Step-down vs. step-up noxious stimulation: differential effects on pain perception and patterns of brain activation

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Background: We hypothesize that pain and brain responses are affected by changes in the presentation sequence of noxious stimuli that are, overall, identical in intensity and duration.

Methods: During functional magnetic resonance imaging (fMRI) scanning, 21 participants experienced three patterns of noxious stimulation: Up-type (step-up noxious stimulation, 15 s), Down-type (step-down noxious stimulation, 15 s), and Down–up-type (decreasing and increasing pattern of noxious stimulation, 15 s). The total intensity and duration of the three noxious stimulation patterns were identical, but the stimulation sequences were different.

Results: Pain and unpleasantness ratings in the Down- and Down–up-type noxious stimulations were lower than in the Up-type noxious stimulation. The left prefrontal cortex [(PFC, BA (Brodmann area) 10, (−45, 50, 1)] was more highly activated in the Down- and Down–up-type noxious stimulations than in the Up-type noxious stimulation. The S1, S2, insula, bilateral PFC (BA 46), and midcingulate cortex were more highly activated in the Up-type noxious stimulation than in the Down-type noxious stimulation. PFC BA 10 was located at an inferior level compared to the bilateral PFC BA 46 (Z axis = 1 for BA 10, compared to 22 and 25 for the right and left BA 46, respectively). When cortisol level was increased, the left hippocampal cortex, along with the left parahippocampal cortex, was greatly activated for the Up-type noxious stimulation.

Conclusion: When pain cannot be avoided in clinical practice, noxious stimuli should be applied to patients in a step-down pattern that delivers the most intense pain first and the least intense pain last.

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Editorial comment: what this article tells us
Aversiveness and memory of pain are influenced by the time course of pain during painful procedures. This study showed that unpleasantness ratings were lower and brain activation patterns indicated less pain, with the more intense pain first.

Pain is a dynamic phenomenon, as evidenced by the continual change in nociceptive perception during surgery and medical procedures.[1] This suggests that pain may change according to the intensity and duration of noxious stimulation. Previous study has shown that less intense pain at the end of a painful procedure leads subjects to remember less intense overall pain.[2] In addition, adding moments of more intense pain to the end of an episode can make the episode worse, while adding moments of less intense pain can make it better.[2] This indicates that memories of an episode are dominated by its final moments. However, noxious stimulation duration and noxious intensity were different in the short and long trials in a previous study.[2] We therefore investigated the effects of changes in noxious stimulation sequence on pain perception and the brain's modulatory system using identical noxious intensity and noxious stimulation duration.

One noxious stimulation method that leads to pain reduction is known as offset analgesia. Offset analgesia is a reduction in perceived pain using a small incremental decrease in noxious stimuli.[3,4] However, previous studies have compared two noxious stimulation methods with different total noxious intensities.[3,5] In those studies, a small incremental decrease in noxious stimulation was compared to constant noxious stimulation. Constant noxious stimulation may cause temporal summation or adaptation that changes pain perception when noxious heat is continuously applied to the skin.[3,6] Therefore, comparison between a small incremental decrease in noxious stimulation and constant noxious stimulation is not suitable, because the two noxious stimulation methods have completely different noxious intensities. A comparison between two noxious stimulation methods should be based on identical noxious intensity and duration. We hypothesized that pain and brain responses are modulated by changes in noxious stimulation sequence for stimuli of identical intensity and duration. If identical intensity of noxious stimulation is perceived as different pain sensation when the stimulation sequences are different, noxious stimulation should be applied in the sequences that pain perception can be decreased when pain cannot be avoided in clinical practice.

Pain perception may be changed by testosterone and cortisol levels.[7–12] During stress, cortisol level is negatively correlated with pain threshold, and testosterone level is positively correlated with pain threshold.[7] This indicates that hormone levels can influence an individual's susceptibility to pain. Therefore, we hypothesized that pain and brain responses to thermal noxious stimulation are modulated by hormones.

Methods

Participants

Twenty-five participants (12 men and 13 women) were recruited for participation in this study. Twenty-one participants (10 men and 11 women) were included in the results, because two men and two women had to be excluded due to excessive head movement (Table S1). Age did not differ between men and women (Table S1). Each participant was paid for participation. All participants provided written informed consent acknowledging the following: (1) they would experience experimental thermal pain; (2) no tissue damage would result from this pain; (3) all the methods and procedures were clearly explained; and (4) they were free to withdraw from the experiment at any time. The Medical Ethics Committee of Yonsei University, Wonju College of Medicine approved this study. Participants with peripheral and central nervous system disease or any other significant clinical conditions were excluded, as were participants using medications that could affect sensory perception, such as neuropsychotropics or analgesics.

