EJN European Journal of Neuroscience

FENS Federation of European Neuroscience Societies

European Journal of Neuroscience, Vol. 44, pp. 2966–2974, 2016 doi:10.1111/ejn.13443

CLINICAL AND TRANSLATIONAL NEUROSCIENCE

Venlafaxine and oxycodone effects on human spinal and supraspinal pain processing: a randomized cross-over trial

D. Lelic,[1] I. W. D. Fischer,[1,2] A. E. Olesen,[1,2] C. D. Mørch,[3] F. G. Arguissain,[3] J. A. B. Manresa,[3] A. Dahan[4] and A. M. Drewes[1,5]

[1]Mech-Sense, Department of Gastroenterology & Hepatology, Aalborg University Hospital, Mølleparkvej 4, 9000 Aalborg, Denmark

[2]Department of Drug Design and Pharmacology, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

[3]Department of Health Science and Technology, Aalborg University, Aalborg, Denmark

[4]Department of Anesthesiology, Leiden University Medical Center, Leiden, The Netherlands

[5]Department of Clinical Medicine, Aalborg University, Aalborg, Denmark

Keywords: brain source localization, EEG, evoked potentials, nociceptive withdrawal reflexes, opioid, serotonin and norepinephrine reuptake

Edited by Michel Barrot

Received 1 July 2016, revised 13 October 2016, accepted 13 October 2016

Abstract

Severe pain is often treated with opioids. Antidepressants that inhibit serotonin and norepinephrine reuptake (SNRI) have also shown a pain relieving effect, but for both SNRI and opioids, the specific mode of action in humans remains vague. This study investigated how oxycodone and venlafaxine affect spinal and supraspinal pain processing. Twenty volunteers were included in this randomized cross-over study comparing 5-day treatment with venlafaxine, oxycodone and placebo. As a proxy of the spinal pain transmission, the nociceptive withdrawal reflex (NWR) to electrical stimulation on the sole of the foot was recorded at the tibialis anterior muscle before and after 5 days of treatment. For the supraspinal activity, 61-channel electroencephalogram evoked potentials (EPs) to the electrical stimulations were simultaneously recorded. Areas under curve (AUCs) of the EMG signals were analyzed. Latencies and AUCs were computed for the major EP peaks and brain source analysis was done. The NWR was decreased in venlafaxine arm ($P = 0.02$), but the EP parameters did not change. Oxycodone increased the AUC of the EP response ($P = 0.04$). Oxycodone also shifted the cingulate activity anteriorly in the mid-cingulate-operculum network ($P < 0.01$), and the cingulate activity was increased while the operculum activity was decreased ($P = 0.02$). Venlafaxine exerts its effects on the modulation of spinal nociceptive transmission, which may reflect changes in balance between descending inhibition and descending facilitation. Oxycodone, on the other hand, exerts its effects at the cortical level. This study sheds light on how opioids and SNRI drugs modify the human central nervous system and where their effects dominate.

Introduction

Pain is one of the most frequently presented symptoms of many diseases. One in five adults in the Western world suffers from chronic pain, leading to decreased quality of life and wide-ranging socioeconomic consequences (Breivik *et al.*, 2006; Sjogren *et al.*, 2009; Langley *et al.*, 2010a,b). Severe pain is often treated with opioids (Liu & Wu, 2007). Antidepressant drugs with effect on serotonin and norepinephrine reuptake inhibition (SNRI) are also used in chronic pain, but the specific mode of action in humans remains vague (Marks *et al.*, 2009). Previous studies have suggested that opioids attenuate the affective components of pain possibly by interaction with the medial pain system (i.e. medial thalamus, anterior cingulate cortex, anterior insula) and brainstem structures exerting a negative feedback on sensory pathways (Price *et al.*, 1985; Sprenger *et al.*, 2006). The role of anterior cingulate cortex (ACC) and insula has been repeatedly reported, in both imaging and cortical evoked potential (EP) brain source localization studies (Wise *et al.*, 2002; Brooks & Tracey, 2005; Petrovic, 2005; Leppa *et al.*, 2006). Evoked potentials (EPs) have been receiving increasing attention due to the excellent temporal resolution on millisecond time scale and brain source localization methods make it possible to estimate the brain areas underlying the EPs. The EPs give good assessment of alterations in the brain following drug administration. However, it is important to also investigate what happens at the spinal level simultaneously to gain understanding of the interaction between the spinal cord and the brain during treatment with analgesics. In this regard, the nociceptive withdrawal reflex (NWR) is a widely used

Correspondence: D. Lelic, as above.
E-mail: dl@mech-sense.com

technique to study spinal pain transmission (Sandrini *et al.*, 2005). The NWR is a spinal reflex that combines afferent input, descending modulatory signals, descending motor commands and incorporates the spinal motor systems to determine the appropriate withdrawal response of the limbs to escape from a potentially painful stimulus (Andersen, 2007). Serotoninergic and noradrenergic pain modulatory systems have shown to interact at the spinal cord level to produce a more powerful antinociception (Pertovaara, 2006). Both, serotoninergic drugs and opioids have been shown to modulate the NWR thresholds (Willer & Bussel, 1980; Willer, 1985; Willer *et al.*, 1985; Sandrini *et al.*, 1986a,b).

In the current randomized, double-blind, three-way crossover trial, we combined NWR and EPs to assess spinal and supraspinal neural activity. We hypothesized that venlafaxine (an SNRI) would primarily induce changes on the modulation of spinal nociceptive transmission and that oxycodone (an opioid) would affect the supraspinal processing to a greater extent. Therefore, the primary objective of this study was to investigate central changes at spinal and supraspinal levels due to oxycodone and venlafaxine. In order to test our hypotheses, we compared changes from the baseline due to placebo, venlafaxine and oxycodone for: (i) area under curve (AUC) of the NWR, (ii) latencies and AUC of the major EP components and (iii) brain networks underlying the EPs.

Methods

The trial was registered with the European Clinical Trials Database (Eudra-CT 2013-000170-30, registration date: 2013-03-04). The study was conducted according to the Declaration of Helsinki. The local Ethics Committee (N-20130011) and the Danish Medicines Agency (201300017030) approved the study. The study was conducted in the laboratories at the Department of Gastroenterology, Aalborg University Hospital according to the rules of Good Clinical Practice and monitored by the Good Clinical Practice unit, Aalborg and Aarhus University Hospitals, Denmark. The volunteers took each drug over 5-day period. Side effects (nausea, vomiting, headache, dizziness, sedation, mouth dryness, rapid heart rate, constipation, itching, low appetite, increased sweating, general discomfort) were recorded on a 5-point Likert scale (i.e. 0 = no side effect, 1 = minimum side effect, 2 = moderate side effect, 3 = high side effect and 4 = very high side effect) each day. Sensory and neurophysiological assessments were done on days one and five of each treatment arm.

Study volunteers

Based on previous studies (using same experimental models) and based on power calculations ($N = ((Z_{\alpha/2} \times SD)/E)^2$) where α was 0.05, the power of statistical analysis was set to 0.95, SD was chosen from pain ratings from previous studies, and E was set to 1, the required number of subjects was: $N = ((1.96 \times 2.3)/1)^2 = 20.3$. Hence, 20 volunteers (all male, mean age 24.6 $\pm$ 2.5) participated in this study.

