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Brain mechanisms of pain relief by transcutaneous electrical nerve stimulation: A functional magnetic resonance imaging study

J.C. Choi[1], J. Kim[2], E. Kang[2], J.-M. Lee[3], J. Cha[3], Y.J. Kim[4], H.G. Lee[4], J.-H. Choi[5], D.-J. Yi[6]

1 Department of Anesthesiology and Pain Medicine, Intensive Care Unit, Brain Research Group, Yonsei University Wonju College of Medicine, South Korea
2 Department of Psychology, Kangwon National University, Chuncheon, South Korea
3 Department of Biomedical Engineering, Hanyang University, Seoul, South Korea
4 Department of Radiology, Yonsei University Wonju College of Medicine, South Korea
5 Julie English Institute, Wonju, South Korea
6 Department of Psychology, Yonsei University, Seoul, Korea

Correspondence
Jae Chan Choi
E-mail: jaechan@yonsei.ac.kr
Do-Joon Yi
E-mail: dojoon.yi@yonsei.ac.kr

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Abstract

Background: Although the exact mechanism of TENS pain relief is unknown, it is believed that TENS impulses interrupt nociceptive signals at the dorsal horn of the spinal cord.
Aims: To evaluate the hypotheses that during pain caused by noxious stimuli, brain responses, temporal summation and brain functional connectivity are modulated by TENS, and that mechanisms of pain relief by TENS differ between men and women.
Methods: During fMRI scanning, the same noxious stimuli were delivered to each participant in pain-only and pain+TENS conditions. In the pain-only condition, noxious stimuli were presented without TENS. In the pain+TENS condition, participants received noxious stimuli and TENS concurrently. Participants were initially presented with TENS at an intensity that was just below that causing discomfort. TENS intensity was presented in a step-wise fashion to prevent temporal summation from repetitive noxious stimuli.
Results: Pain and unpleasantness ratings were significantly higher in the pain-only than the pain+TENS condition. With non-painful TENS, primary and secondary somatosensory and parietal cortices were activated, and temporal summation from repetitive noxious stimuli was prevented. Periaqueductal gray (PAG) and lateral prefrontal cortex functional connectivity was increased by TENS, and modulated by testosterone and cortisol. Women reported greater pain during TENS than men, and showed greater activation in the temporoparietal junction cortex and increased PAG functional connectivity with the orbitofrontal cortex.
Conclusion: TENS led to pain reduction, probably due to activation of the descending pain-inhibitory pathway, indicating that this TENS method may be applied in clinical practice.

1. Introduction

Transcutaneous electrical nerve stimulation (TENS) delivers electric current through the skin to reduce acute and chronic pain with various aetiologies. TENS efficacy has been shown in pressure pain, intense experimental pain and thermal pain models (Claydon et al., 2011). Intensity has been shown to be critical to TENS effectiveness (Vance et al., 2012), and TENS should be delivered at a strong, non-painful intensity level to produce maximum pain relief

What's already known about this topic?

- Two studies investigated post-TENS effects using fMRI. In these, activations of the sensory cortex, caudal anterior cingulate cortex and parahippocampal cortex decreased after TENS application. However, the effects of TENS on pain were not investigated in a counterbalanced manner in either study.

What does this study add?

- Non-painful TENS reduced pain and activated S1, S2 and parietal cortices. TENS prevented temporal summation, increased periaqueductal gray (PAG) and prefrontal cortex (PFC) functional connectivity, and activated the descending pain-inhibitory pathway.
- Women reported more pain during TENS than men, and showed greater activation in the temporoparietal junction cortex and increased PAG functional connectivity with the orbitofrontal cortex.

(Moran et al., 2011). Adequately intense TENS is needed to produce postoperative pain relief as TENS of lower intensity is ineffective for analgesic effects (Bjordal et al., 2003; Rakel and Frantz, 2003).

Although the exact mechanism of TENS pain relief is not known, it is believed that TENS impulses interrupt nociceptive signals at the dorsal horn of the spinal cord (Tashani and Johnson, 2009). Descending spinal pathways from the brain and large-diameter A-beta afferent stimuli may modulate dorsal horn small-fibre nociceptive afferent input such that pain transmission along the ascending spinal pathways is affected (Tashani and Johnson, 2009). Since the 1990s, functional magnetic resonance imaging (fMRI) has helped elucidate pain pathophysiology (Peyron et al., 2000). This suggests that fMRI can provide a means of identifying the brain mechanisms associated with TENS pain relief. Recently, a study measuring laser-evoked potentials (LEPs) in the brain has shown that both acute pain and LEPs are significantly decreased when TENS and noxious stimuli are concurrently applied (Vassal et al., 2013). In two recent studies of post-TENS effects in patients with carpal tunnel syndrome and subacromial impingement syndrome (Kara et al., 2010; Kocyigit et al., 2012), activations of the sensory cortex, caudal anterior cingulate cortex and parahippocampal cortex were decreased after TENS application. However, the effects of TENS on pain and brain responses were not investigated in a counterbalanced manner.

We first hypothesized that pain and brain responses to noxious stimuli are modulated by TENS. We secondly hypothesized that temporal summation from noxious stimulation would be prevented if TENS intensity were increased in proportion to pain duration. Temporal summation in this context is an increase in perceived pain when noxious heat is continuously applied to skin (Tran et al., 2010). When a constant noxious heat stimulus is continuously applied to the skin, pain perception increases during the first 10 s, and remains roughly constant thereafter (Tran et al., 2010). Therefore, a 15-s duration was selected for the current study to insure that maximal temporal summation was induced. Because women report pain more frequently than men and are at substantially higher risk for clinical pain and pain-related distress (Fillingim et al., 2009; Paller et al., 2009; Bartley and Fillingim, 2013), we hypothesized that temporal summation, pain and brain responses to noxious stimuli during TENS would differ between men and women.

Midbrain periaqueductal gray (PAG) integrates top-down and bottom-up influences to modulate pain perception (Millan, 2002; Heinricher and Fields, 2013). Ventral PAG may decrease pain (Fardin et al., 1984), whereas dorsal PAG may increase pain (Behbehani, 1995). Also, because ventrolateral PAG plays an important role in opioid analgesia (Bandler and Shipley, 1994) and antinociception (Basbaum and Fields, 1984; Borszcz, 1999; Munn et al., 2009; Linnman et al., 2012a,b), ventrolateral PAG was selected for the present study. Pain may also be changed by testosterone and cortisol levels (Choi et al., 2012, 2013, 2014). Thus, we hypothesized that functional connectivity of the PAG with other brain areas is modulated by TENS and hormones.

