjects were included in the subsequent statistical analyses. Instantaneous movement was always less than a third of the voxel size in the remaining data (maximum across all participants and runs: x = 0.49 mm, y = 0.43 mm, z = 0.95 mm; pitch = 0.01, roll< 0.01, yaw< 0.01°). In addition, the mean values did not change between baseline and ↑pain intensity runs (p-values of the within-subject comparisons: x = 0.763, y = 0.979, z = 0.221, pitch = .892, roll = 0.354, yaw = 0.835) and did not show significant correlation to the FACS scores of AU 6_7 representing the orbicularis oculi activation (p-values for the correlations were: x = 0.834, y = 0.702, z = 0.205, pitch = .775, roll = 0.547, yaw = 0.273). This ensured that facial responses were not confounded with head motion. The BOLD and structural images were spatially normalized to MNI space using unified segmentation based method, with the normalization parameters determined during the segmentation of the structural images. Spatial smoothing with 6-mm isotropic full width half maximum (FWHM) Gaussian kernel was subsequently applied to the functional images in order to increase signal-to-noise ratio. A high-pass temporal filter (cut-off = 128s) and correction for auto-correlation between successive scans were applied to the time series (AR1).

2.2.5.2. Image statistical analysis. First-level (individual participants) statistical maps were modeled using a canonical hemodynamic response function. Analysis of these data was performed using the general linear

model (GLM) to obtain parameter estimates of stimulus-related activity at each voxel, for each condition, and each subject. Each run was modeled as 3 conditions: "Ramp-up"; "Pain" (including the 5s plateau and the 2s ramp-down), and "Ratings". In order to assess the influence of suggestions for "↑pain intensity" on pain-related BOLD activity, contrasts were obtained by comparing the "Pain" conditions from the "↑pain intensity" runs with the "Pain" conditions from the "baseline" runs ($Pain_{\uparrow intensity} > Pain_{baseline}$). To further account for possible effect of head movements during the scans, the 6 motion correction parameters (3 translational and 3 rotational) were included in the design matrix as nuisance regressors. Additionally, mean signals across voxels from the white matter and the cerebrospinal fluid were also added as covariates of no interest to remove possible physiological noise.

Single-subject contrast images were then used in second-level (group) analyses which included the following:

1. One-sample *t*-test was computed to obtain the group average of suggestion-induced changes in pain-related activation (Avg. $Pain_{\uparrow intensity} > Pain_{baseline}$).
2. Robust regression analyses (Wager et al., 2005) were conducted:
   a) to investigate whether changes in orbicularis oculi activity (AU6_7) can predict activity changes in cortical targets of the spino-thalamo-cortical pathway, especially S1.Predictor: suggestion-induced changes in AU6_7. Criterion: suggestion-induced changes in pain-related brain responses obtained from the first level analysis (ΔAU6_7 x Avg. $Pain_{\uparrow intensity} > Pain_{baseline}$).
   b) to test whether the regression outcomes in a) are specific to AU6_7, and not due to general changes in facial activity, we repeated the regression analysis by controlling for individual changes in "All other AUs" (excluding AU6_7):Predictor: suggestion-induced changes in AU6_7. Criterion: suggestion-induced changes in BOLD responses (ΔAU6_7 ctl "all other AUs" x Avg. $Pain_{\uparrow intensity} > Pain_{baseline}$).

We first examined pain-related responses within bilateral brain areas commonly activated by painful stimuli (for review, see (Apkarian et al., 2005; Duerden and Albanese, 2013)) and targeted by the spinothalamic system in primates (i.e., thalamus, S1, S2, ACC, and INS (Dum et al., 2009)). Given our very specific hypotheses, a directed search based on these previously established pain-related regions was performed to reveal changes in pain-related activity induced by suggestions with a Bonferroni-correction adjusted threshold of p-corrected<0.05 (one-tail) i.e. uncorrected-p < 0.004. In the between-subject regression analyses, since our primary interest lies within S1 (Hofbauer et al., 2001)), a small-volume correction ($p < 0.05$, one tail, FDR-corrected; 15-mm radius sphere) was performed on the right S1 area based on the coordinate of the highest peak of the putative leg area of S1 observed in our previous study using similar noxious thermal stimulation of the leg ($x = 28$, $y = -28$, $z = 62$) and demonstrating changes in pain-evoked activity consistent with the overall pain facial responses observed (Kunz et al., 2011).