Before inclusion, a medical doctor responsible for enrolling the volunteers conducted a routine health screening for each participant, ruling out any pain-related conditions and history of abuse (participant and closest family). Moreover, before enrollment, the volunteers gave written informed consent acknowledging that all methods and procedures used in the experiment were understood, that they were aware that they were going to experience pain and were free to withdraw from the experiment at any time. Inclusion criteria for the study were: (i) normal medical examination; (ii) age between 20 and 35 years; (iii) male; (iv) able to read and understand Danish; and (v) Scandinavian origin.

Drug and placebo administration

This was a randomized, double-blind, three-way crossover study, with minimum 1 week 'wash-out' intervals. Oxycodone has a plasma half-life of 4–6 h and the plasma half-life of venlafaxine is 5 h (pro.medicin.dk), thus 1-week wash-out interval is long enough for the drug to be out of the system.

Oxycodone and venlafaxine were formulated as orally administered tablets similar to each other and to placebo. The dosages for oxycodone were 10 mg extended release and for venlafaxine, the dosages were 37.5 mg extended release. These are the lowest clinical therapeutic dosages (pro.medicin.dk). A steady-state plasma concentration is reached within 24 h for oxycodone and within 72 h for venlafaxine treatment. Hence, 4 days of treatment was considered appropriate. All drugs followed the same administration: on day 1 and day 5 once, and on day 2–4 b.i.d. in total eight doses. The medications were taken at 8.30 in the morning and 8.30 in the evening (i.e. every 12 h). The tablets were produced by the pharmacy at Aarhus University Hospital. The pharmacy generated a randomization list by www.randomization.com, where all participants were randomized to receive venlafaxine, oxycodone or placebo for specific periods. Mirror randomization was employed in case of participant drop-outs. The staff at the Hospital Pharmacy packed and labeled the medication to ensure that all participants received correct medication for specific periods. The medication was labeled as period 1, 2 or 3. Thus, the experimenters and the participant were fully blinded for randomization.

Electrical stimulation

Electrical stimulation of the plantar skin (site of innervation of the medial plantar nerve) was applied through surface electrodes to evoke the NWR and EPs. The cathode was placed in the arch of the sole of the right foot (15 mm $\times$ 15 mm, Neuroline 700; Ambu A/S, Denmark) (Jensen *et al.*, 2015). The anode for stimulation was an electrode placed on the foot dorsum (50 mm $\times$ 90 mm, Synapse; Ambu A/S). The stimulus was delivered by a computer-controlled electrical stimulator (Noxitest IES 230, Aalborg, Denmark) as a constant current burst of five square-wave pulses, with 1 ms duration and 5 ms between pulses. Subjects felt each of these bursts as a brief, single stimulus. A custom-made LABVIEW software (Center for Sensory-Motor Interaction, Aalborg University, Denmark) was used to control the electrical stimulation. For each of the experimental days, sensory threshold and NWR threshold (RT) were found by slowly increasing the stimulus intensity in 1 mA steps. The sensory threshold was detected as the stimulation intensity at which the subjects first felt the stimulus. The RT was defined as the initial simultaneous flexion of the ankle, knee and hip. Once the RT was found, the volunteers were asked to rate the RT, 1.3 $\times$ RT and 1.6 $\times$ RT on pain and unpleasantness scales. Each scale ranged from 0 to 10, 0 meaning no pain/unpleasantness and 10 meaning maximum imaginable pain/unpleasantness. Subsequently, 18 stimuli (six times each of the three intensities in random order) were applied with a varying inter-stimulus interval of 8–12 s.

EMG recordings

The NWR was evaluated by surface EMG recordings of the ipsilateral tibialis anterior (TA) muscle. Two surface electrodes (15 mm $\times$ 15 mm, Neuroline 700; Ambu A/S) were placed on the belly of the right tibialis anterior (TA), 1/3 on the line from the tip of the fibula to the tip of the medial malleolus. The skin was lightly

abraded before the placement of electrodes. The ground electrode (50 mm × 90 mm, Synapse; Ambu A/S) was placed just under the right knee. The EMG responses were amplified (20 000–50 000 times), bandpass filtered (5–500 Hz), sampled (2 kHz) and stored (100 ms before to 900 ms after stimulation onset) for later analysis.

EP recordings

A 61 surface electrode EEG cap (MEQNordic A/S, Jyllinge, Denmark) was used. The reference electrode was just above AFz. Electrode gel was applied to reduce the electrode impedance below 10 kΩ. During reflex stimulations, the subjects relaxed quietly with eyes open. The volunteers were asked to minimize the eye and body movement. The EP signals were recorded in continuous mode with a sampling rate of 1000 Hz (SynAmp; Neuroscan, El Paso, TX, USA) and stored offline for further analysis.

Experimental procedure

On day 1, experiment was conducted before drug administration (baseline recording) and on day 5, the experiment was conducted after the volunteer took the last dose of the medication. The participants were placed in an arm chair allowing them to comfortably sit in a supine position with back support inclined 120° relative to the horizontal level. Pillows were placed under the volunteer's right knee to flex the knee joint approximately 30°.

Data analysis

EMG analysis

The quantification of NWR was performed by applying area under curve (AUC) calculation on the rectified EMG signal in the interval between 60 and 160 ms after the stimulation onset. This time interval was chosen based on conduction velocity studies of nociceptive afferents; if evoked, any activity before 60 ms would be not nociceptive and after 160 ms would likely be due to voluntary movement (Andersen, 2007). The quantification was performed on each single NWR and the AUC values were then averaged for each of the experimental days and for each subject for final analysis.

EP analysis

The EPs from each session were analyzed offline. The data were pre-processed using Neuroscan software (v 4.3.1; Neuroscan) as follows: (i) bandpass filtered between 1 and 30 Hz, (ii) epoched from 50 ms before the stimulus to 950 ms after and (iii) averaged.

Latencies of EP peaks at the central electrode (Cz) were identified and compared between the three treatments with respect to their baselines. This electrode was favored because of its central location and maximal EP amplitude due to the electrical stimulation on the foot. Then, the AUC of the global field power (GFP) of the main EP peak was computed. GFP was utilized in order to account for all the electrodes on the scalp. Finally, brain source network analysis was done on the 61-channel recording in order to study the underlying brain networks generating the EPs. The brain source network analysis has been described in detail elsewhere (Lelic *et al.*, 2012b), but briefly: (i) EP data were decomposed by multichannel matching pursuit (MMP) into components well defined in time and frequency (Durka *et al.*, 2005). Then, similar MMP components in time-frequency for each subject (six datasets, three baselines and three treatments) were clustered together by an in-house developed clustering method (Lelic *et al.*, 2011). MMP decomposition and clustering were done in MATLAB (version 8.4.0; The Mathworks Inc., Natick, MA, USA). The clusters which were similar between subjects were then visually identified and used for final analysis; (ii) Brain source localization was applied to the MMP components. As MMP components are mono-frequency and have single topographies, each component is either generated by a single source or by a set of sources that operate synchronously. Inverse modeling on the MMP components was done in Brain Electrical Source Modelling (BESA) (BESA research 5.3; MEGIS software GmbH, Gräfelfing, Germany); and (iii) Brain source strengths were computed for each of the brain sources in the network by calculating the AUC of the rectified source waveforms. Then, the contribution of each brain source to the network was calculated as follows: (i) source strengths of all brain sources in the network were summed up. Let this sum be characterized by k and let m represent the AUC of each single source; and (ii) percentage of each source strength was calculated as $(m/k) \times 100$. This percentage represents how much each source contributes to the network.