2. Methods

2.1 Participants

Twenty-four participants (12 men and 12 women) were recruited for this study (Table 1); each was paid for participation (200,000 KRW, or approximately 150 EUR). Age did not differ between men and women (Table 1). Thirteen women were initially included, but one was excluded due to excessive head movement. All provided written informed consent acknowledging the following: (1) all of the

Table 1 Differences between men and women with regard to demographic data, hormone levels and other parameters measured before fMRI scanning. (n = 24 including 12 men and 12 women).

	Average (n = 24)	Men (n = 12)	Women (n = 12)	p-value
Age (years)	22.46 ± 3.92	22.92 ± 3.03	22.00 ± 4.75	0.579
Weight (kg)	63.63 ± 9.72	66.42 ± 8.83	60.83 ± 10.12	0.164
Height (cm)	169.67 ± 9.44	177.33 ± 5.21	162.00 ± 5.58	0.001
Body mass index (kg/m^2)	22.05 ± 2.45	21.07 ± 2.14	23.03 ± 2.43	0.047
Testosterone (ng/mL)	3.38 ± 3.30	6.45 ± 1.46	0.31 ± 0.10	<0.001
Cortisol (μg/dL)	9.82 ± 3.96	11.74 ± 3.74	7.89 ± 3.29	0.014
T/C × 1000	321.79 ± 318.75	592.23 ± 226.50	51.35 ± 39.57	<0.001
TENS intensity (mA)	16.83 ± 1.76	17.92 ± 1.56	15.75 ± 1.22	0.001

Data shown are mean ± SD. T/C ratio = testosterone/cortisol. Data were analysed using an independent samples t-test. TENS intensity (mA) = the TENS intensity level that participants identified as strong, but comfortable.

methods and procedures were clearly explained to them; (2) they would experience experimental thermal pain; (3) no tissue damage would result from this pain; 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 those using medications that could affect sensory perception, such as neuropsychotropics or analgesics.

2.2 Apparatus

Noxious thermal stimuli (all were 45 °C, applied for 15 s) were delivered with a computerized thermal contact stimulator (CHEPS, Medoc Advanced Medical Systems Ltd., Ramat Yishai, Israel) with a 27-mm-diameter thermode. Two-channel TENS (SM-920, SamsonMed, Seoul, Korea) was applied to the left lower leg skin using a silver chloride electrode (Hurev Co., Ltd., Wonju, South Korea). The cathode electrode of one channel was attached to the lateral aspect of the left lateral lower leg over the fibular neck, and the anode electrode of that same channel was attached to the skin immediately above the lateral malleolus. The cathode electrode of the second channel was attached to the skin of the posterior aspect of the left lower leg at the fibular head level, and the anode electrode of that same channel was attached to the posterior aspect of the left ankle. One channel stimulated the common peroneal nerve, and the other stimulated the tibial nerve, both of which innervate the lateral lower leg. Thermal stimuli were applied to the lateral left lower leg through a thermode attached between the cathodes (fibular head level) and anodes (ankle level) using a Velcro strap. Once inside the MRI, participants determined TENS intensity levels (mA) that they perceive as strong, but that did not cause pain or discomfort. This comfortable TENS intensity (CTI, mA) was established for each individual participant at TENS settings under 80 Hz and 60 μs (pulse duration).

2.3 fMRI scanning

During scanning, the same painful stimuli (45 °C, 15 s) were delivered to each participant in the pain-only and pain+TENS conditions. In the pain-only condition (pain-only session), these noxious stimuli were presented without TENS. In the pain+TENS condition (pain+TENS session), participants received noxious stimuli and TENS concurrently for 15 s (Fig. 1). The pain+TENS and pain-only sessions were composed of 10 repetitions of noxious stimuli during each session. The pain+TENS and pain-only conditions in this manuscript refer to the pain+TENS and pain-only sessions, respectively. Temperature in the pain-only and pain+TENS conditions was increased from baseline (32 °C) to the target (45 °C) at 25 °C/s. Target temperature was maintained for 15 s, then returned to 32 °C at a rate of 25 °C/s.

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 stimulation. Participants then received 15 s of 45 °C noxious stimuli concurrent with a visual white horizontal bar cue, followed by a 30-s resting period during which a visual white cross cue was presented. This 47-s block (2 + 15 + 30) was repeated 10 times for both conditions. To familiarize participants with the noxious stimuli and visual cues, a practical session was perfomed before the experiment began.

In the pain+TENS condition, TENS (15 s) and noxious stimuli were presented concurrently, and TENS

A Pain-only condition (session)

Cueing (2 s)	Pain (45 °C, 15 s)	Rest (30 s)
No pain, no TENS	Pain, no TENS	No pain, no TENS

B Pain+TENS condition (session)

Cueing (2 s)	Pain (45 °C, 15 s)	Rest (30 s)
No pain, no TENS	Pain+TENS	No pain, no TENS

Figure 1 Stimulation paradigm. During scanning, fMRI experiments (sessions) using the same noxious stimuli (45 °C) were conducted twice for each participant, once with TENS (pain+TENS session) and once without TENS (pain-only session). Participants in both sessions received noxious stimulation 10 times (45 °C, 15 s). The pain+TENS and pain-only conditions in this manuscript indicate the pain+TENS and pain-only sessions, respectively. A visual cue was displayed 2 s prior to noxious stimulation in both conditions, and participants then received 15 s of 45 °C noxious stimulation, followed by a 30-s resting period. This 47-s block (2 + 15 + 30) was repeated 10 times for each condition. Participants received neither noxious stimulation nor TENS during the cueing or resting periods in either condition. Participants received noxious stimuli but not TENS during the noxious stimulation period of the pain-only condition. Participants received noxious stimulation (45 °C, 15 s) and TENS (15 s) concurrently during the pain stimulation period of the pain+TENS condition. TENS intensity during the noxious stimulation period of the pain+TENS condition was increased in a step-wise fashion. The 15-s TENS application period was divided into three 5-s periods; For the first 5 s, participants received a comfortable TENS intensity (CTI), for the second 5-s period, they received CTI plus 1 mA, and for the third 5-s period, CTI plus 2 mA. Pain-only (Fig. 1A) and pain+TENS (Fig. 1B) sessions were counterbalanced.