3. Connectivity analysis. As a complementary step in identifying brain regions implicated in the effects of increased pain suggestion, we conducted a within-session psycho-physiological interactions (PPI) analyses to examine context-specific changes in S1 functional connectivity throughout the brain during ↑intensity vs. baseline. Volumes from each run were concatenated to create a single time-series volume that contains the BOLD data from each scan, where the between-run conditions (suggestion to ↑intensity, ↑unpleasantness and baseline) are modeled as within-session trials: $Ramp\text{-}Up_{baseline}$, $Pain_{baseline}$, $Ratings_{baseline}$; $Ramp\text{-}Up_{\uparrow intensity}$, $Pain_{\uparrow intensity}$, $Ratings_{\uparrow intensity}$; $Ramp\text{-}Up_{\uparrow unpleasantness}$, $Pain_{\uparrow unpleasantness}$, $Ratings_{\uparrow unpleasantness}$. Session regressors were included to account for inter-scan differences. Contrast of "$Pain_{\uparrow intensity} > Pain_{baseline}$" was used as the effect of interest, and the S1 response specific to AU 6_7 was selected as the seed region ($x = 18$; $y = -30$; $z = 72$).

PPI analysis was conducted using SPM8. First, the time course of the BOLD signal of the S1 VOI from each subject was extracted and then deconvolved using Bayesian estimation to create the time series representing the neural signal in the ROI. The interaction term (PPI regressor) was then generated as the element-by-element product of the task time course ($Pain_{\uparrow intensity} > Pain_{baseline}$) and the deconvolved seed S1 time course, and used as regressors in the subsequent 1st level GLM analysis. The resulting images of contrast estimates showing areas of connectivity to the S1 seed region due to suggestion were entered into the 2nd level random effects group analysis (one-sample *t*-test) to examine both the positive and negative interactions with S1 at a statistical significance level of cluster-wise FDR of 0.05.

3. Results

The mean (±SD) pain threshold across subjects was 45.2 °C (±0.72), and the average stimulation temperature used to induce pain in all functional runs was 48.2 °C (i.e. +3 °C above the individual pain threshold).

3.1. Effect of suggestions for ↑pain intensity on perceptual and neural responses

VAS ratings: Suggestions targeting the sensory dimension of pain resulted in a significant interaction effect between conditions and pain dimension ($F(1,21) = 6.447$; $p = 0.019$). As expected, VAS pain intensity ratings significantly increased in the ↑pain intensity runs (mean 76.9 (SD 9.9)) compared to baseline (mean 71.0 (SD 13.3)) ($p < 0.001$). In contrast, VAS unpleasantness ratings were not affected significantly by these suggestions ($p = 0.261$) (↑pain intensity: mean 59.1 (SD 20.0) vs. baseline: mean 62.7 (SD 18.7)). When directly comparing the changes in pain intensity and unpleasantness ratings (from baseline to ↑pain intensity runs), we also found a significant difference ($T(21) = 4.83$; $p < 0.001$). Thus, suggestions for ↑pain intensity produced selective changes in ratings of pain intensity, as intended.

Brain activation: Functional imaging data analysis contrasting the painful stimulation in the ↑pain intensity runs with baseline runs conditions showed increased activity in S1, cingulate cortex and insula (see supplement 3; Table_B.1). Thus, the suggestions for ↑pain intensity led to an increase in neural activity within areas generally activated by noxious stimulation and associated with pain perception.

3.2. Effect of suggestions for ↑pain intensity on facial responses

Table 1 lists the frequency of occurrence of all Action Units (in percentage) observed separately for the baseline and the ↑pain intensity runs. As can be seen, AU6_7 (contraction of the muscles surrounding the eyes) was the most frequent facial response to pain across runs and participants. More importantly, AU 6_7 was displayed significantly more frequently in the ↑pain intensity compared to the baseline runs, with a large effect size (Cohen's $d = 1.4$). In contrast, no other AU increased in frequency after suggestions for ↑pain intensity.

For further analyses, the combined frequency and intensity values of "AU6_7" and of "All other AUs" were used. Fig. 2 shows the average as well as individual scores for "AU6_7" and "All other "AUs" across baseline and ↑pain intensity runs.[1] Suggestions targeting the sensory dimension of pain resulted in a significant interaction between conditions and type of facial response ($F(1,21) = 13.60$; $p = 0.001$). AU6_7

[1] *The relatively small values for "All other AUs" are due to the fact that this variable represents the average of all Action Units, except AU6_7, and thus, also includes Action Units with low frequency of occurrence.*

Table 1
Comparing the frequency of occurrence of facial Action Units (AUs) in response to painful stimulation between baseline runs and ↑pain intensity runs.