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fMRI scanning

Noxious thermal stimuli were delivered with a computerized thermal contact stimulator (CHEPS, Medoc Advanced Medical Systems Ltd., Ramat Yishai, Israel) with a 27-mm-diameter thermode. The thermode was attached to the medial aspect of the left lower leg skin using a Velcro strap. In the previous study, temperatures less than 46°C (44.5, 45, 45.5, and 46°C) are used as low noxious thermal stimulations.[13] It is known that the normal pain thresholds for a hot stimulus are 44–47°C. Therefore, we used temperatures less than 46°C as noxious stimulation.[14,15] The temperature of the thermode in three noxious stimulation patterns was increased from baseline (32°C) to the target temperature at 25°C/s. The target temperature was maintained for 15 s and then returned to 32°C at 25°C/s. Target temperatures of the three noxious stimulation types were as follows: Up-type noxious stimulation pattern (15 s) = 44.5°C for the first 5 s + 45°C for the second 5 s + 46°C for the third 5 s; Down-type noxious stimulation pattern (15 s) = 46°C for the first 5 s + 45°C for the second 5 s + 44.5°C for the third 5 s; and Down–up-type noxious stimulation pattern (15 s) = 46°C for the first 5 s + 44.5°C for the second 5 s + 45°C for the third 5 s. Participants were told that three noxious stimulation types would be applied during fMRI scanning (Fig. 1). The intensity and duration of the three sets of noxious stimuli were identical, but the sequence of stimulus presentation varied.

The present pain experiment was composed of three runs. Three noxious stimulation patterns were randomly repeated 10 times during each run. The three runs were randomly applied to participants (Fig. 1). During scanning, visual cues were projected onto a screen located in the MRI console. Participants viewed these through a mirror mounted on a head-coil. A red cross was displayed 2 s prior to noxious stimulation, indicating that noxious stimulation was to begin. Participants then received noxious stimulation (15 s), followed by a resting period (30 s, white cross). Visual cues of 15-s noxious stimulation for the three noxious stimulation patterns were the following: Up-type, ↑; Down-type, ↓; Down–up-type, ↕. This 47-s (2 + 15 + 30) block was repeated 10 times in one run. To familiarize participants, the noxious stimulus and visual cues were presented before the experiment began. To minimize the possibility of habituation or sensitization, the thermode was moved a short distance to the adjacent medial left lower leg skin between runs.

A Up-type pain stimulation pattern

Cueing (2 s)	Pain (15 s)	Rest (30 s)
No pain	44.5 °C (1st 5 s) + 45°C (2nd 5 s) + 46°C (3rd 5 s)	No pain

B Down-type pain stimulation pattern

Cueing (2 s)	Pain (15 s)	Rest (30 s)
No pain	46°C (1st 5 s) + 45°C (2nd 5 s) + 44.5°C (3rd 5 s)	No pain

C Down-up-type pain stimulation pattern

Cueing (2 s)	Pain (15 s)	Rest (30 s)
No pain	46°C (1st 5 s) + 44.5°C (2nd 5 s) + 45°C (3rd 5 s)	No pain

Fig. 1. Study protocol. Total noxious intensity in the three stimulation patterns was identical, but the stimulation sequence was varied. This pain experiment was composed of three runs. Three noxious stimulation patterns [Up-type noxious stimulation (UTS), Down-type noxious stimulation (DTS), and Down–up-type noxious stimulation (DUTS)] were randomly repeated 10 times during each run. The sequences of the three runs were as follows: DUTS-DTS-DTS-UTS-DTS-UTS-DUTS-DTS-UTS-DUTS; UTS-DUTS-DTS-UTS-DUTS-UTS-DUTS-DTS-UTS-DTS; DTS-DUTS-UTS-DUTS-DUTS-UTS-DTS-DTS-UTS-DUTS. The three runs were randomly applied to participants. A red cross was displayed 2 s prior to noxious stimulation; then, participants received noxious stimulation (15 s), followed by a resting period (30 s). This 47-s (2 + 15 + 30) block was repeated 10 times during each run.

At the completion of fMRI scanning, participants were asked to rate their average pain and unpleasantness during each run. The final average pain and unpleasantness ratings of the three noxious stimulation patterns were calculated as the mean of pain and unpleasantness ratings during the three runs. Ratings were assessed using a numerical rating scale where 0 = no pain and unpleasantness and 100 = maximum imaginable pain and unpleasantness.

Hormones

To measure testosterone and cortisol levels, venous blood samples were drawn from an antecubital vein with a 21-gauge needle. Participants were instructed to avoid eating for 2 h before blood sampling. Blood samples were collected before fMRI scanning. Testosterone

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and cortisol levels were quantified using the COBRA 5010 Quantum γ-counter (Packard, Meriden, CT, USA) with Coat-A-count Testosterone (Siemens, Los Angeles, CA, USA) and Coat-A-count Cortisol (Siemens) kits.[10] The intra- and inter-assay coefficients of variation were 5.5% and 6.3% for testosterone and 5.5% and 6.3% for cortisol, respectively.

Statistical analyses of behavioral and hormonal data

In choosing the sample size, we relied on two previous fMRI studies in which brain activation was measured.[16,17] These were performed using 20 patients, a sample size that was exceeded in the present study. Group data were analyzed using repeated measures ANOVA with PASW (Predictive Analytics Software) Statistics version 20 (SPSS Inc., Chicago, IL, USA). Post hoc tests were performed using the Bonferroni correction. Differences between men and women were analyzed using an independent samples *t*-test. Pearson correlation coefficients were calculated. $P < 0.05$ was considered statistically significant.