[80 Hz, pulse duration (60 μs), constant pulse pattern, rectangular waveform] was run continuously for 15-s periods. When participants received a comfortable TENS intensity (CTI) during the noxious stimulation period, they did not report discomfort associated with TENS itself. However, when participants in pilot testing received CTI during rest without noxious stimulation, they reported discomfort caused by TENS alone. TENS has been reported to be uncomfortable, even when administered at intensities below the pain threshold (Hughes et al., 2013). Vibration may function in a manner similar to TENS, and may cause nerve dysfunction or exacerbate pain during prolonged exposure (Lundborg, 1994; Smith et al., 2004). Therefore, TENS was applied only during noxious stimulation.

Studies using high-frequency TENS (>50 Hz) in healthy humans have shown that strong, but comfortable, intensities produce greater pain reduction (Vance et al., 2012). Accordingly, we selected TENS intensities that were strong, but comfortable to participants. Constant TENS results in a reduction in the effect (Spielholz and Nolan, 1995). In pilot testing, constant TENS resulted in a reduction in the pain-reducing effect when applied during the 15-s noxious stimulation period. Therefore, a step-wise increase in TENS intensity during the 15 s of noxious stimulation was used to overcome this habituation effect. For this part of the study, the 15-s TENS period was divided into three 5-s periods. For the first 5 s of the pain+TENS condition, participants received CTI; for the second 5-s period, they received CTI plus 1 mA; for the third 5-s period, CTI plus 2 mA. This method was also applied to investigate whether this TENS application method effectively relieves temporal summation caused by 15-s of continuous noxious stimulation.

In the pain-only condition, noxious stimuli were applied to participants in the same way as in the pain+TENS condition, but without TENS application. To control for any variation between the pain-only and pain+TENS conditions resulting from TENS interference with the fMRI, TENS in the pain-only condition was applied to rolled pig skin positioned between the lower legs of the participants. TENS electrical current was applied to the rolled pig skin only during the noxious stimulation period of the pain-only condition, whereas TENS was applied to the skin of the participants' lower left leg only during that of the pain+TENS condition.

To reduce magnetic susceptibility caused by TENS electrical current, an electric wire was passed through a hollow passage in the fMRI CHEPS filter. Due to electromagnetic fields emitted by the electrodes, a flexible copper-alloy sheet (diameter: 110 mm; length: 690 mm; wall thickness: 0.5 mm) was used as a shield (Gasser et al., 2005). To minimize the possibility of habituation or sensitization, the thermode was moved a short distance to the adjacent lateral left lower leg skin between sessions.

Pain+TENS (Fig. 1B) and pain-only (Fig. 1A) sessions were counterbalanced. Twelve participants (six men and six women) first received pain-only followed by pain+TENS, whereas the other 12 participants (six men and six women) first received pain+TENS followed by pain-only. Participants remained in the scanner for the entire study. At the completion of each condition, they were asked to rate average pain and unpleasantness experienced during the 15-s noxious stimulation periods. Because TENS intensity was increased in a step-wise fashion (every 5 s), they were also asked to rate pain experienced in the first, second, and third 5-s periods of the total 15-s noxious stimulation period. Ratings were assessed with a numerical rating scale (0 = no

pain or unpleasantness; 100 = maximum imaginable pain and unpleasantness).

2.4 Hormones

To assess testosterone and cortisol levels, venous blood samples were drawn before fMRI scanning from the antecubital vein with a 21-gauge needle. Participants were instructed to avoid eating 2 h before blood sampling. Testosterone 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 (Choi et al., 2013). 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.

2.5 Statistical analyses of behavioural and hormonal data

In a pilot study of men, the difference in pain ratings between the pain-only and pain+TENS conditions was 18.33. The required sample size for having 80% power (i.e. $\beta = 0.2$) at $\alpha = 0.05$ (SD = 20) can be obtained by normal approximation as $n = [(Z\alpha/2 + Z\beta)_*\sigma/\mu D]^2 \approx 10$ (Shein-Chung et al., 2007). When we consider a 10% drop rate, the required sample size was 11. Since the current study consisted of both men and women, we selected 24 participants (12 men and 12 women). In choosing the sample size, we also relied on two previous fMRI studies in which brain activation was measured after TENS application (Kara et al., 2010; Kocyigit et al., 2012). These were performed using 20 patients (TENS group = 10, sham group = 10), a sample size that was exceeded in the present study.

Group data were analysed using paired-sample *t*-tests with PASW (Predictive Analytics Software) Statistics version 20 (SPSS Inc., Chicago, IL, USA). Differences between men and women were analysed using an independent samples *t*-test. Pearson's correlation coefficients were calculated. Effects of temporal summation were analysed using repeated measures ANOVA. $p < 0.05$ was the criterion for statistical significance.

2.6 Image acquisition

Before scanning, participants were instructed to stay awake and to refrain as much as possible 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 each participant, 33 slices were acquired to cover the whole brain volume, except for one participant from whom only 35 slices were acquired. A structural T1-weighted image was obtained using 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).

2.7 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 pain+TENS and pain-only sessions 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 (cut-off: 128-s period) and an autocorrelation correction were applied to the resulting time series.

Preprocessed images were analysed using a general linear model (GLM) in which four events, cue presentation and noxious stimulation delivery in the pain-only and pain+TENS conditions, were modelled using a canonical hemodynamic function. Cue events were modelled with no time duration, whereas pain events were modelled with 15-s durations. To account for potential pain habituation effects, first- and second-order time modulation functions were included for each event. 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 participant as a random effect. To prevent false positives, a statistical criterion of eight or more continuous voxels with a voxel-wise threshold of uncorrected $p < 0.001$, which corresponds to an experiment-wise threshold of $p < 0.05$, corrected for multiple comparisons was used (Slotnick et al., 2003). This threshold was applied to all statistical analyses of fMRI data in this study.