Action Unit		Frequency of occurrence (in %)		Difference in frequency of occurrence baseline < ↑pain intensity	
number	name	baseline	↑pain intensity	p (Wilcoxon)	Effect size (Cohen's d)
1/2	Inner/outer brow raiser	1	4	.527	.42
4	Brow lowerer	24	21	.101	.27
5	Upper lid raiser	5	5	.680	.23
6/7	Contraction of the muscles surrounding the eyes (cheek raiser and lid compressor/lid tightener)	68	100	**.004****	**1.4**
9/10	Nose wrinkler/ upper lip raiser	36	32	.235	.39
12	Lip corner puller	1	2	.391	.36
14	Dimpler	2	2	.713	.09
15	Lip Corner depressor	3	1	.380	.23
16	Lower lip depressor	3	3	1.00	.00
17	Chin raiser	2	2	.257	.29
18	Lip pucker	3	5	.142	.46
22	Lip funneler	2	<1	.129	.49
23	Lip tightener	4	1	.068	.45
24	Lip pressor	2	1	.157	.46
25/26/ 27	Opening of the mouth	37	35	.669	.07
28	Lip suck	3	2	.546	.23
30	Jaw sideways	1	<1	.102	.48
32	Lip biting	1	1	1.00	.08
38	Nostril dilator	1	1	.739	.07
43	Eyes closed	26	25	.975	.07

**$p < 0.01$.

significantly increased in the ↑pain intensity runs compared to baseline (see Fig. 2) ($p < 0.001$), whereas "All other AUs" were not significantly affected by the suggestions ($p = 0.376$).

To confirm that this increase in AU6_7 was specific to the ↑pain intensity runs, we conducted the same type of analysis comparing baseline to the ↑pain unpleasantness runs (the corresponding figure and table can be found in supplement 2). Here, neither AU6_7 ($p = 0.934$) nor "All other AUs" ($p = 0.511$) increased significantly.

3.3. Association between suggestion-induced changes in AU6_7 and changes in brain activation

The magnitude of change in AU6_7 (between ↑pain intensity and baseline runs) was related to an activity increase in target areas of the spino-thalamo-cortical pathway (see Table 2). Most notably, we found a significant positive association between changes in AU6_7 and changes in S1 (putative leg area, corresponding to the stimulation site; see Fig. 3a). This indicates that individuals showing the largest increase in orbicularis oculi activity also showed the largest increase in pain-evoked S1 activity following the suggestions for increased pain intensity. Moreover, changes in AU6_7 were also significantly associated with changes in the putative face area of M1 bilaterally, consistent with facial motor activity.

Significant negative effects were also observed between changes in AU6_7 and brain response to the painful stimuli with notable peaks in the frontal cortex, especially in prefrontal areas (see Fig. 4a and supplement 3). Furthermore, several other brain areas, including cingulate cortex, secondary somatosensory cortex, insula and striatum, also showed a negative association with AU6_7.

3.4. Controlling for changes in all other AUs

Suggestion-induced changes in "All other AUs" was added to the analysis model as a covariate into the regression between changes in AU6_7 and changes in brain activity. The significant positive association between AU6_7 and the putative leg area of S1 was confirmed in this analysis (see Table 3 and Fig. 3b). The association between M1 activity and AU6_7 was no longer significant after controlling for "All other AUs".

Many prefrontal peaks negatively associated with AU 6_7 remained significant after controlling for "All other AUs" (see Fig. 4b and supplement 4 (Table_C.1)). Moreover, suggestion-induced changes in AU6_7 still showed a significant negative association with changes in areas of the cingulate cortex, secondary somatosensory cortex, insula and striatum.

3.5. Brain connectivity analysis (PPI) on the peak S1 response specific to AU6_7 during suggestion to increase pain intensity vs. baseline

We found significant negative associations between S1 and the prefrontal cortex, as well as the caudate nucleus (see Fig. 5, Table 4). No regions showed significant increase in connectivity with S1 under the context of suggestion to increase pain intensity compared to baseline.

4. Discussion

Using suggestions targeting the sensory magnitude of pain resulted in a selective increase in pain intensity ratings, whereas unpleasantness ratings remained unchanged compared to the baseline runs. Moreover, the suggestions also led to significant changes in pain-related brain areas, including S1. With regard to the key variable of interest, namely the facial expression, suggestions for increased pain intensity produced a remarkably selective modulation in the muscles surrounding the eyes (orbicularis oculi muscle), whereas all other facial responses remained unaltered. This selective orbicularis oculi activity increase was significantly associated with changes in S1, which prevailed even when controlling for changes in all other facial responses.

4.1. Using suggestions to modulate the sensory dimension of pain

We used suggestions to specifically increase the sensory dimension of pain and hereby experimentally disentangle the affective and sensory dimensions, which are most often highly correlated. The use of suggestions for increased pain intensity led to a very specific increase in orbicularis oculi activity, which we expected based on our previous study (Kunz et al., 2012). All other facial responses were not affected by the suggestions. Moreover, this increase in orbicularis oculi activity was not observed when using suggestions to selectively increase pain affect, which further supports the specificity of the association between orbicularis oculi activity and pain intensity.