Image acquisition

Before scanning, participants were instructed to stay awake and to refrain from moving throughout the imaging session. After being placed in a comfortable position, the head was immobilized with padded ear muffs and a foam headrest, and a plastic bar was placed across the bridge of the nose. MRI data were acquired using a 3T MRI scanner (Philips Medical Systems, Best, The Netherlands). Functional images were acquired using echo-planar imaging with the following imaging parameters: echo time = 35 ms, repetition time = 3000 ms, flip angle = 90°, matrix size = 128 × 128, field of view = 220 × 220 mm^2, voxel size = 1.72 × 1.72 × 4.5 mm^3, gap = 0.5 mm, and slice thickness = 4 mm. For most participants, 33 slices were acquired to include the entire brain volume; 35 slices were acquired in one participant. A structural T1-weighted image was obtained using a gradient echo sequence (echo time = 4.6 ms, repetition time = 9.9 ms, flip angle = 8°, matrix size = 220 × 220, field of view = 220 × 220 mm^2, and voxel size = 1 mm^3).

Statistical analyses of fMRI data

Preprocessing and basic statistical analyses were conducted using Statistical Parametric Mapping (SPM8; Wellcome Department of Imaging Neuroscience, University College, London). Functional volumes for three runs were concatenated, corrected for slice timing, realigned, normalized (resampling voxel size, 3 × 3 × 3 mm), and smoothed (Gaussian kernel, 8 × 8 × 8 mm). A high-pass filter (cutoff: 128-s period) and an autocorrelation correction were applied to the resulting time series.

Preprocessed images were analyzed using a general linear model (GLM) in which three 15-s noxious stimulation events in each of three noxious stimulation patterns were modeled using a canonical hemodynamic function. In addition, six movement parameters and session means were included as covariates. Analyses were performed for each individual, and resulting contrast images were entered into second-level analyses, treating the participant as a random effect. To prevent false positives, we used a statistical criterion of 22 or more continuous voxels with a voxel-wise uncorrected threshold of $P < 0.001$, which corresponds to an experiment-wise threshold of $P < 0.05$, corrected for multiple comparisons. This threshold was applied to all statistical analyses of fMRI data in this study.[18] We used AlphaSim in REST software (http://restfmri.net/forum/REST) to correct for multiple comparisons to $P < 0.05$. The AlphaSim (REST toolbox implementation) employs Monte Carlo simulations for the control of type I and II errors. This program was run by the mask that was used to analyze the present fMRI data in SPM8.

To investigate the relationships between brain activation and covariates for the Up-type noxious stimulation, we performed a multiple regression analysis using SPM8. Cortisol (first regressor) and testosterone/cortisol ratio (second regressor) were included as regressors. When cortisol level was increased [contrast (0 1 0)], brain activation areas in the Up-type noxious stimulation were identified. When the testosterone/cortisol ratio was decreased [contrast (0 0 −1)], brain activation areas in the Up-type noxious stimulation were identified.

The time series analysis of three noxious stimulation patterns was calculated using MarsBaR

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[radius of ROI (region of interest) = 5 mm] (http://marsbar.sourceforge.net/). Using a repeated measures ANOVA test, average % MR signal changes were compared among three noxious stimulation patterns between 0 and 30 s after onset of noxious stimulation [noxious stimulation period (0–15 s); resting period (15–30 s)]. Although we recognize the danger of double dipping,[19] this ROI analysis is performed to graphically explain the time series of brain activations to those who are relatively unfamiliar with brain imaging studies.

Results

Behavioral and hormonal data

Testosterone, cortisol, and T/C ratio were higher in men than in women (Table S1). Average pain and unpleasantness ratings in three noxious stimulations were significantly lower in men than in women (Table S2).

In a repeated measures ANOVA test of behavioral data (Table 1), average pain ratings differed significantly among Up-, Down-, and Down–up-types [$F(2, 40) = 88.20$, $P < 0.001$]. Post hoc tests using the Bonferroni correction revealed that pain ratings in the Up-type noxious stimulation were significantly higher than those in the Down- and Down–up-type noxious stimulations. However, there was no significant difference between the Down- and Down–up-type noxious stimulations. Average unpleasantness ratings differed significantly among Up-, Down-, and Down–up-types [$F(2, 40) = 80.127$, $P < 0.001$]. Post hoc tests using the Bonferroni correction revealed that unpleasantness ratings in the Up-type noxious stimulation were significantly higher than those in the Down- and Down–up-type noxious stimulations. However, there was no significant difference between the Down- and Down–up-type noxious stimulations.

The testosterone/cortisol (T/C) ratio was negatively correlated with average pain rating in the three noxious stimulation patterns [Up-type ($r = -0.704$, $P < 0.001$), Down-type ($r = -0.571$, $P = 0.007$), Down–up-type ($r = -0.506$, $P = 0.019$)]. The testosterone/cortisol (T/C) ratio was also negatively correlated with average unpleasantness rating in the three noxious stimulation patterns [Up-type ($r = -0.684$, $P = 0.001$), Down-type ($r = -0.570$, $P = 0.007$), Down–up-type ($r = -0.543$, $P = 0.011$)].