Statistical analysis

Descriptive statistics are reported as mean ± SD. To compare data of the three treatments from their baselines, analyses were done using two-way repeated measures analysis of variance (RM-ANOVA) with treatment (placebo, oxycodone, venlafaxine) and time (day 1, day 5) as the two main factors. The interaction between these two factors was assessed to investigate the following variables: (i) stimulation intensity (in mA) required to evoke NWR; (ii) unpleasantness and pain scores for the three stimulation intensities (1 × RT, 1.3 × RT, 1.6 × RT); (iii) AUCs of NWR; (iv) latencies and AUCs of EPs; (v) source locations, and (vi) source strengths. If significant interaction was seen, pairwise multiple comparison procedures (Holm-Sidak method) were done in order to see due to which treatment the difference between days occurred. $P \leq 0.05$ was considered significant. The software package SIGMA STAT v.3.0 (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis.

Results

The first subject was enrolled on 21/11/2013 and the last subject's last visit was on 12/12/2014. NWR and EPs were recorded in all 20 volunteers. The electrical stimulations were well tolerated and all subjects completed the study without any complications.

Sensory parameters

Electrophysiological data and statistics are presented in Table 1. The sensory and NWR thresholds were not significantly different between treatments ($F_{2,38} = 1.0$; $P = 0.4$ for sensory; $F_{2,38} = 2.3$; $P = 0.1$ for NWR). The pain scores did not differ between treatments for either of the intensities (all $F_{2,38} < 1.2$; $P > 0.1$). Although unpleasantness scores decreased on day 5 for all three treatments ($P < 0.01$), they were not significantly different between treatments for either of the intensities (all $F_{2,38} < 1.3$; $P > 0.1$).

Nociceptive withdrawal reflexes

Two of the subjects had noisy NWR recordings in at least one visit where the peaks could not be detected and hence these data were discarded from the final analysis. The NWR AUC results are shown in Table 2. The drug effect on the NWR was significant

TABLE 1. (a) Sensory data. (b) Pain and unpleasantness scores on numeric rating scale

(a)

	NWR threshold (mA)					
	Placebo		Oxycodone		Venlafaxine	
	Baseline	Treatment	Baseline	Treatment	Baseline	Treatment
Sensory	2.7 ± 0.7	2.4 ± 0.5	2.8 ± 0.7	2.6 ± 0.8	2.4 ± 0.7	2.6 ± 0.7
NWR	14.4 ± 7.8	14.5 ± 9.4	12.6 ± 7.8	12.7 ± 7.0	13.9 ± 8.3	14.2 ± 7.1

(b)

	Pain (1*RT)	Unpleasant (1*RT)	Pain (1.3*RT)	Unpleasant (1.3*RT)	Pain (1.6*RT)	Unpleasant (1.6*RT)
Placebo						
Baseline	2.3 ± 2.1	3.3 ± 1.6	3.2 ± 2.5	4.3 ± 2.3	3.9 ± 2.5	5.0 ± 1.9
Treatment	1.7 ± 1.6	3.0 ± 2.1	2.5 ± 1.9	**3.5 ± 2.0**$^{+}$	3.2 ± 2.0	**3.9 ± 2.1**$^{+}$
Oxycodone						
Baseline	2.1 ± 1.8	3.6 ± 2.0	2.4 ± 1.8	3.9 ± 2.1	3.4 ± 2.0	4.5 ± 1.9
Treatment	1.7 ± 1.8	**2.6 ± 1.9**$^{+}$	2.3 ± 2.0	3.4 ± 1.9	3.0 ± 2.4	**3.8 ± 2.2**$^{+}$
Venlafaxine						
Baseline	1.5 ± 1.6	3.0 ± 2.1	2.5 ± 2.0	4.2 ± 2.1	3.1 ± 2.0	4.7 ± 1.7
Treatment	1.5 ± 1.0	**2.3 ± 1.5**$^{+}$	2.5 ± 1.6	**3.4 ± 2.0**$^{+}$	3.4 ± 2.1	4.3 ± 2.3

The significant values have bold caption. NWR, nociceptive withdrawal reflex; RT, reflex threshold. $^{+}P < 0.05$.

TABLE 2. Nociceptive withdrawal reflex results

	Placebo AUC	Oxycodone AUC	Venlafaxine AUC
Baseline	27.0 ± 15.7	22.9 ± 15.8	23.7 ± 11.9
Treatment	28.3 ± 17.9	24.1 ± 17.0	**17.6 ± 10.2**$^{+}$

The significant values have bold caption. AUC, area under curve. $^{+}P < 0.05$.

($F_{2,38} = 4.5$; $P = 0.02$). The *post hoc* tests showed that venlafaxine decreased the NWR AUC ($P < 0.05$), whereas there were no differences in placebo or oxycodone experimental arms.

Evoked potentials

The EP in response to electrical stimulation had a triphasic shape. The most prominent peak occurred around 100 ms (see Fig. 1 and Table 3 for EP details). All latencies remained unchanged in all three treatment arms (all $F_{2,38} < 1.3$; $P > 0.2$). The AUC of the triphasic potential was increased due to treatment ($F_{2,38} = 3.3$; $P = 0.04$) and the *post hoc* tests revealed that oxycodone increased the size of the EPs ($P < 0.05$), whereas no differences in the AUC were observed due to placebo and venlafaxine treatment.

Brain source localization

Two main brain networks underlying the EPs that were consistent between volunteers were found: the anterior cingulate – operculum network at 2.4 Hz (Delta band) and mid cingulate – operculum network at 4.2 Hz (Theta band). Throughout this article, we use the term operculum to represent the brain structures insula and secondary somatosensory cortex, as they are anatomically very close. These two networks were analyzed in detail, in order to observe how they were modified due to each treatment and the results are shown in Table 4 and visualized in Fig. 2.

No changes were seen in the anterior cingulate – operculum network for any of the arms (all $F_{2,38} < 2.7$; $P > 0.1$). In the mid cingulate – operculum network, there was a shift of cingulate activity following drug treatment ($F_{2,38} = 4.9$; $P < 0.01$) and the *post hoc* analysis revealed that oxycodone treatment resulted in a more anterior shift of the cingulate activity ($P < 0.05$), while there were no changes due to placebo or venlafaxine. There was also a difference in the contribution of each source to the network ($F_{2,38} = 5.2$; $P = 0.02$). The *post hoc* analysis revealed that the contribution of cingulate activity to the network increased while the contribution of the operculum activity decreased following oxycodone treatment ($P < 0.05$). There were no significant differences due to placebo or venlafaxine.