4.2. Positive association between orbicularis oculi activity and brain activation during pain

The most important finding of the present study was that the increase in orbicularis oculi activity (following suggestions for increased pain intensity) was positively associated with changes in primary somatosensory cortex (S1) activity. More precisely, the location of the significant association with orbicularis oculi activity was consistent with the leg area of S1 (the site of pain stimulation). The role of S1 in nociceptive processing has been the source of controversy (Apkarian et al., 2005; Bushnell et al., 1999). Nevertheless, evidence from studies in primates corroborates the role of S1 in nociception, with the spino-thalamo-cortical pathways including a projection to S1 (Dum et al., 2009; Gingold et al., 1991). Moreover, a magnetoencephalography (MEG) study investigating the role of S1 in pain processing in humans (Omori et al., 2013) has shown that the nociceptive input to S1 is

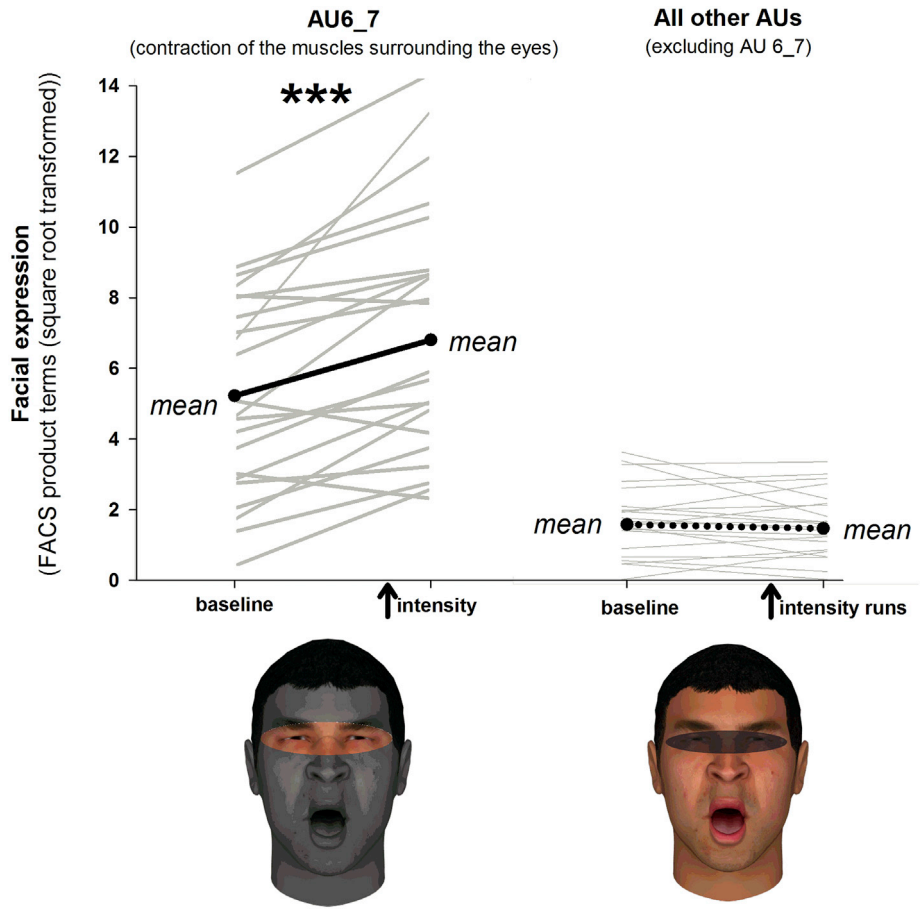

Fig. 2. Facial responses to painful heat stimulation during baseline and ↑intensity (suggestions for increased intensity were given) runs. Individual scores and mean scores are given for AU6_7 and for All other AUs.

Table 2
Robust Regression Analysis: positive associations between suggestion-induced changes (Δ↑ pain intensity vs. baseline) in AU6_7 and in brain activation.

BRAIN AREA	COORDINATES			LOCAL PEAK t-value
	x	*y*	*z*	
Postcentral Gyrus (S1 putative leg area)	18	−30	72	4.32
Precentreal Gyrus (M1 putative face area)	−64	0	26	5.91
	−42	−16	38	6.18
	42	−18	44	3.75
	42	−14	38	3.63
Precentreal Gyrus (M1 putative leg area)	20	−16	74	4.33
Thalamus	4	−6	2	6.74
Superior Temporal	62	−4	2	4.02
	−54	−4	2	4.57
	−64	−8	−4	10.44

FDR-corrected q = 0.05.

somatotopically organized, which (according to the authors) suggests that this area is mainly involved in processing of the sensory/discriminative dimension of pain (Omori et al., 2013). Furthermore, a previous study using positron emission tomography also found significant changes in S1 activity following similar suggestions to alter pain intensity (Hofbauer et al., 2001). Thus, the present finding of a close association between changes in orbicularis oculi and S1 activity strongly supports the idea that the orbicularis oculi activation during pain mirrors the sensory dimension of the pain experience. The specificity of this facial encoding was supported by the fact that the association between orbicularis oculi and S1 activity remained significant even when statistically controlling for changes in all other facial responses.