Table 1 Average pain and unpleasantness ratings measured during the 15-s pain period for the three noxious stimulation types.

Ratings	Ratings	*P* value
Pain ratings		
Up-type (87.25 ± 14.85)	Down-type (63.97 ± 18.48)	< 0.001
Up-type (87.25 ± 14.85)	Down–up-type (68.38 ± 16.95)	< 0.001
Down-type (63.97 ± 18.48)	Down–up-type (68.38 ± 16.95)	0.085
Unpleasantness ratings		
Up-type (86.87 ± 14.54)	Down-type (64.00 ± 17.78)	< 0.001
Up-type (86.87 ± 14.54)	Down–up-type (67. 54 ± 17.31)	< 0.001
Down-type (64.00 ± 17.78)	Down–up-type (67. 54 ± 17.31)	0.322

Data shown are mean ± SD. Data were analyzed using a repeated measures ANOVA test. Up-type = step-up noxious stimulation. Down-type = step-down noxious stimulation. Down–up-type = decreasing and increasing pattern of noxious stimulation.

fMRI data

There was no difference in brain activation between men and women in the two sample *t*-test or repeated measures ANOVA in the threshold selected in this study.

Repeated measures ANOVA

The primary somatosensory cortex (S1), secondary somatosensory cortex (S2), insula, midcingulate cortex (MCC), and frontal cortex were activated in repeated measures ANOVA (Table S3). The S1, S2, insula, PFC (prefrontal cortex, BA 46), MCC, and dorsal anterior cingulate cortex (dACC) were more highly activated in the Up-type noxious stimulation than in the Down-type noxious stimulation (Table 2). The left lateral frontopolar cortex [BA 10, (−45, 50, 1)] was more highly activated in the Down–up-type noxious stimulation than in the Up-type noxious stimulation (Table S4).

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Table 2 Brain areas that were more highly activated in the Up-type noxious stimulation than in the Down-type noxious stimulation.

Region of activation	Voxels	Coordinates	*T* value
Rt precentral cortex	1002	9, −13, 70	5.71
Rt supplementary motor cortex, MCC including dACC		3, −1, 49	5.55
Rt S1		12, −31, 73	5.41
Lt dorsolateral prefrontal cortex (BA 46)	73	−30, 47, 25	5.19
Lt central opercular cortex	84	−57, 5, 1	4.90
Lt insula		−48, 2, 4	4.35
Rt central opercular cortex including insula (45, 1, 2)	421	51, 2, 4	4.87
Rt S2		60, −19, 19	4.57
Lt S2	150	−63, −25, 16	4.71
Rt dorsolateral prefrontal cortex (BA 46)	29	30, 50, 22	4.43

Coordinates = peak MNI coordinates (x, y, z). MCC = midcingulate cortex. dACC = dorsal anterior cingulate cortex. S1 = primary somatosensory cortex. S2 = secondary somatosensory cortex. Voxels = number of voxels. The brain areas that do not have listed voxels are included in the cluster. Up-type noxious stimulation = step-up noxious stimulation. Down-type noxious stimulation = step-down noxious stimulation.

Multiple regression analysis of fMRI data

When cortisol level was increased, the left hippocampal cortex, along with the left parahippocampal cortex was greatly activated for the Up-type noxious stimulation [left (−21, −13, −20), 3.87 (*t*-value), 37 (voxel)].

Percentage MR signal change in fMRI data

These findings are shown in Fig. 2 and in the supplementary materials.

Percentage MR signal change in the left PFC

In a time series analysis of three noxious stimulation patterns using MarsBaR, a repeated measures ANOVA determined that percentage signal change in the left prefrontal cortex differed among the three noxious stimulation patterns (Fig. 2A and Table S5–S7, radius of ROI = 5 mm, (−45, 50, 1) and supplementary materials). Post hoc tests revealed that between 9 and 12 s after onset of noxious stimulation, percentage signal change was higher in the Down–up-type noxious stimulation than in the Up-type noxious stimulation. Between 12 and 15 s, percentage signal change in the Down- and Down–up-type noxious stimulation patterns was significantly higher than that in the Up-type noxious stimulation pattern. Between 15 and 18 s, percentage signal change in the Down- and Down–up-type noxious stimulation patterns was significantly higher than that in the Up-type noxious stimulation pattern. This means that, between 12 s and 18 s, activation of the left prefrontal cortex in the Down- and Down-up-type noxious stimulation patterns was greater than in the Up-type noxious stimulation pattern. This data indicate that activation of the left prefrontal cortex localized around the coordinate (BA 10, −45, 50, 1) might lead to decreased pain ratings in the Down- and Down–up-type noxious stimulation patterns compared to the Up-type noxious stimulation pattern.