To analyse functional connectivity, a generalized psychophysiological interaction (gPPI) analysis was conducted (Friston et al., 1997; McLaren et al., 2012). Preprocessed images of individual participants were reanalysed using a GLM in which noxious stimulation events were modelled using a canonical hemodynamic function, as well as first- and second-order time modulation functions. Then, seed regions (3-mm radius spheres) were defined based on previous literature (Linnman et al., 2012a). Because the ventrolateral PAG plays an important role in opioid analgesia (Bandler and Shipley, 1994) and antinociception (Basbaum and Fields, 1984; Borszcz, 1999; Munn et al., 2009; Linnman et al., 2012a,b), bilateral ventrolateral PAG were selected as seeds in this study [right MNI peak coordinate (4, −26, −16), left MNI peak coordinate (−4, −26, −16)]. For each seed, a separate gPPI analysis was conducted to investigate changes in functional connectivity between the seed region and remaining brain regions. PPI regressors were calculated as interactions between the mean time course of seed voxels and each experimental condition. Resulting PPI regressors were then included in the GLM to contrast the two critical conditions. These contrast images were entered into second-level random effect analyses using the same statistical threshold as for amplitude analyses.

To investigate the relationships between brain activation and covariates (hormonal and behavioural data), simple regression (correlation) analysis was performed using SPM8. Brain activation areas in the pain-only and pain+TENS conditions were acquired by simple regression analysis. Also, difference images between the pain-only and pain+TENS conditions were acquired. With regard to these difference images and covariates, simple regression analysis was conducted.

To assess TENS and hormone covariate effects on functional connectivity, simple regression (correlation) analysis was performed between covariates and PAG (seed) functional connectivity with the remaining brain regions. The TENS effect was calculated as pain rating for the pain-only condition minus that in the pain+TENS condition. TENS effect was positive because pain ratings in the pain-only condition were higher than those in the pain+TENS condition. A 3-mm spherical volume of interest (VOI) was selected in brain areas identified with simple regression analysis. The correlation coefficient (r) between the covariate and eigenvalue acquired from the activated brain area was calculated. Although there are dangers of double dipping with this statistical method (Kriegeskorte et al., 2009), it was used to graphically explain the relationships between brain activations and covariates.

3. Results

3.1 Behavioural and hormonal data

Testosterone, cortisol and TENS intensity levels were higher in men than women (Table 1). Average pain ratings ($n = 24$) were significantly higher in the pain-only compared to pain+TENS condition (Table 2). Average pain rating in the pain-only condition was significantly lower in men (65.00 ± 19.66) than in women (86.50 ± 8.39, $p = 0.003$). Also, average pain rating in the pain+TENS condition was significantly lower in men (36.08 ± 23.38) than in women (63.17 ± 13.89, $p = 0.003$). Average unpleasantness ratings ($n = 24$) were significantly higher in the pain-only compared to the pain+TENS condition. Average unpleasantness ratings in the pain-only condition were significantly lower in men (62.00 ± 19.94) than in women (85.50 ± 8.82, $p = 0.002$). Also, average unpleasantness ratings for the pain+TENS condition were significantly lower in men (31.50 ± 20.90) than in women (62.75 ± 15.01, $p < 0.001$).

3.2 2 × 2 factorial analysis [sex (men vs. women) x treatment (pain-only condition vs. pain+TENS condition)]

There were main effects of sex ($p = 0.003$) and TENS treatment ($p < 0.001$). That is, pain ratings in men differed from those in women, and pain ratings in the pain-only condition differed from those in the pain+TENS condition. However, there was not a significant interaction between sex (men vs. women) and treatment (pain-only condition vs. pain+TENS condition; $p = 3.351$).

3.3 Temporal summation (Table 3)

To investigate temporal summation, pain and unpleasantness, ratings in the first, second and third 5-s periods were measured. Temporal summation existed in the pain-only condition, whereas temporal summation was prevented in the pain+TENS condition.

In the pain-only condition ($n = 24$), a repeated measures ANOVA with Greenhouse–Geisser correction showed that pain ratings differed significantly among the first, second and third 5-s periods [F

Table 2 Average pain and unpleasantness ratings measured during the 15-s noxious stimulation period for pain-only and pain+TENS conditions.

	Pain-only condition	Pain+TENS condition	*p*-value
Average pain ratings			
12 men + 12 women	75.75 ± 18.41	49.63 ± 23.35	<0.001
12 men	65.00 ± 19.66	36.08 ± 23.38	<0.001
12 women	86.50 ± 8.39	63.17 ± 13.89	<0.001
Average unpleasantness ratings			
12 men + 12 women	74.00 ± 19.12	47.13 ± 23.91	<0.001
12 men	62.00 ± 19.94	31.50 ± 20.90	<0.001
12 women	85.50 ± 8.82	62.75 ± 15.01	<0.001

Table 3 Pain ratings measured in the first, second and third 5-s periods of the total 15-s noxious stimulation period in pain-only and pain+TENS conditions.

	First 5-s pain ratings	Second 5-s pain ratings	Third 5-s pain ratings	*F* values, *p*-values
Pain-only condition				
12 men + 12 women	62.71 ± 20.95	71.46 ± 18.68	79.46 ± 18.28	161.543, <0.001
12 men	52.92 ± 24.44	61.67 ± 20.60	69.92 ± 20.76	55.652, <0.001
12 women	72.50 ± 10.55	81.25 ± 9.80	89.00 ± 8.31	132.445, <0.001
Pain+TENS condition				
12 men + 12 women	48.46 ± 24.43	42.79 ± 24.89	37.25 ± 23.34	20.816, <0.001
12 men	33.75 ± 26.21	29.33 ± 24.31	25.75 ± 20.55	4.438, =0.058
12 women	63.17 ± 13.89	56.25 ± 17.60	48.75 ± 20.68	23.464, <0.001

(1.294, 29.763) = 161.543, $p < 0.001$]. Bonferroni *post hoc* tests showed that the third 5-s pain ratings (79.46 ± 18.28) were significantly higher than the second 5-s (71.46 ± 18.68, $p < 0.001$) and first 5-s pain ratings (62.71 ± 20.95, $p < 0.001$). The pain ratings of the second 5 s were significantly higher than those of the first 5 s ($p < 0.001$).

In the pain-only condition for men ($n = 12$), *post hoc* tests using the Bonferroni correction revealed that third 5-s pain ratings (69.92 ± 20.76) were significantly higher than those of the second 5 s (61.67 ± 20.60, $p < 0.001$) and first 5 s (52.92 ± 24.44, $p < 0.001$). The pain ratings of the second 5 s were significantly higher than those of the first 5 s ($p = 0.001$).