In an additional step, we performed a functional connectivity analysis (PPI) with S1 as the seed region to reveal areas more or less strongly co-activated with S1 during pain in the increased intensity condition compared to the baseline pain condition. We found negative associations in the prefrontal cortex and the caudate nucleus. This pattern of negative connectivity extends previous findings of our group where we found that frontostriatal activity was inversely associated with the degree of facial expressiveness to pain (Kunz et al., 2011). The more facially expressive a person was, the higher was the activity decrease in the prefrontal cortex and the caudate nucleus, consistent with a role of frontostriatal networks in inhibiting facial displays. Thus, the inverse connectivity between the peak S1 response specific to AU6_7 and the frontostriatal areas (found in the present study) could reflect a suggestion-induced disinhibition of pain responses, especially of orbicularis oculi activity. We elaborate on the role of the frontostriatal network when discussing the negative associations between orbicularis oculi activity and brain activation.

Moreover, changes in orbicularis oculi were also significantly associated with activity increase in the putative face area of M1. This is in accordance with previous findings suggesting that M1 is involved in facial responses (Blair, 2003; Kunz et al., 2011; Morecraft et al., 2004). However, this association was no longer found when controlling for changes in all other facial responses. Thus, as expected, M1 activation seems to be associated with facial responses in general and not specifically with changes in orbicularis oculi activity. Furthermore, suggestion-induced changes in orbicularis oculi were significantly associated with changes in the superior temporal sulcus (STS). We can only speculate about the meaning of this association. Given that the STS has

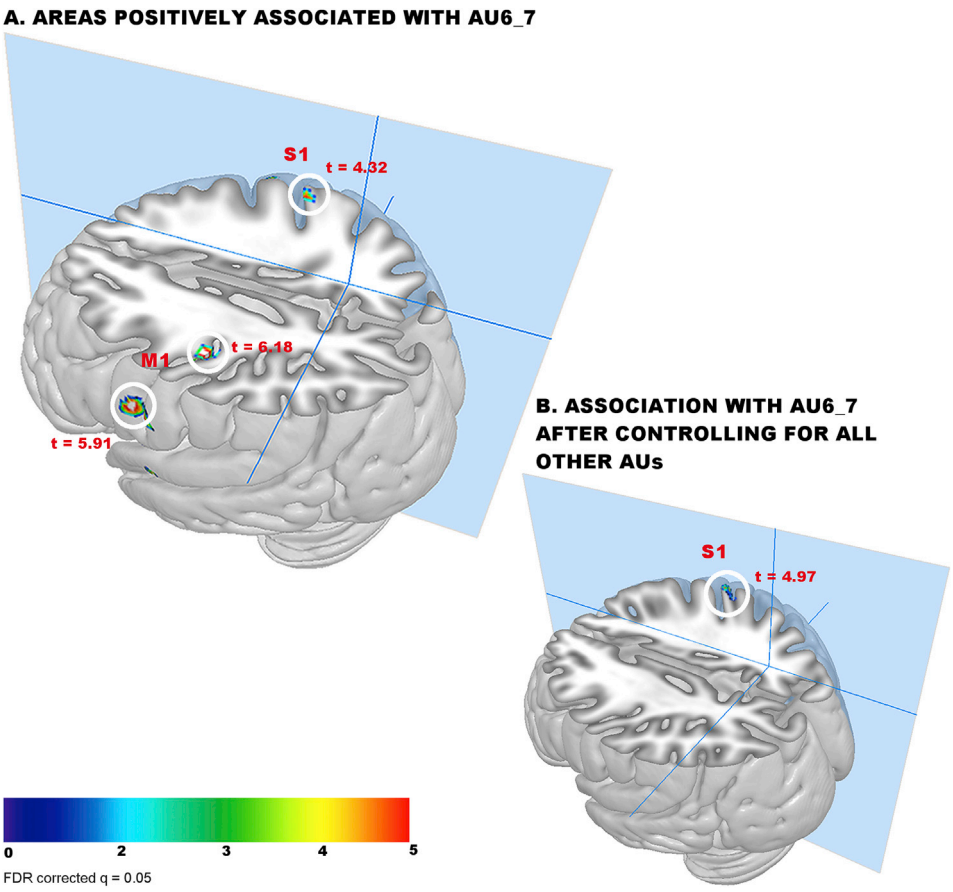

Fig. 3. Suggestion-induced changes in orbicularis oculi activity (AU6_7) predicts changes in pain-associated brain activity.
A. Suggestion-induced changes in AU6_7 positively predict activity changes in S1 and M1. B. When controlling for "All other AUs", the positive association between AU6_7 and S1 remain significant.