Percentage MR signal change in the left PFC between 18 and 21 s after onset of noxious stimulation

Percentage signal change in the left prefrontal cortex between 18 and 21 s differed among the three noxious stimulation patterns [$F(2, 40) = 3.905$, $P = 0.028$], shown in Fig. 2B and Table S8, radius of ROI = 5 mm, (−30, 47, 25). Post hoc tests revealed that percentage signal change in the Up-type noxious stimulation pattern was significantly higher than that in the Down-type noxious stimulation pattern. This indicates that, between 18 and 21 s, activation of the left prefrontal cortex in the Up-type noxious stimulation pattern was greater than in the Down-type noxious stimulation pattern. This suggests that activation of the left prefrontal cortex localized around coordinate (BA 46, −30, 47, 25) might lead to increased pain ratings in the Up-type noxious stimulation pattern compared to the Down-type noxious stimulation pattern.

Percentage MR signal change in the right PFC between 18 and 21 s after onset of noxious stimulation

Percentage signal changes in the right prefrontal cortex between 18 and 21 s differed among the three noxious stimulation patterns [$F(2, 40) = 3.345$, $P = 0.045$], shown in Fig. 2C and Table S9, radius of ROI = 5 mm, (30, 50, 22).

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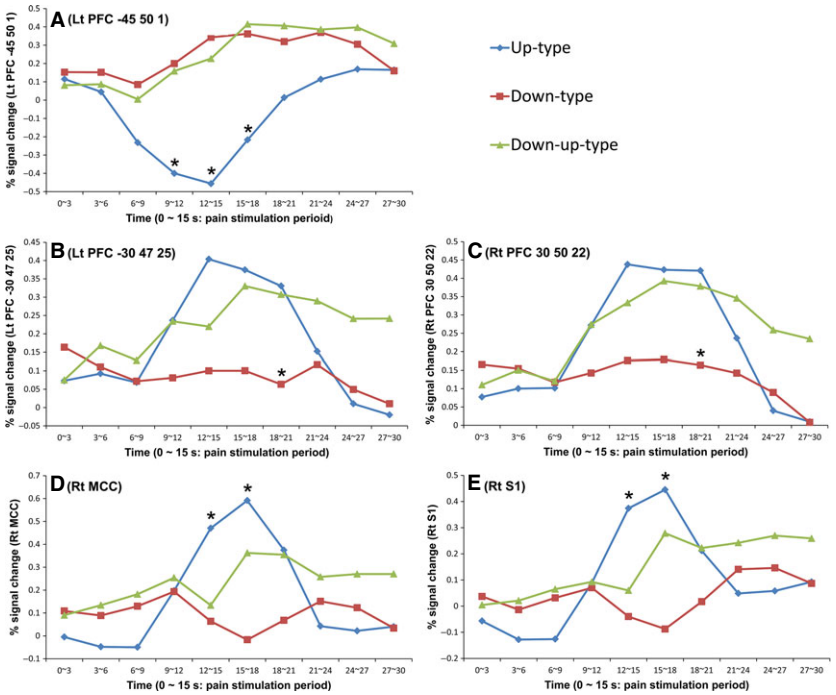

Fig. 2. Average% MR signal change [noxious stimulation period (0–15 s); resting period (15–30 s)] (supplementary materials). (A) [Percentage MR signal change in the left PFC (−45, 50, 1)]. 9 s ~ 12 s (Down–up-type > Up-type; This indicates that, between 9 and 12 s, activation of the left prefrontal cortex in the Down–up-type noxious stimulation was greater than in the Up-type noxious stimulation), 12–15 s (Down- and Down–up-types > Up-type), 15–18 s (Down- and Down–up types > Up-type). (B) [Percentage MR signal change in the left PFC (−30, 47, 25)]. 18–21 s (Up-type > Down-type). (C) [Percentage MR signal change in the right PFC (30, 50, 22)]. 18–21 s (Up-type > Down-type). (D) [Percentage MR signal change in the right MCC (6, −4, 43)]. 12–15 s (Up-type > Down-type), 15–18 s (Up- and Down–up-types > Down-type). (E) [Percentage MR signal change in the right S1 (15, −40, 67)]. 12–15 s (Up-type > Down- and Down–up-types), 15–18 s (Up- and Down–up-types > Down–up-type). Stars indicate $P < 0.05$ when post hoc tests were conducted using the Bonferroni correction. Up-type = step-up noxious stimulation. Down-type = step-down noxious stimulation. Down–up-type = decreasing and increasing pattern of noxious stimulation. PFC = prefrontal cortex, MCC = midcingulate cortex, S1 = primary somatosensory cortex, Lt = left, Rt = right.

Post hoc tests revealed that percentage signal change in the Up-type noxious stimulation pattern was significantly higher than those in the Down-type noxious stimulation pattern. This indicates that, between 18 and 21 s, activation of the right prefrontal cortex in the Up-type noxious stimulation pattern was greater than in the Down-type noxious stimulation pattern. This suggests that activation of the right prefrontal cortex localized around coordinate (BA 46, 30, 50, 22) might lead to increased pain ratings in the Up-type noxious stimulation pattern compared to the Down-type noxious stimulation pattern. Similar PFC areas in both hemispheres [BA 46, left (−30, 47, 25), right (30, 50, 22)] were more highly activated in the Up-type noxious stimulation pattern compared to the Down-type noxious stimulation pattern.