Side effects

All volunteers experienced different, non-serious side effects as listed in Table 5. However, none of these were of a degree that resulted in an interruption of the experiment.

Discussion

This study investigated how oxycodone and venlafaxine modify the nociceptive withdrawal reflexes and the corresponding brain networks as compared to placebo. We showed that the spinal nociceptive withdrawal reflex was decreased in venlafaxine arm. Oxycodone, on the other hand, modified the cortical evoked response to the electrical stimulation. Hence, venlafaxine induced changes in the modulation of spinal nociceptive transmission to a greater extent, whereas oxycodone induced larger changes at the cortical level.

Sensory properties and nociceptive withdrawal reflexes

None of the three treatments significantly changed the stimulation intensity required to evoke a NWR or the pain scores to electrical stimulation. This is in line with previous studies that investigated the effects of low dosage opioids on the NWR. Willer (1985) investigated reflex and pain thresholds before and after four doses of intravenous (IV) administered morphine chlorhydrate (0.05, 0.1, 0.2

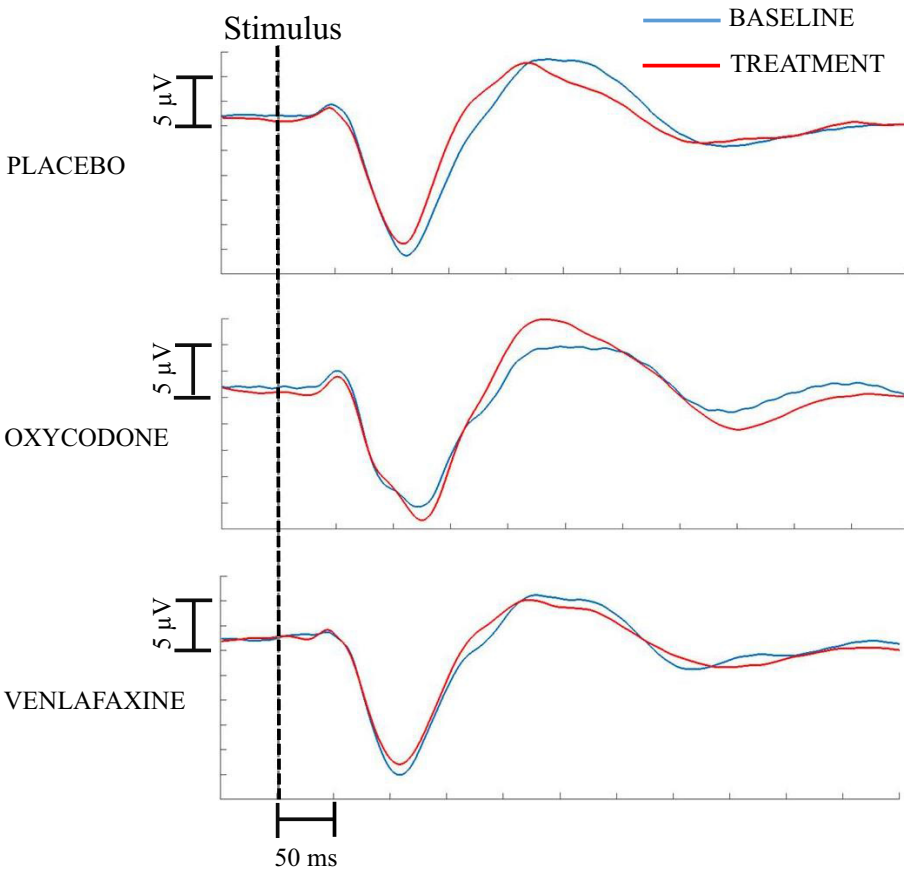

FIG. 1. Cortical evoked potentials at central scalp electrode (Cz) from one representative subject. It can be seen that the latencies are similar between the three arms as compared to placebo, whereas the amplitude in the oxycodone arm is increased. [Colour figure can be viewed at wileyonlinelibrary.com].

TABLE 3. Evoked potentials

	Placebo	Oxycodone	Venlafaxine
Peak 1 Latency (ms)			
Baseline	53.8 ± 11.5	51.0 ± 4.8	51.2 ± 6.1
Treatment	50.4 ± 5.0	51.4 ± 5.9	51.7 ± 6.8
Peak 2 Latency (ms)			
Baseline	108.1 ± 14.3	105.8 ± 13.2	106.6 ± 14.2
Treatment	104.6 ± 12.4	104.6 ± 13.0	107.9 ± 14.2
Peak 3 Latency (ms)			
Baseline	192.7 ± 27.7	194.3 ± 23.5	192.2 ± 25.5
Treatment	192.5 ± 23.1	193.7 ± 23.9	190.8 ± 22.9
AUC			
Baseline	86.9 ± 24.6	86.6 ± 24.2	90.7 ± 32.0
Treatment	87.7 ± 22.0	***93.4 ± 25.5***$^{+}$	89.2 ± 25.5

AUC, area under curve; $^{+}P < 0.05$. The significant values have bold and italic caption.

and 0.3 mg/kg) and found that low dose of morphine (0.05 mg/kg) left the thresholds unchanged. On the other hand, higher doses increased the thresholds in a linear fashion (i.e. very slight increase for 0.1 mg/kg and larger increases for 0.2 and 0.3 mg/kg), indicating a close relationship between the effects of morphine and the NWR. Bossard *et al.* (2002), instead, found that IV administration of 0.1 mg/kg of morphine did not change the RT unless it was used in combination with ketamine. In our study, we used 10 mg of orally administered oxycodone in all the volunteers, which is equal to approximately 5 mg IV (i.e. 0.07 mg/kg for a 70 kg person) administered morphine. Hence, our results are in agreement with the above studies where low dose of IV administered morphine did not affect the RT. Using higher dosage of oxycodone was not feasible in this study. Previous studies that used higher dosage of opioids and IV infusion were done in 1 day per treatment and hence, once the treatment was done, the drug was out of the bloodstream within hours and the healthy volunteers could continue with their normal life. The present oxycodone treatment lasted 5 days, in which the volunteers experienced side effects such as drowsiness, nausea, etc. Increasing the dosage would likely increase these side effects and decrease the volunteers' quality of life during the time of the treatment. Thus, ethical constraints also prevent an increase of the dosage.