been closely linked to voice and speech processing (Rämä et al., 2004), it is possible that the STS activity reflects a trace of memory maintenance of the verbal suggestions given before the trial. Following this line of thinking, the found association might mean that stronger verbal memory maintenance of the suggestions is positively associated with stronger orbicularis oculi increase.

4.3. Negative association between orbicularis oculi activity and brain activation during pain

With regard to negative associations, we found that the suggestion-induced increase in orbicularis oculi activity was inversely correlated with changes in various brain areas brain. Interestingly, among these were brain areas commonly associated with pain processing (e.g. insula, mid cingulate cortex, S2). Thus, finding this clear dissociation of orbicularis oculi activity being positively associated with S1 activity on the one hand while being negatively associated to other pain processing areas on the other hand, further stresses the possibility of a specific positive relationship between orbicularis oculi and S1 activity during pain.

Strong negative associations were also found with activity in frontal areas, especially in the medial prefrontal cortex (mPFC): the more the orbicularis oculi activity increased due to the suggestions, the greater the decrease in mPFC activity-even after statistically controlling for changes in all other facial responses. As mentioned above (PPI analysis) this observation replicates findings from our previous studies using fMRI (Kunz et al., 2011) as well as repetitive transcranial magnetic stimulation (rTMS) (Karmann et al., 2016). In these studies, the mPFC was shown to play a key role in down-regulating facial responses to painful stimuli. The down-regulation of facial responses to pain is believed to be socially learned (Craig et al., 2001; Hadjistavropoulos et al., 2011; Karmann et al., 2014; Kunz et al., 2018). Whereas during the first months of life, facial expressions are rather stimulus-driven and reflexive (Grunau and Craig, 1987), children gradually learn to regulate their facial expressions according to social display rules (Larochette et al., 2006). This is accompanied by a maturation of prefrontal areas, which continues from early childhood up to late adolescence (Fuster, 2001). Thus, the maturation of prefrontal areas might facilitate or promote the development of facial expression control. In addition, we also found negative associations between orbicularis oculi and other brain areas, including the cingulate cortex (e.g. mid cingulate) and the striatum (e.g. putamen, caudate nucleus). This is in line with findings from a recent review article on the role of the prefrontal cortex in emotion (Dixon et al., 2017). The authors propose that inhibition of overt emotional responses (like facial expressions) involve the cingulate cortex in connection with the rostral medial prefrontal cortex (rmPFC). Moreover, if this regulation is overtrained (a plausible assumption for the regulation of facial expressions), this process should also be associated with the recruitment of the dorsolateral striatum (Dixon et al., 2017). Thus, our finding of a negative association between suggestion-induced changes in orbicularis oculi and changes within this "medial prefrontal - cingulate - striatal" - network, suggests that this emotion-expression governing system reduces its inhibitory control in response to the suggestion of more pain intensity.

4.4. Orbicularis oculi activity during pain

In general, the activation of the orbicularis oculi muscle serves several purposes; it ensures protection and moistening of the eyes, but also plays