In the pain-only condition for women ($n = 12$), *post hoc* tests using the Bonferroni correction revealed that third 5-s pain ratings (89.00 ± 8.31) were significantly higher than second 5-s (81.25 ± 9.80, $p < 0.001$) and first 5-s pain ratings (72.50 ± 10.55, $p < 0.001$). The pain ratings of the second 5 s were significantly higher than those of the first 5 s ($p < 0.001$).

In the pain+TENS condition ($n = 24$), a repeated measures ANOVA with Greenhouse–Geisser correction determined that pain ratings were significantly different among first, second and third 5-s periods [F (1.083, 24.905) = 20.816, $p < 0.001$]. *Post hoc* tests using the Bonferroni correction revealed that first 5-s pain ratings (48.46 ± 24.43) were significantly higher than second 5-s pain ratings (42.79 ± 24.89, $p < 0.001$) and third 5-s pain ratings (37.25 ± 23.34, $p < 0.001$). Second 5-s pain ratings were significantly higher than third 5-s pain ratings ($p = 0.003$).

In the pain+TENS condition for men ($n = 12$), a repeated measures ANOVA with Greenhouse–Geisser correction determined that pain ratings did not significantly differ among the first 5 s, second 5 s, and third 5 s [F(1.024, 11.262) = 4.438, $p = 0.058$].

In the pain+TENS condition for women ($n = 12$), *post hoc* tests using the Bonferroni correction revealed that first 5-s pain ratings (63.17 ± 13.89) were significantly higher than second 5-s pain ratings (56.25 ± 17.60, $p = 0.009$) and third 5-s pain ratings (48.75 ± 20.68, $p = 0.001$). Second 5-s pain ratings were significantly higher than third 5-s pain ratings ($p < 0.001$).

3.4 Correlation between hormone levels and pain rating (Table 1)

Testosterone levels were significantly negatively correlated with average pain rating in the pain-only ($r = -0.691$, $p < 0.001$, $n = 24$) and pain+TENS conditions ($r = -0.524$, $p = 0.009$, $n = 24$). The testosterone/cortisol ratio was significantly negatively correlated with average pain rating in the pain-only

($r = -0.582$, $p = 0.003$, $n = 24$) and pain+TENS conditions ($r = -0.431$, $p = 0.036$, $n = 24$).

3.5 fMRI data

3.5.1. 2 × 2 factorial analysis [sex (men vs. women) × treatment (pain-only condition vs. pain+TENS condition)]

There was no significant main effect of sex. Brain activations in the pain-only condition differed from those in the pain+TENS condition. For main effects of brain activations, activated brain regions were the right superior parietal lobule, right postcentral cortex (S1), bilateral parietal operculum (S2), dorsal anterior cingulate cortex (dACC), left supramarginal cortex, dorsal anterior cingulate cortex (dACC), and left anteroventral thalamus (Table S1). There was not a significant interaction between sex (men vs. women) and treatment (pain-only condition vs. pain+TENS condition).

3.5.2 Comparison between the pain-only and pain+TENS conditions in 24 participants (paired *t*-test in SPM8, Table S2 and Fig. 2)

The left anteroventral thalamus, bilateral anterior cingulate cortex, right hippocampal cortex, right cerebellum, left middle frontal cortex and right inferior parietal cortex were more highly activated in the pain-only compared to pain+TENS condition. The thalamus, rostral anterior cingulate cortex and hippocampal cortex, all of which are related to pain and pain affect, were significantly more activated in the pain-only than the pain+TENS condition. The right superior parietal lobule, bilateral supramarginal cortex, right postcentral cortex, and bilateral parietal operculum were more highly activated in the pain+TENS than the pain-only condition.

3.5.3 Comparison between men and women in the pain-only and pain+TENS conditions (two sample t-test in SPM8)

The right temporoparietal junction cortex [TPJ, (48, −55, 25), 4.10 (*t*-value), 18 (voxel)] in the pain+TENS condition was more highly activated in women ($n = 12$) than in men ($n = 12$), whereas there was no significant difference in activation between men and women in the pain-only condition.

3.5.4 Simple regression (correlation) analysis

Increased pain ratings in the pain-only condition were associated with more highly activated brain areas compared to the pain+TENS condition, including the left thalamus [(−6, −5, 10), 5.52 (*t*-value), 46 (voxel)] and right S1 [(60, −10, 40), 4.05 (*t*-value), 14 (voxel)] ($n = 24$). This indicates that increased pain ratings for the pain-only compared to pain+TENS condition was associated with activation of the thalamus and S1. This also suggests that activation of the thalamus and S1 in the pain-only compared to pain+TENS condition was associated with brain responses to noxious stimulation.

When TENS intensity (mA) was increased, brain areas more highly activated in the pain+TENS compared to pain-only condition were the left S1 [(−54, −25, 55), 5.52 (*t*-value), 46 (voxel)], right S1 [(30, −43, 67), 4.04 (*t*-value), 12 (voxel), $r = 0.646$, $p = 0.001$, Fig. 3A], right ventrolateral prefrontal cortex [VLPFC (54, 29, 1, BA 45), 4.05 (*t*-value), 14 (voxel)] and right supramarginal cortex [(63, −28, 34), 3.85 (*t*-value), 8 (voxel)] (r = the r between TENS intensity (mA) and eigenvalues acquired from the right S1)] ($n = 24$). This suggests that activation of the bilateral S1, right VLPFC and right supramarginal cortex in the pain+TENS compared to pain-only condition was associated with brain responses to non-painful TENS as TENS intensity (mA) was increased.

The increased testosterone level in men was associated with more highly activated brain areas in the pain+TENS compared to pain-only condition, including the left precentral cortex [(−51, 2, 37), 8.52 (*t*-value), 32 (voxel)], right supramarginal cortex [(42, −37, 34), 6.04 (*t*-value), 51 (voxel)], left supramarginal cortex [(−39, −34, 40), 5.91 (*t*-value), 43 (voxel)] and right superior frontal cortex [premotor area (21, 2, 55, BA 6), 5.52 (*t*-value), 21 (voxel)] ($n = 12$ men). As the testosterone/cortisol ratio in the pain-only condition decreased, activation in the left orbitofrontal cortex [(−18, 11, −17), 6.10 (*t*-value), 30 (voxel)] and right parahippocampal cortex [(24, −25, −23), 5.50 (*t*-value), 21 (voxel)] increased ($n = 12$ men). As the testosterone/cortisol ratio in the pain+TENS condition decreased, activation of the right temporoparietal junction (TPJ) [(57, −58, 25), 4.02 (*t*-value), 20 (voxel)] increased ($n = 24$).