Venlafaxine did not change the RT either. This is in line with Matthey *et al.*'s (2013) study who investigated the effects of Milnacipran's (an SNRI) on the NWR over a 3-week treatment period with increasing dosage. In this study, however, venlafaxine changed the magnitude of the NWR recorded at tibialis anterior, which did not occur due to oxycodone or placebo treatment. This observation probably suggests that, at the given dose, venlafaxine influenced the modulation of spinal nociceptive transmission, despite the lack of change in RT. Venlafaxine may inhibit serotonin and noradrenaline reuptake in a disproportionate manner and a change in either of the two systems due to venlafaxine could affect the other. It has been demonstrated that low doses of venlafaxine (75 mg/day) did not inhibit the noradrenaline reuptake process in healthy volunteers

TABLE 4. Brain source details

	Network 1						Network 2					
	Anterior Cingulate (Brain source 1)			Operculum (Brain source 2)			Mid-cingulate (Brain source 1)			Operculum (Brain source 2)		
	X	Y	Z	X	Y	Z	X	Y	Z	X	Y	Z
Baseline												
MEAN (mm)	−2.3	24.7	24.6	±41.6	−3.7	1.6	−0.2	4.3	33.1	±41.9	−1.3	3.8
SD	3.6	8.3	6.2	3.0	8.1	4.4	3.8	14.8	6.5	3.9	9.0	4.7
AUC		34.8±14.4			22.1±21.1			16.1±10.4			15.0±5.9	
Contribution(%)		64.4±10.9			35.6±10.9			49.1±14.8			51.0±14.8	
Frequency (Hz)			2.4±0.6						4.3±1.0			
Placebo												
MEAN (mm)	−3.6	22.1	24.4	±42.1	−1.2	0.4	0.7	2.6	35.8	±40.7	2.1	2.1
SD	4.1	13.5	10.6	4.0	8.2	4.3	4.9	15.9	7.8	4.5	8.3	3.9
AUC		33.5 ± 14.2			19.0 ± 11.8			15.6 ± 10.5			15.4 ± 7.4	
Contribution(%)		62.4 ± 16.1			37.6 ± 16.1			47.1 ± 17.4			53.0 ± 17.4	
Frequency (Hz)			2.4 ± 0.7						4.3 ± 0.9			
Oxycodone^{+}												
MEAN (mm)	−1.3	21.1	24.2	±35.6	−0.1	0.2	−1.9	***14.0***$^{+}$	30.8	±42.2	−2.9	4.6
SD	3.6	12.9	6.9	19.0	7.6	6.1	7.2	14.3	7.7	5.2	7.9	3.5
AUC		32.5 ± 16.9			21.8 ± 13.3			16.5 ± 8.2			13.1 ± 9.0	
Contribution(%)		59.4 ± 16.2			40.6 ± 16.2			***55.4*** ± ***10.6***$^{+}$			***44.6*** ± ***10.6***$^{+}$	
Frequency (Hz)			2.4 ± 0.6						4.2 ± 0.9			
Venlafaxine												
MEAN (mm)	−1.3	19.6	26.1	±41.1	−1.6	0.2	−0.8	8.9	33.1	±41.2	−3.0	4.4
SD	4.1	11.4	5.9	4.4	7.0	4.6	5.4	12.6	7.6	5.7	8.0	3.8
AUC		34.8 ± 16.7			26.1 ± 28.8			17.0 ± 10.1			13.3 ± 5.6	
Contribution(%)		59.9 ± 12.9			40.1 ± 12.9			54.1 ± 15.2			45.9 ± 15.2	
Frequency (Hz)			2.6 ± 0.6						4.3 ± 0.9			

SD – standard deviation; AUC – area under curve; *Dipole coordinates:* X – lateral/medial, Y - anterior/posterior, Z – inferior/superior; $^{+}$ - $P < 0.05$. The significant values have bold caption.

(Harvey *et al.*, 2000; Blier *et al.*, 2007). However, peripheral rather than central measures were used to assess drug effects on noradrenaline function. Thus, knowledge on central measures in humans is still lacking. Moreover, there is a dual role of serotonergic mechanisms in the expression of descending inhibition and descending facilitation (Millan, 2002). Thus, the net effect will be a balance between pro- and antinociceptive roles of descending serotonergic pathways. Hence, the changes in the NWR due to venlafaxine administration possibly reflect a change in balance between descending inhibition and descending facilitation. The lack of change of the activity recorded at TA due to oxycodone is in line with previous low dosage opioid studies (Willer, 1985; Bossard *et al.*, 2002). Hence, although it has been established that opioids exert their effects at the spinal level, the doses in this study were not sufficient to see these effects.

Although the reflex thresholds were not significantly changed due to either treatments, the subjective unpleasantness ratings were decreased in all three treatments. As this reduction in ratings occurred in all three arms, it is likely that there was a time effect as the volunteers were more comfortable and used to the electrical stimulation on day 5.

Evoked potentials

The EPs in the placebo and venlafaxine arms were unchanged compared to the baseline EPs, whereas they were increased in the oxycodone arm. Studies involving EPs to electrical stimulation eliciting a NWR in combination with opioids are scarce. Nonetheless, the increase in amplitudes following oxycodone administration was to authors' surprise. A number of studies involving somatosensory EPs (i.e. median nerve, tibial nerve) and opioids in humans normally showed a decrease in amplitudes (Freye *et al.*, 1986; McPherson *et al.*, 1986; Schubert *et al.*, 1987; Kalkman *et al.*, 1988; Kimovec *et al.*, 1990). However, these studies have typically explored higher dosage of anesthetic opioids and oxycodone is an analgesic opioid. The central effect of opioids likely varies depending not only on the type of the opioid (i.e. analgesic or anesthetic), but also on the dosage. A study investigating dose-dependent effect of remifentanil on somatosensory EPs in patients undergoing elective surgery, showed that after tracheal intubation, remifentanil increased the early EP amplitudes, whereas high doses decreased them (Crabb *et al.*, 1996). Another aspect to take into consideration is the specificity of the type of stimulation used in this study. Non-invasive, high-current electrical