Percentage MR signal change in the right midcingulate cortex (MCC)

A repeated measures ANOVA determined that percentage signal change in the right MCC differed among the three noxious stimulation patterns, as shown in Fig. 2D, Table S10 and S11, radius of ROI = 5 mm, (6, −4, 43). Post hoc tests revealed that between 12 and 15 s after onset of noxious stimulation, percentage signal change in the Up-type noxious stimulation pattern was significantly higher than that in the Down-type noxious stimulation pattern. Between 15 and 18 s, percentage signal change in the Up- and Down–up-type noxious stimulation patterns was significantly higher than that in the Down-type noxious stimulation pattern. This means that, between 12 s and 18 s, activa-

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tion of the right MCC in the Up-type noxious stimulation pattern was greater than that in the Down-type noxious stimulation pattern. This data indicate that activation of the right MCC might lead to increased pain ratings in the Up-type noxious stimulation pattern compared to the Down-type noxious stimulation pattern.

Percentage MR signal change in the right S1

A repeated measures ANOVA determined that percentage signal change in the right S1 differed among the three noxious stimulation patterns, as shown in Fig. 2E, Table S12 and S13, radius of ROI = 5 mm, (15, −40, 67). Post hoc tests revealed that between 12 and 15 s after onset of noxious stimulation, percentage signal change in the Up-type noxious stimulation pattern was significantly higher than that in the Down- and Down–up-type noxious stimulation patterns. Between 15 and 18 s, percentage signal change in the Up- and Down–up-type noxious stimulation patterns was significantly higher than that in the Down noxious stimulation pattern. This indicates that, between 12 and 18 s, activation of the right S1 in the Up-type noxious stimulation pattern was significantly greater than in the Down-type noxious stimulation pattern. Therefore, pain ratings might be higher in the Up-type noxious stimulation patterns than in the Down-type noxious stimulation pattern.

Discussion

Pain and unpleasantness ratings in the Down- and Down–up-type noxious stimulations were lower than those in the Up-type noxious stimulation. The brain areas that are usually activated by noxious stimulation were more highly activated in the Up-type noxious stimulation than in the Down-type noxious stimulation. The left PFC located around coordinate (BA 10, −45, 50, 1) was more highly activated in the Down- and Down–up-type noxious stimulations than in the Up-type noxious stimulation.

Although the prefrontal cortices are activated in both Down- [(PFC, BA 10, (−45, 50, 1)] and Up-type [BA 46, (30, 50, 22), (−30, 47, 25)] noxious stimulations, the PFC (BA 10) activated in the Down-type noxious stimulation was located at an inferior level compared to those (BA 46) activated in the Up-type noxious stimulation (*Z* axis = 1 for BA 10, compared to 22 and 25 for the right and left BA 46, respectively). Pain and unpleasantness ratings in the Down- and Down–up-type noxious stimulations were significantly lower than those in the Up-type noxious stimulation. The lateral PFC has been implicated in endogenous pain inhibition.[20] The Down-type noxious stimulation may result in higher activation of the left PFC (−45, 50, 1), leading to decreased pain perception in the Down- and Down–up-type noxious stimulations compared to the Up-type noxious stimulation.

The S1, S2, and bilateral midinsula were significantly activated in the Up-type noxious stimulation compared to the Down-type noxious stimulation. Pain and unpleasantness ratings in the Up-type noxious stimulation were higher than in the Down-type noxious stimulation. Between 12 and 18 s after onset of noxious stimulation, activation of the right S1 in the Up-type noxious stimulation pattern was significantly greater than in the Down-type noxious stimulation pattern. Because S1 and S2 contain neurons activated by noxious somatosensory stimuli,[20] they are related to the sensory discriminative dimension of noxious somatosensory stimuli. Activation in the anterior insula is correlated with the subjective evaluation of heat pain, while activation in the posterior insula is correlated with the objective intensity of heat pain[21,22]. The insula is strongly interconnected with the cingulate cortex as well as the frontal, parietal, and temporal lobes.[23] The anterior insula and midcingulate cortex are commonly coactivated in studies of emotional processing.[21,23] In the present study, the insula and midcingulate cortex were coactivated in the Up-type noxious stimulation compared to Down-type noxious stimulation. Therefore, activation of S1, S2, and the bilateral midinsula may contribute to increased pain perception in the Up-type noxious stimulation compared to the Down-type noxious stimulation.

The MCC was more highly activated in the Up-type noxious stimulation than in the Down-type noxious stimulation. Between 12 and 18 s after onset of noxious stimulation, activation of the MCC was significantly higher in the Up-type noxious stimulation than in the Down-type noxious stimulation. It is known that neural responses to acute pain in the MCC have

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attentional and affective functions in relation to painful stimuli.[24] Activation of the MCC may lead to increased pain perception in the Up-type noxious stimulation compared to the Down-type noxious stimulations.