3.5.5 Differences between men and women with regard to functional connectivity (two sample *t*-test in SPM8)

For the pain+TENS condition, right PAG functional connectivity with the orbitofrontal cortex [(0, 32, −20), 4.80 (*t*-value), 12 (voxel)], right occipital pole [(3, −97, 10), 4.70 (*t*-value), 12 (voxel)] and

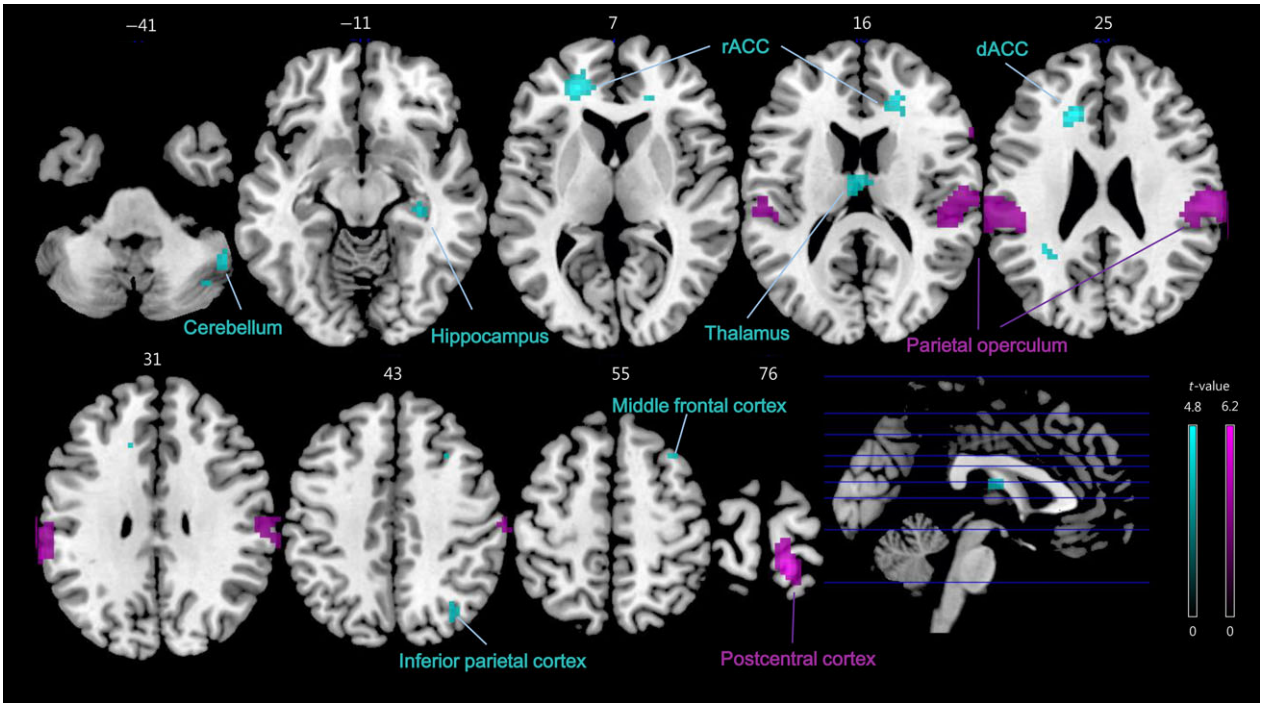

Figure 2 Comparison between the pain-only and pain+TENS conditions in 24 participants. Activation of brain areas in the pain-only condition compared to in the pain+TENS condition (cyan colour = pain-only condition > pain+TENS) and activation of brain areas in the pain+TENS condition compared to in the pain-only condition (violet colour = pain+TENS > pain-only condition). The left anteroventral thalamus, bilateral anterior cingulate cortex, right hippocampal cortex, right cerebellum, right middle frontal cortex and right inferior parietal cortex were more highly activated in the pain-only compared to pain+TENS condition. The bilateral parietal operculum and right postcentral cortex were more highly activated in the pain+TENS compared to pain-only condition. Contrast maps are superimposed on nine axial slices at z = −41, −11, 7, 16, 25, 31, 43, 55, 76.

left lateral occipital cortex [(−27, −79, 43), 4.25 (*t*-value), 13 (voxel)] was significantly increased in women compared to men. Right PAG functional connectivity with the right cerebellum [(46, −61, −47), 4.49 (*t*-value), 12 (voxel)] in the pain+TENS compared to the pain-only condition was significantly increased in women compared to men. Left PAG functional connectivity with the bilateral cerebellums [right (42, −61, −47), 4.57 (*t*-value), 31 (voxel); left (−39, −55, −47), 4.49 (*t*-value), 28 (voxel)] in the pain+TENS compared to the pain-only condition was significantly increased in women compared to men.

3.5.6 Covariate (TENS effect and hormones) effects on brain functional connectivity (Table S3)

As the TENS effect increased, left PAG functional connectivity with the left superior frontal (DLPFC) and left precentral cortices was significantly increased for pain+TENS compared to pain-only condition (Table S3 and Fig. 3B). As the TENS effect increased in women, left PAG functional connectivity with left middle frontal (VLPFC), left superior frontal (DLPFC) and right lateral occipital cortices, and right precuneus were significantly increased in the pain+TENS compared to the pain-only condition. TENS increased the functional connectivity of the PAG with the lateral prefrontal cortex (DLPFC and VLPFC). As the TENS effect increased in men, right PAG functional connectivity with the right cerebellum, left rACC (Fig. 3C), right orbitofrontal cortex (OFC) and left S1 decreased in the pain+TENS compared to the pain-only condition. These results suggest that increased functional connectivity of the right PAG with the cerebellum, rACC, OFC and S1 was associated with brain responses to noxious stimulation.