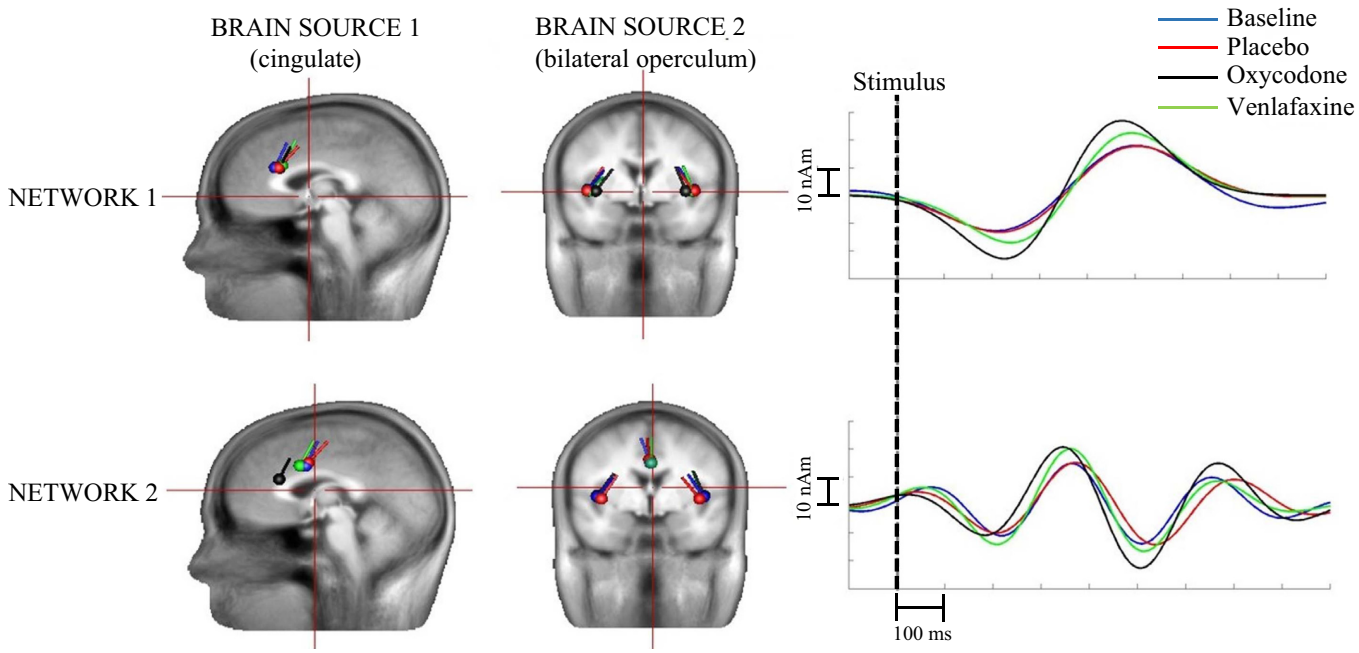

FIG. 2. Brain networks underlying the cortical evoked potentials. The grand mean data are shown, although analysis was done on individual basis. There were two dominant brain networks: anterior cingulate-operculum network (Network 1 on top of the figure) and mid cingulate-operculum network (Network 2) on bottom of the figure. The left part of the figure shows the source locations and the right part of the figure shows the time-course of the network multichannel matching pursuit (MMP component at a single electrode/Cz). Each brain source in the network had the same time waveform as the MMP component, the only difference between the source waveforms was the amplitude (source strength) which is presented in Table 4 as area under curve (AUC) of the source waveform. There were no significant changes in brain source locations in Network 1. Frontal shift of the cingulate source in the oxycodone arm can be seen in Network 2. [Colour figure can be viewed at wileyonlinelibrary.com].

TABLE 5. Side effects. The numbers represent the number of volunteers that had the side effect present. All of the volunteers that had side effects reported them to be ≤3 on 5-point Likert scale (i.e. 0 = no side effect, 1 = minimum side effect, 2 = moderate side effect, 3 = high side effect and 4 = very high side effect)

Side effect	Placebo	Oxycodone	Venlafaxine
Nausea	0	3	10
Vomiting	0	0	0
Headache	2	2	4
Dizziness	0	4	6
Sedation	2	8	5
Mouth dryness	0	0	8
Rapid heart rate	0	1	1
Constipation	0	1	0
Itching	0	6	0
Low appetite	1	0	2
Increased sweating	1	2	0
General discomfort	0	5	8

stimulation concomitantly activates both non-nociceptive Aβ and nociceptive Aδ fibers, and EPs consequently reflect the processing of both afferents. In line with this, it has been shown that the EPs in response to electrical stimulation at intensities above RT do not carry enough information to distinguish between different levels of stimulation intensity, which could also explain why there were changes in the NWR following venlafaxine treatment, but not in the EPs (Arguissain *et al.*, 2015). Furthermore, a number of studies suggest that EPs, regardless of the type of stimulation used, might largely reflect activity related to the detection of salient, potentially threatening sensory stimuli (Iannetti *et al.*, 2008; Mouraux & Iannetti, 2009).

To authors' knowledge, previous research in somatosensory/pain EPs and SNRI drugs has not been done in humans and hence this is the first study ever to report SNRI effect on cortical EPs. The lack of changes in the EPs in combination with the observed changes in the NWR suggests that venlafaxine mainly exerts its effects at the spinal level. It is possible that in order to observe the venlafaxine effects in the brain, the treatment should have been longer than 5 days (i.e. at least 2 weeks) (pro.medicin.dk).

Brain Networks

The brain network analysis method used in this study has been validated before and has been shown to be sensitive to detect changes due to analgesics (Lelic *et al.*, 2012a,b, 2014). The anterior cingulate-operculum and mid cingulate-operculum networks were the two dominant brain networks in all three arms. Cingulate cortex together with operculo-insular cortex is the most often reported brain area in pain studies (Mauguiere, 2004; Olesen *et al.*, 2010; Wiech *et al.*, 2010; Brock *et al.*, 2012). Cingulate cortex has an important role in emotional and attentional processing of pain stimulus and operculo-insular cortex is said to be the first activated cortical region that sets off the brain networks involved in pain experience (Isnard *et al.*, 2011). Due to its importance in pain processing, Garcia-Larrea *et al.* (2010) refer to the operculo-insular cortex as the third somatosensory area processing pain. Brain network analysis revealed that after oxycodone administration, there was a forward shift in the mid cingulate-operculum network. This finding is in line with previous studies where the effect of morphine on esophageal EPs (Lelic *et al.*, 2012a) and rectal EPs (Lelic *et al.*, 2014) revealed a frontal shift of cingulate activity at low frequencies (2–4 Hz). Additionally, after 5 days of oxycodone treatment, the cingulate activity was increased in mid-cingulate-operculum network, whereas the opercular activity was decreased. Hence, the increased AUC of the EPs is likely due to the increased synchronization of brain activity within

cingulate cortex following oxycodone treatment. Previous studies in animals and humans point to prefrontal cortex and cingulate cortex having a high density of mu opioid receptors (Wamsley *et al.*, 1982; Sadzot *et al.*, 1991; Schoell *et al.*, 2010). Therefore, the frontal shift of the cingulate activity is likely due to activation of mu opioid receptors in this brain region. Additionally, as the frontal shift of the cingulate activity makes network two almost identical in location to the anterior cingulate/operculum network 1, this alteration in activity could also imply a change of frequency content of network 1 due to oxycodone. In the placebo and venlafaxine arms, analysis revealed consistent brain networks between baseline and treatment conditions. The absence of an effect of venlafaxine on EPs and brain networks could be because the changes are mainly exerted at the brainstem level. This makes it extremely difficult to observe with the current setup, as usually thousands of trials are required to detect activity in such deep brain areas, whereas the number of stimuli that are delivered in order to elicit the NWR is normally limited to less than a hundred per session due to the pain and discomfort that the stimulation evokes. Moreover, as SNRI drugs mainly affect descending inhibition (Marks *et al.*, 2009), which is a spinal occurrence, it seems reasonable that the changes were primarily seen in the NWR. In contrast to previous studies, in this study, we recorded both spinal and supraspinal activity simultaneously and hence could separate spinal from supraspinal activity and exclude that any change in spinal activity is due to cortical change and vice versa.

Study considerations

Although this study shed some light on how the central nervous system (CNS) is affected by oxycodone and venlafaxine treatment, some considerations need to be noted. It can be argued that the central changes seen in this study are questionable because there were no changes in pain ratings in response to the electrical stimulation in either of the experimental arms. However, the pain scores of the brief, phasic electrical stimuli on day 1 and 5 are subjective verbal ratings. It has been previously reported that the sensitivity of the numerical verbal scales is generally low (Williamson & Hoggart, 2005) and recalling pain ratings is less reliable in large time spans (Erskine *et al.*, 1990; Biurrun Manresa *et al.*, 2011). On the other hand, both EPs and the NWR are objective measures of central activity, which may better reflect changes in the CNS due to drug administration.