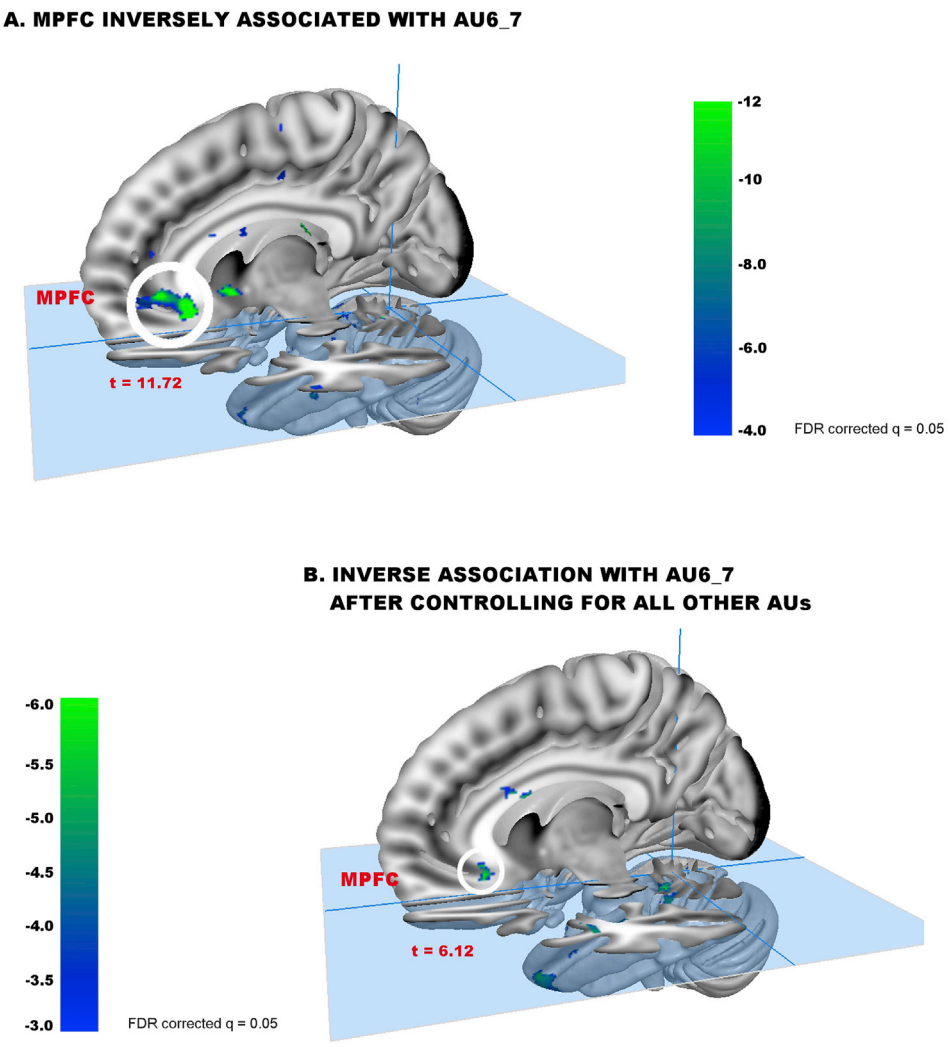

Fig. 4. Suggestion-induced changes in orbicularis oculi activity (AU6_7) are negatively associated with changes in prefrontal areas.
A. Suggestion-induced changes in AU6_7 negatively predict activity changes in medial prefrontal cortex (mPFC). B. When controlling for "All other AUs", the negative association between AU6_7 and mPFC remain significant.

Table 3
Robust Regression Analysis: positive associations between suggestion-induced changes (Δ↑ pain intensity vs. baseline) in AU6_7 and in brain activation after controlling for "All other Action Units".

BRAIN AREA	COORDINATES			LOCAL PEAK t-value
	x	*y*	*z*	
Postcentral Gyrus (S1 putative leg area)	22	−30	74	4.97[a]
OTHER AREAS				
Superior Frontal	18	2	70	4.42
Superior Temporal	−64	−8	−4	5.24

FDR-corrected q = 0.05.
[a] FDR-corrected at q = 0.05 (p = 0.00067) within right S1 ROI. The ROI for the right S1 was defined as the intersection between the S1 (broadmann areas 1, 2 and 3) mask in the Juelich Histological Atlas, and a 15-mm radius sphere centered at the seed S1 (28, −28, 62) region from our previous study (Kunz et al., 2011).

an important role in emotion expression. It is one of the key facial responses in the expression of happiness (Ekman and Friesen, 1982; Wolf et al., 2005), disgust (Kunz et al., 2013; Wolf et al., 2005) and anger (Kohler et al., 2004). Thus, activation of this muscle is not specific to the experience of pain. However, in the context of pain, activity of the orbicularis oculi muscle has been discussed to reflect efforts to protect the eyes (by narrowing the opening) while simultaneously still maintaining enough vision to engage in protective behavior if necessary (Craig et al., 2001). Non-verbal pain behavior, including facial responses to pain, has been suggested to serve two main purposes: a pain management/protection function and/or a communicative function (Prkachin, 1986). The close association of the orbicularis oculi activity with the sensory dimension of pain combined with the assumption that this movement reflects efforts to protect the eyes from noxious stimuli, suggests that orbicularis oculi activity might mainly serve a pain protection function. In contrast, other pain-indicative facial responses (e.g. contraction of the eyebrows, lifting the upper lip) might mainly serve a communicative function. Consistent with this assumption, observers do not rely on orbicularis oculi when differentiating pain from other emotions, but on the frown line and the mouth area (thus, demonstrating the communicative function of these areas (Blais et al., 2019; Roy et al., 2015)).