Multiple regression analysis indicates that when cortisol level increased, the left hippocampal cortex, along with the left parahippocampal cortex was greatly activated for the Up-type noxious stimulation. The parahippocampal cortex is activated in response to pain and is thought to contribute to the negative effects associated with pain and aversive drive mediation.[25,26] In a pain experiment conducted during stress conditions, salivary cortisol level was negatively correlated with pain threshold.[7] The right hippocampal cortex was greatly activated in more painful condition in the previous study.[8] Therefore, pain perception in the Up-type noxious stimulation might be increased due to activation of the hippocampal cortex with increased cortisol.

Our findings of greater pain and brain responses to noxious stimulation in the Up-type noxious stimulation than in the Down- and Down–up-type noxious stimulations may influence clinical practice in this area. Articular cartilage is less sensitive to pain than the highly pain-sensitive periosteum.[27] When the periosteum and articular cartilage are sequentially manipulated and incised in orthopedic surgery, pain perception might be reduced if the periosteum is manipulated before the articular cartilage. This is because manipulation of articular cartilage causes less pain than periosteal manipulation.[28] Pain-sensitive visceral organs such as the gut, bladder, and ureters are differentiated from less pain-sensitive visceral organs such as the liver.[29,30] If the liver and pain-sensitive visceral organs are sequentially manipulated and incised in surgery, pain perception might be decreased if pain-sensitive visceral organs are manipulated before the liver.

Conclusion

This study is the first to investigate brain mechanisms that modulate pain perception by changing noxious stimulation sequences under identical noxious intensity and duration. Pain and unpleasantness ratings in the Down- and Down–up-type noxious stimulation were lower than in the Up-type noxious stimulation. The Up-type noxious stimulation resulted in greater activation of the brain areas that are usually activated by noxious stimulation, which led to increased pain perception. Down- and Down–up-type noxious stimulations resulted in greater activation of the left PFC [(BA 10, (−45, 50, 1)], which led to decreased pain perception in the Down- and Down–up-type noxious stimulations compared to the Up-type noxious stimulation. In clinical practice where pain cannot be avoided, noxious stimulation must be applied to patients in a step-down pattern in which the most intense pain applied before the least intense pain.

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Author contributions

JCC, JK, and EK designed the study. JCC and JHC collected data. JCC, JK, CJH, WYP, YSC, JC, CH, SKP, MHK, GHL, HJD, SWJ, and JML conducted the data analysis. JCC wrote the manuscript. All authors read and approved the final manuscript.

References

1. Bennett GJ. What is spontaneous pain and who has it? J Pain 2012; 13: 921–9.
2. Kahneman D, Fredrickson BL, Schreiber CA, Redelmeier DA. When more pain is preferred to less: adding a better end. Psychol Sci 1993; 4: 401–5.