The venlafaxine treatment was given over a 5-day period and treatment with SNRIs should be at least 2 weeks in order to observe maximal clinical effect (pro.medicin.dk). As mentioned before, giving a drug like venlafaxine to healthy volunteers for 2 weeks or longer is not ethically feasible. Moreover, effects of venlafaxine on the pain system have been observed already after few days of treatment (Enggaard *et al.*, 2001). Although a longer treatment would be desirable to observe the maximal clinical effect, the 5-day treatment in this study was long enough to detect changes in the CNS.

Conclusions

This study showed differences in pain processing of NWR between venlafaxine and oxycodone as compared to placebo at spinal and cortical levels. Venlafaxine exerts its effects on the processes modulating spinal nociceptive transmission to a greater extent, which may reflect changes in balance between descending inhibition and descending facilitation. Oxycodone, on the other hand, exerts its effects at the cortical level (frontal activity shift within cingulate cortex) to a greater extent. This study sheds light on how opioids and SNRI drugs modify the human CNS and where their effects dominate. As there is an unmet need for human models to explore the mechanisms of drugs with effect on the CNS, the current approach could be used to explore the consequences to treatment with analgesics and other drugs.

Acknowledgements

The study was supported by Innovation Fund Denmark – Individuals, Disease and Society (#10-092786/DSF), The Obel Family Foundation, Karen Elise Jensens Foundation and a free grant from Grünenthal.

References

Andersen, O.K. (2007) Studies of the organization of the human nociceptive withdrawal reflex – focus on sensory convergence and stimulation site dependency. *Acta Physiol.*, **189**, 1–35.

Arguissain, F.G., Manresa, J.A.B., Morch, C.D. & Andersen, O.K. (2015) On the use of information theory for the analysis of synchronous nociceptive withdrawal reflexes and somatosensory evoked potentials elicited by graded electrical stimulation. *J. Neurosci. Methods*, **240**, 1–12.

Biurrun Manresa, J.A., Neziri, A.Y., Curatolo, M., Arendt-Nielsen, L. & Andersen, O.K. (2011) Test-retest reliability of the nociceptive withdrawal reflex and electrical pain thresholds after single and repeated stimulation in patients with chronic low back pain. *Eur. J. Appl. Physiol.*, **111**, 83–92.

Blier, P., Saint-Andre, E., Hebert, C., de Montigny, C., Lavoie, N. & Debonnel, G. (2007) Effects of different doses of venlafaxine on serotonin and norepinephrine reuptake in healthy volunteers. *Int. J. Neuropsychopharmacol.*, **10**, 41–50.

Bossard, A.E., Guirimand, F., Fletcher, D., Gaude-Joindreau, V., Chauvin, M. & Bouhassira, D. (2002) Interaction of a combination of morphine and ketamine on the nociceptive flexion reflex in human volunteers. *Pain*, **98**, 47–57.

Breivik, H., Collett, B., Ventafridda, V., Cohen, R. & Gallacher, D. (2006) Survey of chronic pain in Europe: prevalence, impact on daily life, and treatment. *Eur. J. Pain*, **10**, 287–333.

Brock, C., Olesen, S., Valeriani, M., Arendt-Nielsen, L. & Drewes, A. (2012) Brain activity in rectosigmoid pain: unravelling conditioning pain modulatory pathways. *Clin. Neurophysiol.*, **123**, 829–837.

Brooks, J. & Tracey, I. (2005) From nociception to pain perception: imaging the spinal and supraspinal pathways. *J. Anat.*, **207**, 19–33.

Crabb, I., Thornton, C., Konieczko, K.M., Chan, A., Aquilina, R., Frazer, N., Dore, C.J. & Newton, D.E. (1996) Remifentanil reduces auditory and somatosensory evoked responses during isoflurane anaesthesia in a dose-dependent manner. *Br. J. Anaesth.*, **76**, 795–801.

Durka, P.J., Matysiak, A., Montes, E.M., Sosa, P.V. & Blinowska, K.J. (2005) Multichannel matching pursuit and EEG inverse solutions. *J. Neurosci. Methods*, **148**, 49–59.

Enggaard, T.P., Klitgaard, N.A., Gram, L.F., Arendt-Nielsen, L. & Sindrup, S.H. (2001) Specific effect of venlafaxine on single and repetitive experimental painful stimuli in humans. *Clin. Pharmacol. Ther.*, **69**, 245–251.

Erskine, A., Morley, S. & Pearce, S. (1990) Memory for pain: a review. *Pain*, **41**, 255–265.

Freye, E., Buhl, R. & Ciaramelli, F. (1986) Opioids with different affinity for subreceptors induce different effects on early and late sensory evoked potentials (SEP) in man. *NIDA Res. Monogr.*, **75**, 551–554.

Garcia-Larrea, L., Perchet, C., Creac'h, C., Convers, P., Peyron, R., Laurent, B., Mauguiere, F. & Magnin, M. (2010) Operculo-insular pain (parasylvian pain): a distinct central pain syndrome. *Brain*, **133**, 2528–2539.

Harvey, A.T., Rudolph, R.L. & Preskorn, S.H. (2000) Evidence of the dual mechanisms of action of venlafaxine. *Arch. Gen. Psychiatry*, **57**, 503–509.

Iannetti, G.D., Hughes, N.P., Lee, M.C. & Mouraux, A. (2008) Determinants of laser-evoked EEG responses: pain perception or stimulus saliency? *J. Neurophysiol.*, **100**, 815–828.

Isnard, J., Magnin, M., Jung, J., Mauguiere, F. & Garcia-Larrea, L. (2011) Does the insula tell our brain that we are in pain? *Pain*, **152**, 946–951.

Jensen, M.B., Biurrun, M.J. & Andersen, O.K. (2015) Reliable estimation of nociceptive withdrawal reflex thresholds. *J. Neurosci. Methods*, **253**, 110–115.