Across both men and women participants, as testosterone level increased, right PAG functional connectivity with the left superior frontal cortex (DLPFC) and right medulla significantly increased for the pain+TENS compared to the pain-only condition. As testosterone level increased in women, right PAG functional connectivity with the left insula was significantly decreased for the pain+TENS compared to the pain-only condition (Fig. 3D). As T/C ratio

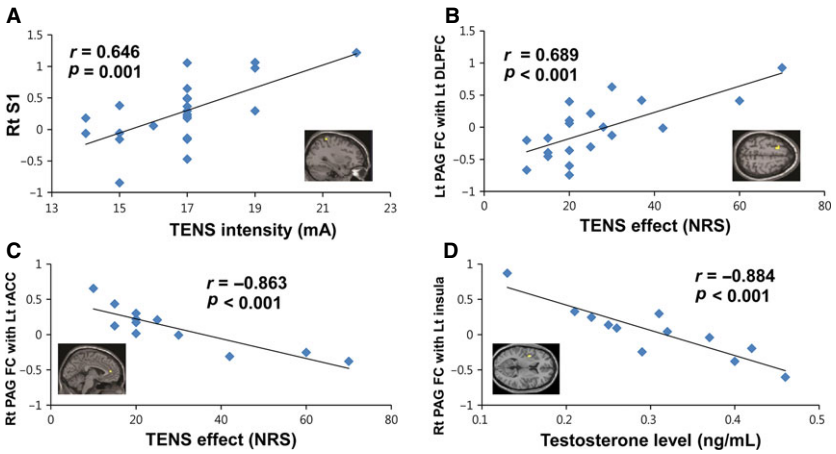

Figure 3 Graphic explanation of simple regression (correlation) analysis. (A) As TENS intensity (mA) increased for all participants, right S1 was more highly activated in the pain+TENS compared to pain-only condition [*r* (correlation coefficient) between TENS intensity (mA) and eigenvalue acquired from the right S1 = 0.646]. (B) As the TENS effect increased for all participants, left PAG functional connectivity with the left superior frontal cortex (DLPFC) was significantly increased in the pain+TENS condition compared to the pain-only condition (*r* between TENS effect and the eigenvalue acquired from the left DLPFC = 0.689). (C) In men, as the TENS effect increased, right PAG functional connectivity with the left rACC decreased in pain+TENS compared to the pain-only condition (*r* between TENS effect and eigenvalue acquired from the left rACC = −0.863). This suggests that increased right PAG functional connectivity with the left rACC was associated with brain responses to noxious stimulation. (D) For women, as testosterone level increased, right PAG functional connectivity with the left insula was significantly decreased in pain+TENS compared to the pain-only condition (*r* between testosterone level and eigenvalue acquired from the left insula = −0.884). Pain ratings were assessed using a numerical rating scale (NRS) in which 0 = no pain, and 100 = the maximum imaginable pain. The TENS effect was calculated as the NRS pain rating in the pain-only condition minus that of the pain+TENS condition. The TENS effect was always positive because pain ratings in the pain-only condition were higher than those in the pain+TENS condition.

increased in women, right PAG functional connectivity with the bilateral orbitofrontal cortex significantly decreased for the pain+TENS compared to the pain-only condition. For both men and women, as cortisol levels increased, bilateral PAG functional connectivity with the left DLPFC was significantly decreased for pain+TENS compared to pain-only condition.

4. Discussion

TENS reduced pain and prevented temporal summation from repetitive noxious stimuli when TENS intensity was increased in a step-wise fashion. The TENS effect increased PAG functional connectivity with the lateral prefrontal cortex, probably resulting in activation of the descending pain-inhibitory pathway.

The superior parietal lobule, S1, S2, supramarginal cortex and right VLPFC were significantly activated in the pain+TENS compared to the pain-only condition. Because S1 and S2 contain neurons activated by innocuous and noxious somatosensory stimuli (Apkarian et al., 2013), they are related to the sensory discriminative dimension of innocuous and noxious somatosensory stimuli. Because thermal pain intensity (45 °C) was the same for both the pain-only and pain+TENS conditions, and because pain ratings and pain-related unpleasantness ratings were lower for pain+TENS compared to the pain-only condition, greater activation of the superior parietal cortex, S1, S2, supramarginal cortex and right VLPFC might have resulted from non-painful TENS. Simple regression analysis corroborated this, showing that the supramarginal cortex, bilateral S1 and right VLPFC were more highly activated in the pain+TENS compared to the pain-only condition as TENS intensity (mA) was increased. Several studies have shown that S1, S2, parietal and frontal cortices are activated by non-painful electrical stimulation (Forss et al., 1994; Boakye et al., 2000; Yuan et al., 2010). In this study, activation of S1, S2, supramarginal cortex and right VLPFC by non-painful TENS suggests that large-diameter afferent A-beta fibres were effectively stimulated, resulting in reduced pain sensations. Therefore, activation of S1, S2, supramarginal cortex and right VLPFC by non-painful TENS may have reduced pain perception in the pain+TENS condition. Although we were not able to infer a causal relationship between TENS effects and brain activation patterns, brain activation changes caused by TENS could contribute to pain relief during TENS.

In the present study, the left anteroventral thalamus and bilateral rACC were more highly activated in the pain-only compared to pain+TENS condition. It is known that anterior thalamic nuclei provide numerous projections to the ACC (Shibata and Naito, 2005). The ACC has been shown to process the affective-motivational dimension of pain (Apkarian et al., 2013). The right hippocampal cortex was more activated in the pain-only compared to pain+TENS condition in this study. The hippocampal cortex is activated in response to noxious stimulation, and is thought to contribute to the negative affect associated with pain and aversive drive mediation (Apkarian et al., 2005; Roy et al., 2009). As the T/C ratio in men decreased, right parahippocampal cortex was more activated in the pain-only condition. Pain and pain-related unpleasantness increased as T/C ratio decreased (Choi et al., 2014). Therefore, activation of the thalamus, rACC and hippocampal cortex may have led to greater pain and pain-related unpleasantness in the pain-only compared to the pain+TENS condition.

The right temporoparietal junction (TPJ) was more highly activated in the pain+TENS condition for women compared to men. Also, as the T/C ratio decreased, right TPJ activation increased in the pain+TENS condition. These results can be explained by the observation that the T/C ratio was lower in women than in men. The TPJ is involved in directing attention to salient events, and in detecting behaviourally relevant stimuli that are unexpected, novel, salient and potentially dangerous (Corbetta and Shulman, 2002). If women did attend more strongly to the noxious stimuli than men, their right TPJ cortices would have been more highly activated than those of men.