4.5. Limitations

In the present study we used experimental heat stimuli to induce pain and it cannot be ruled out that our findings would have been different if clinical pain conditions or other pain modalities had been investigated. Moreover, our sample was highly selective. We only included individuals

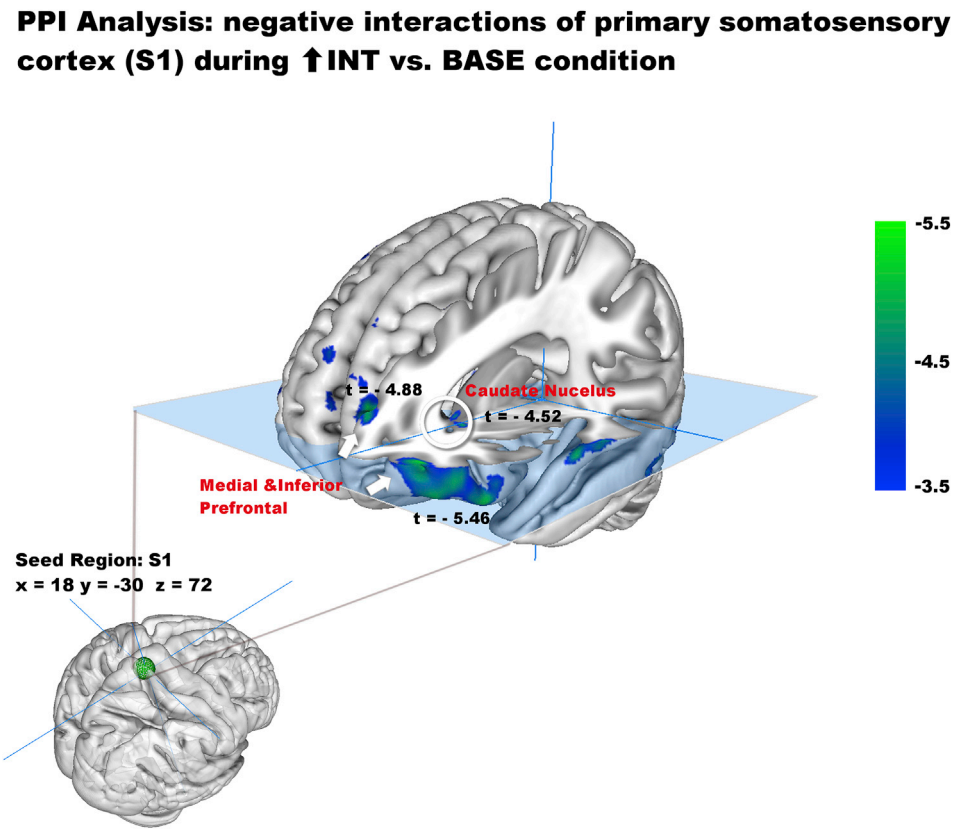

Fig. 5. PPI analysis on the peak S1 response specific to AU6_7 during increased pain intensity vs. baseline condition.
PPI analysis. The psychological variable for the interaction is the contrast between the suggestion for increased pain intensity and the baseline condition (INTvsBASE).
The right S1, where response was higher during suggestion to increase pain intensity in subjects with higher AU6_7 scores, served as the physiological variables (seed region) for the PPI. Areas in the prefrontal cortex, together with the caudate nucleus (marginal significance) exhibit higher decrease in connectivity (negative association) with S1 seed region (cluster-level threshold FDR of q = 0.05.
Images are shown at p = 0.001 uncorrected for display purposes).

Table 4
PPI analysis of S1 during ↑ INT vs. BASE condition.

BRAIN AREA	x	COORDINATES y	z	LOCAL PEAK t-value
Negative Association*	−48	46	−16	−5.46
Lat. Inferior Frontal	−32	58	−4	−4.88
Caudate Nucleus	−12	16	−2	−4.52**

FDR-corrected at q = 0.05 cluster threshold (p = 7.8021e-06).
** Marginal significance (p = 0.0003039).

who were both suggestible and facially responsive to pain, and thus, we cannot know to what extent findings can be generalized to the population at large and to other experimental and clinical contexts. These limitations are inherent to a proof-of-concept study establishing a plausible functional association but not intended to test generalizability.

5. Conclusion

Our findings provide evidence that the contractions of the muscles surrounding the eyes mirror specifically the sensory dimension of pain. These results are consistent with the notion that the facial expression of pain differentially encodes the multi-dimensional pain experience that reflects partly the activity of the spino-thalamo-cortical pathway targeting the primary somatosensory cortex. This study further demonstrates the importance of understanding facial expressions of pain as a meaningful output channel that provides some valid information about central nociceptive processes and is dependent on the regulatory control exerted by fronto-striatal circuits.

Authorcontribution

MK and PR designed the study. MK and JIC conducted the study and analyzed the data. MK, JIC and PR all wrote the article together.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neuroimage.2020.116885.

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