Kalkman, C.J., Leyssius, A.T. & Bovill, J.G. (1988) Influence of high-dose opioid anesthesia on posterior tibial nerve somatosensory cortical evoked

potentials: effects of fentanyl, sufentanil, and alfentanil. *J. Cardiothorac. Anesth.*, **2**, 758–764.
Kimovec, M.A., Koht, A. & Sloan, T.B. (1990) Effects of sufentanil on median nerve somatosensory evoked potentials. *Br. J. Anaesth.*, **65**, 169–172.
Langley, P., Muller-Schwefe, G., Nicolaou, A., Liedgens, H., Pergolizzi, J. & Varrassi, G. (2010a) The impact of pain on labor force participation, absenteeism and presenteeism in the European Union. *J. Med. Econ.*, **13**, 662–672.
Langley, P., Muller-Schwefe, G., Nicolaou, A., Liedgens, H., Pergolizzi, J. & Varrassi, G. (2010b) The societal impact of pain in the European Union: health-related quality of life and healthcare resource utilization. *J. Med. Econ.*, **13**, 571–581.
Lelic, D., Gratkowski, M., Hennings, K. & Drewes, A. (2011) Multichannel matching pursuit validation and clustering – a simulation and empirical study. *J. Neurosci. Methods*, **196**, 190–200.
Lelic, D., Olesen, A., Brock, C., Staahl, C. & Drewes, A. (2012a) Advanced pharmaco-EEG reveals morphine induced changes in the brain's pain network. *J. Clin. Neurophysiol.*, **29**, 219–225.
Lelic, D., Olesen, S., Valeriani, M. & Drewes, A. (2012b) Brain source connectivity reveals the visceral pain network. *NeuroImage*, **60**, 37–46.
Lelic, D., Olesen, A.E., Gregersen, H., Dahan, A., Kolesnikov, Y. & Drewes, A.M. (2014) Morphine modifies the cingulate-operculum network underlying painful rectal evoked potentials. *Neuropharmacology*, **77**, 422–427.
Leppa, M., Korvenoja, A., Carlson, S., Timonen, P., Martinkauppi, S., Ahonen, J., Rosenberg, P., Aronen, H. *et al.* (2006) Acute opioid effects on human brain as revealed by functional magnetic resonance imaging. *NeuroImage*, **31**, 661–669.
Liu, S.S. & Wu, C.L. (2007) The effect of analgesic technique on postoperative patient-reported outcomes including analgesia: a systematic review. *Anesth. Analg.*, **105**, 789–808.
Marks, D.M., Shah, M.J., Patkar, A.A., Masand, P.S., Park, G.Y. & Pae, C.U. (2009) Serotonin-norepinephrine reuptake inhibitors for pain control: premise and promise. *Curr. Neuropharmacol.*, **7**, 331–336.
Matthey, A., Cedraschi, C., Piguet, V., Besson, M., Chabert, J., Daali, Y., Courvoisier, D., Montagne, A. *et al.* (2013) Dual reuptake inhibitor milnacipran and spinal pain pathways in fibromyalgia patients: a randomized, double-blind, placebo-controlled trial. *Pain Physician*, **16**, E553–E562.
Mauguiere, F. (2004) The role of secondary somatosensory cortex and insula in pain. *Suppl. Clin. Neurophysiol.*, **57**, 62–71.
McPherson, R.W., Sell, B. & Traystman, R.J. (1986) Effects of thiopental, fentanyl, and etomidate on upper extremity somatosensory evoked potentials in humans. *Anesthesiology*, **65**, 584–589.
Millan, M.J. (2002) Descending control of pain. *Prog. Neurobiol.*, **66**, 355–474.
Mouraux, A. & Iannetti, G.D. (2009) Nociceptive laser-evoked brain potentials do not reflect nociceptive-specific neural activity. *J. Neurophysiol.*, **101**, 3258–3269.
Olesen, S., Frokjaer, J., Lelic, D., Valeriani, M. & Drewes, A. (2010) Pain-associated adaptive cortical reorganisation in chronic pancreatitis. *Pancreatology*, **10**, 742–751.
Pertovaara, A. (2006) Noradrenergic pain modulation. *Prog. Neurobiol.*, **80**, 53–83.
Petrovic, P. (2005) Opioid and placebo analgesia share the same network. *Semin. Pain Med.*, **3**, 31–36.
Price, D., Vondergruen, A., Miller, J., Rafii, A. & Price, C. (1985) A psychophysical analysis of morphine analgesia. *Pain*, **22**, 261–269.
Sadzot, B., Price, J., Mayberg, H., Douglass, K., Dannals, R., Lever, J., Ravert, H., Wilson, A. *et al.* (1991) Quantification of human opiate receptor concentration and affinity using high and low specific activity [C-11] diprenorphine and positron emission tomography. *J. Cereb. Blood Flow Metab.*, **11**, 204–219.
Sandrini, G., Alfonsi, E., Derysky, C., Marini, S., Facchinetti, F. & Nappi, G. (1986a) Evidence for serotonin-S2 receptor involvement in analgesia in humans. *Eur. J. Pharmacol.*, **130**, 311–314.
Sandrini, G., Degliuberti, E.C., Salvadori, S., Margutti, A., Trasforini, G., Tomatis, R., Nappi, G. & Pansini, R. (1986b) Dermorphin inhibits spinal nociceptive flexion reflex in humans. *Brain Res.*, **371**, 364–367.
Sandrini, G., Serrao, M., Rossi, P., Romaniello, A., Cruccu, G. & Willer, J.C. (2005) The lower limb flexion reflex in humans. *Prog. Neurobiol.*, **77**, 353–395.
Schoell, E.D., Bingel, U., Eippert, F., Yacubian, J., Christiansen, K., Andresen, H., May, A. & Buechel, C. (2010) The effect of opioid receptor blockade on the neural processing of thermal stimuli. *PLoS One*, **5**, e12344.
Schubert, A., Drummond, J.C., Peterson, D.O. & Saidman, L.J. (1987) The effect of high-dose fentanyl on human median nerve somatosensory-evoked responses. *Can. J. Anaesth.*, **34**, 35–40.
Sjogren, P., Ekholm, O., Peuckmann, V. & Gronbaek, M. (2009) Epidemiology of chronic pain in Denmark: an update. *Eur. J. Pain*, **13**, 287–292.
Sprenger, T., Valet, M., Boecker, H., Henriksen, G., Spilker, M.E., Willoch, F., Wagner, K.J., Wester, H.J. *et al.* (2006) Opioidergic activation in the medial pain system after heat pain. *Pain*, **122**, 63–67.
Wamsley, J., Zarbin, M., Young, W. & Kuhar, M. (1982) Distribution of opiate receptors in the monkey brain – an autoradiographic study. *Neuroscience*, **7**, 595–613.
Wiech, K., Lin, C., Brodersen, K., Bingel, U., Ploner, M. & Tracey, I. (2010) Anterior insula integrates information about salience into perceptual decisions about pain. *J. Neurosci.*, **30**, 16324–16331.
Willer, J.C. (1985) Studies on pain – effects of morphine on a spinal nociceptive flexion reflex and related pain sensation in man. *Brain Res.*, **331**, 105–114.
Willer, J.C. & Bussel, B. (1980) Evidence for a direct spinal mechanism in morphine-induced inhibition of nociceptive reflexes in humans. *Brain Res.*, **187**, 212–215.
Willer, J.C., Bergeret, S. & Gaudy, J.H. (1985) Epidural morphine strongly depresses nociceptive flexion reflexes in patients with postoperative pain. *Anesthesiology*, **63**, 675–680.
Williamson, A. & Hoggart, B. (2005) Pain: a review of three commonly used pain rating scales. *J. Clin. Nurs.*, **14**, 798–804.
Wise, R., Rogers, R., Painter, D., Bantick, S., Ploghaus, A., Williams, P., Rapeport, G. & Tracey, I. (2002) Combining fMRI with a pharmacokinetic model to determine which brain areas activated by painful stimulation are specifically modulated by remifentanil. *NeuroImage*, **16**, 999–1014.