As the TENS effect increased, left PAG functional connectivity with the left, but not the right, lateral PFC (DLPFC and VLPFC) significantly increased in the pain+TENS compared to the pain-only condition. The lateral PFC and PAG have been implicated in endogenous pain inhibition (Apkarian et al., 2013). It is known that PAG is an important component of a supraspinal opioidergic circuit associated with pain modulation (Taylor et al., 2012). When repetitive transcranial magnetic stimulation is applied to the left DLPFC, an analgesic effect is produced (Taylor et al., 2012). It has been proposed that opioidergic analgesia is mediated by left, but not right, DLPFC activation (de Andrade et al., 2011; Taylor et al., 2012). It has been reported that elevated left DLPFC activity is significantly negatively correlated with pain affect (Lorenz et al., 2003). During the resting state fMRI, functional connectivity of the PAG with PFC is decreased in migraineurs with allodynia compared to migraineurs without allodynia (Mainero et al., 2011). Greater spontaneous pain in fibromyalgia patients results in less functional connectivity of the PAG with the executive attention network, including the DLPFC and posterior parietal regions (Napadow et al., 2010). When the TENS effect was increased, pain perception might be decreased by increasing PAG functional connectivity with the left lateral PFC and probably by activation of the descending pain-inhibitory pathway.

When cortisol was increased, bilateral PAG functional connectivity with the left DLPFC was significantly decreased in the pain+TENS compared to the pain-only condition. In a pain experiment conducted during stress conditions, salivary cortisol level was negatively correlated with pain threshold (Choi et al., 2012). As cortisol increases, pain perception might increase due to decreased bilateral PAG functional connectivity with the left DLPFC and, probably, decreased activation of the descending pain-inhibitory pathway.

With increased testosterone levels, right PAG functional connectivity with the DLPFC and right medulla was significantly increased in the pain+-TENS compared to pain-only condition. For male participants, higher testosterone levels were associated with an increased activation of the left precentral, right superior frontal (premotor area) and bilateral supramarginal cortices for the pain+TENS condition in comparison to the pain-only condition. Testosterone has anti-anxiety and analgesic effects (Edinger and Frye, 2005), and modulates opioid analgesia (Craft et al., 2004). During the follicular phase of the menstrual cycle, which is associated with lower pain compared to the luteal phase, left precentral cortex activation is associated with decreased unpleasantness rating and increased blood testosterone level (Choi et al., 2006). As the TENS effect increased, left PAG functional connectivity with the left precentral cortex significantly increased in the pain+TENS compared to the pain-only condition. Bilateral premotor areas in healthy men are activated by increased testosterone levels under placebo conditions (Choi et al., 2011).

For higher testosterone levels in women, right PAG functional connectivity with the left insula was significantly decreased in the pain+TENS compared to pain-only condition. There is significant cortical input to the PAG from pain-related brain cortices that include the insula, somatosensory areas and ACC (Linnman et al., 2012b). Functional con-

nectivity of the PAG with the anterior insula is increased for self-experienced pain as compared to observation of pain in others (Zaki et al., 2007). Pain perception is decreased when testosterone level is increased (Choi et al., 2012, 2014). Testosterone levels in this study were significantly negatively correlated with average pain rating. A high testosterone level might decrease pain perception by increasing right PAG functional connectivity with the DLPFC and right medulla, and by decreasing right PAG functional connectivity with the left insula.

For men, as the TENS effect increased, right PAG functional connectivity with the cerebellum, rACC, OFC and S1 decreased in the pain+TENS compared to pain-only condition. It has been reported that the cerebellar regions that are involved in pain processing show functional connectivity with the PAG, insula, ACC and S2 (Moulton et al., 2011). PAG functional connectivity with the cerebellum was significantly increased in women compared to men. Also, right PAG functional connectivity with the OFC was significantly increased in women compared to men. Because pain ratings in the pain+TENS condition were higher in women than in men, PAG functional connectivity with the cerebellum and right PAG functional connectivity with the OFC might be increased in women compared to men. The OFC is known to play a role in pronociception, although it has also been reported to have an antinociceptive role (Baliki et al., 2003). OFC activity has been positively correlated with pain (Lorenz et al., 2003). The TENS effect might decrease pain perception by decreasing right PAG functional connectivity with the cerebellum, rACC, OFC and S1.

As the T/C ratio increased in women, right PAG functional connectivity with the bilateral OFC was significantly decreased in the pain+TENS compared to the pain-only condition. As the T/C ratio in the pain-only condition decreased, left OFC activation increased. A high T/C ratio may enhance sensitivity to reward compared to punishment (Glenn et al., 2011). The T/C ratio in this study was significantly negatively correlated with average pain ratings. A high T/C ratio might decrease pain perception by decreasing right PAG functional connectivity with the OFC.

One limitation of this study is that we did not include a TENS control group, which could have allowed brain regions activated in responses to TENS per se to be directly identified. However, by subtracting brain regions activated in the pain-only condition from those activated in the pain+TENS condition, we indirectly identified those activated in response to TENS itself. These regions were also identified by simple regression (correlation) analysis. When TENS intensity (mA) was increased, the brain areas that were more highly activated in the pain+-TENS than the pain-only condition were the bilateral S1, right ventrolateral prefrontal cortex (VLPFC) and right supramarginal cortex, suggesting that these regions were activated in response to TENS per se.

5. Conclusion

This study is the first to investigate brain mechanisms of TENS pain relief using fMRI scanning. S1, S2 and parietal cortices were activated by non-painful TENS. The TENS effect increased PAG functional connectivity with the lateral prefrontal cortex, probably resulting in activation of the descending pain-inhibitory pathway, thus producing pain relief. Temporal summation from repetitive noxious stimuli was effectively prevented when TENS intensity was increased in a step-wise fashion, suggesting that pain may be reduced by this TENS application method.

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Author contributions

J.C.C., E.J.K. and J.H.K. designed the study. J.C.C., H.G.L., J.H.C. and Y.J.K. collected data. J.C.C., D.J.Y., J.H.K., J.M.L. and J.H.C. conducted the data analysis. J.C.C. and D.J.Y. wrote the manuscript. All authors read and approved the final manuscript.

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Supporting Information

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

Table S1. Main effects of brain activations in 2 × 2 factorial analysis [sex (men vs. women) x treatment (pain-only condition vs. pain+TENS condition)] (ANOVA in SPM8).
Table S2. Comparison between the pain-only and pain+-TENS conditions in 24 participants (paired t-test in SPM8).
Table S3. Covariate (TENS effect and hormones) effects on functional connectivity.