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3. Grill JD, Coghill RC. Transient analgesia evoked by noxious stimulus offset. J Neurophysiol 2002; 87: 2205–8.
4. Niesters M, Hoitsma E, Sarton E, Aarts L, Dahan A. Offset analgesia in neuropathic pain patients and effect of treatment with morphine and ketamine. Anesthesiology 2011; 115: 1063–71.
5. Derbyshire SWG, Osborn J. Offset analgesia is mediated by activation in the region of the periaqueductal grey and rostral ventromedial medulla. NeuroImage 2009; 47: 1002–6.
6. Yelle MD, Oshiro Y, Kraft RA, Coghill RC. Temporal filtering of nociceptive information by dynamic activation of endogenous pain modulatory systems. J Neurosci 2009; 29: 10264–71.
7. Choi JC, Chung MI, Lee YD. Modulation of pain sensation by stress-related testosterone and cortisol. Anaesthesia 2012; 67: 1146–51.
8. Choi JC, Kim J, Kang E, Lee JM, Cha J, Kim YJ, Lee HG, Choi JH, Yi DJ. Brain mechanisms of pain relief by transcutaneous electrical nerve stimulation: a functional magnetic resonance imaging study. Eur J Pain 2015. doi: 10.1002/ejp.696. [Epub ahead of print].
9. Choi JC, Lee JH, Choi E, Chung MI, Seo SM, Lim HK. Effects of seasonal differences in testosterone and cortisol levels on pain responses under resting and anxiety conditions. Yonsei Med J 2014; 55: 216–23.
10. Choi JC, Min S, Kim YK, Choi JH, Seo SM, Chang SJ. Changes in pain perception and hormones pre- and post-kumdo competition. Horm Behav 2013; 64: 618–23.
11. Choi JC, Park SK, Kim YH, Shin YW, Kwon JS, Kim JS, Kim JW, Kim SY, Lee SG, Lee MS. Different brain activation patterns to pain and pain-related unpleasantness during the menstrual cycle. Anesthesiology 2006; 105: 120–7.
12. Choi JC, Yi DJ, Han BS, Lee PH, Kim JH, Kim BH. Placebo effects on analgesia related to testosterone and premotor activation. NeuroReport 2011; 22: 419–23.
13. Hashmi JA, Davis KD. Effects of temperature on heat pain adaptation and habituation in men and women. Pain 2010; 151: 737–43.
14. Saeki Y. Effect of local application of cold or heat for relief of pricking pain. Nurs Health Sci 2002; 4: 97–105.
15. Verdugo R, Ochoa JL. Quantitative somatosensory thermotest – a key method for functional-evaluation of small caliber afferent channels. Brain 1992; 115: 893–913.
16. Kara M, Ozcakar L, Gokcay D, Ozcelik E, Yorubulut M, Guneri S, Kaymak B, Akinci A, Cetin A. Quantification of the effects of transcutaneous electrical nerve stimulation with functional magnetic resonance imaging: a double-blind randomized placebo-controlled study. Arch Phys Med Rehabil 2010; 91: 1160–5.
17. Kocyigit F, Akalin E, Gezer NS, Orbay O, Kocyigit A, Ada E. Functional magnetic resonance imaging of the effects of low-frequency transcutaneous electrical nerve stimulation on central pain modulation: a double-blind, placebo-controlled trial. Clin J Pain 2012; 28: 581–8.
18. Song XW, Dong ZY, Long XY, Li SF, Zuo XN, Zhu CZ, He Y, Yan CG, Zang YF. REST: a toolkit for resting-state functional magnetic resonance imaging data processing. PLoS ONE 2011; 6: e25031.
19. Kriegeskorte N, Simmons WK, Bellgowan PS, Baker CI. Circular analysis in systems neuroscience: the dangers of double dipping. Nat Neurosci 2009; 12: 535–40.
20. Apkarian AV, Bushnell MC, Schweinhardt P. Representation of pain in the brain. In: McMahon SB, Koltzenburg M, Tracey I, Turk DC, eds. Wall & Melzacks Textbook of Pain, 6th edn. Philadelphia, PA: Elsevier Saunders, 2013: 111–28.
21. Craig AD. How do you feel–now? The anterior insula and human awareness. Nat Rev Neurosci 2009; 10: 59–70.
22. Kong J, White NS, Kwong KK, Vangel MG, Rosman IS, Gracely RH, Gollub RL. Using fMRI to dissociate sensory encoding from cognitive evaluation of heat pain intensity. Hum Brain Mapp 2006; 27: 715–21.
23. Wiech K, Lin CS, Brodersen KH, Bingel U, Ploner M, Tracey I. Anterior insula integrates information about salience into perceptual decisions about pain. J Neurosci 2010; 30: 16324–31.
24. Brown CA, Jones AK. A role for midcingulate cortex in the interruptive effects of pain anticipation on attention. Clin Neurophysiol 2008; 119: 2370–9.
25. Apkarian AV, Bushnell MC, Treede RD, Zubieta JK. Human brain mechanisms of pain perception and regulation in health and disease. Eur J Pain 2005; 9: 463–84.
26. Roy M, Piche M, Chen JI, Peretz I, Rainville P. Cerebral and spinal modulation of pain by emotions. Proc Natl Acad Sci USA 2009; 106: 20900–5.

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27. Sophia Fox AJ, Bedi A, Rodeo SA. The basic science of articular cartilage: structure, composition, and function. Sports Health 2009;1:461–8.
28. Completo A, Fonseca F, Simoes JA, Ramos A, Relvas C. A new press-fit stem concept to reduce the risk of end-of-stem pain at revision TKA: a pre-clinical study. Knee 2012; 19: 537–42.
29. Cervero F. Visceral pain. In: Dubner R, Gebhart GF, Bond MR eds. Proceedings of the Vth World Congress on Pain. Amsterdam: Elsevier, 1988: 216–26.
30. Cervero F. Visceral versus somatic pain: similarities and differences. Dig Dis 2009; 27 (Suppl. 1): 3–10.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Differences between men and women with regard to demographic data and hormone levels measured before fMRI scanning.
Table S2. Differences between men and women with regard to average pain and unpleasantness ratings measured during fMRI scanning in the three noxious stimulations.
Table S3. Brain areas activated in repeated measures ANOVA.
Table S4. Brain areas that were more highly activated in the Down–up-type noxious stimulation than in the Up-type noxious stimulation.
Table S5. Percentage MR signal change in the left PFC between 9 s and 12 s after onset of noxious stimulation in the left PFC.
Table S6. Percentage MR signal change in the left PFC between 12 s and 15 s after onset of noxious stimulation in the left PFC.
Table S7. Percentage MR signal change in the left PFC between 15 s and 18 s after onset of noxious stimulation in the left PFC.
Table S8. Percentage MR signal change in the left PFC between 18 s and 21 s after onset of noxious stimulation.
Table S9. Percentage MR signal change in the right PFC between 18 s and 21 s after onset of noxious stimulation.
Table S10. Percentage MR signal change in the right MCC between 12 s and 15 s after onset of noxious stimulation in the right MCC.
Table S11. Percentage MR signal change in the right MCC between 15 s and 18 s after onset of noxious stimulation in the right MCC.
Table S12. Percentage MR signal change in the right S1 between 12 s and 15 s after onset of noxious stimulation in the right S1.
Table S13. Percentage MR signal change in the right S1 between 15 s and 18 s after onset of noxious stimulation in the right S1.

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