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Using fMRI to evaluate the effects of milnacipran on central pain processing in patients with fibromyalgia

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HIGHLIGHTS

- This study has innovative design with psychophysical measures and fMRI for mechanistic inquiries in randomized trial of milnacipran for fibromyalgia.
- Results indicate altered central pain inhibitory processing after milnacipran treatment.
- The results give directions for future hypothesis-testing of possible treatment mechanisms in fibromyalgia.

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ABSTRACT

Background: In recent years, the prescription of serotonin-noradrenalin reuptake inhibitors (SNRIs) for treatment of fibromyalgia (FM) has increased with reports of their efficacy. The SNRI milnacipran is approved by the U.S. Food and Drug Administration (FDA) for treatment of FM, yet, the mechanisms by which milnacipran reduces FM symptoms are unknown. A large number of neuroimaging studies have demonstrated altered brain function in patients with FM but the effect of milnacipran on central pain processing has not been investigated. The primary objective of this study was to assess the effect of milnacipran on sensitivity to pressure-evoked pain in FM. Secondary objectives were to assess the effect of milnacipran on cerebral processing of pressure-evoked pain using fMRI and the tolerability and safety of milnacipran 200 mg/day in FM.

Methods: 92 patients were randomized to either 13-weeks milnacipran treatment (200 mg/day) or placebo in this double-blind, placebo-controlled multicenter clinical trial. Psychophysical measures and functional MRI (fMRI) assessments were performed before and after treatment using a computer-controlled pressure-pain stimulator. Here, we present the results of several a priori defined statistical analyses.

Results: Milnacipran-treated patients displayed a trend toward lower pressure-pain sensitivity after treatment, compared to placebo, and the difference was greater at higher pain intensities. A single group fMRI analysis of milnacipran-treated patients indicated increased pain-evoked brain activity in the caudatus nucleus, anterior insula and amygdala after treatment, compared to before treatment; regions implicated in pain inhibitory processes. A 2×2 repeated measures fMRI analysis, comparing milnacipran and placebo, before and after treatment, showed that milnacipran-treated patients had greater

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pain-evoked activity in the precuneus/posterior cingulate cortex after treatment; a region previously implicated in intrinsic brain function and FM pathology. This finding was only significant when uncorrected for multiple comparisons. The safety analysis revealed that patients from both treatment groups had treatment-emergent adverse events where nausea was the most common complaint, reported by 43.5% of placebo patients and 71.7% of milnacipran-treated patients. Patients on milnacipran were more likely to discontinue treatment because of side effects.

Conclusions: Our results provide preliminary indications of increased pain inhibitory responses in milnacipran-treated FM patients, compared to placebo. The psychophysical assessments did not reach statistical significance but reveal a trend toward higher pressure-pain tolerance after treatment with milnacipran, compared to placebo, especially for higher pain intensities. Our fMRI analyses point toward increased activation of the precuneus/posterior cingulum in patients treated with milnacipran, however results were not corrected for multiple comparisons. The precuneus/posterior cingulum is a key region of the default mode network and has previously been associated with abnormal function in FM. Future studies may further explore activity within the default mode network as a potential biomarker for abnormal central pain processing.

Implications: The present study provides novel insights for future studies where functional neuroimaging may be used to elucidate the central mechanisms of common pharmacological treatments for chronic pain. Furthermore, our results point toward a potential mechanism for pain normalization in response to milnacipran, involving regions of the default mode network although this finding needs to be replicated in future studies.

1. Introduction

Fibromyalgia (FM) is a chronic pain syndrome characterized by widespread pain, disturbed sleep, fatigue and tenderness. The recent preliminary diagnostic criteria of the American College of Rheumatology (ACR 2010) thus now require the presence of chronic widespread pain and a combination of symptoms and core features including measures of symptom severity [1]. Effective treatment for FM is scarce but recent clinical trials have demonstrated efficacy for several pharmacological treatments [2]. In 2007, the U.S. Food and Drug Administration (FDA) approved the anticonvulsant pregabalin for treatment of FM, and in 2008 and 2009 the serotonin-noradrenalin reuptake inhibitors (SNRIs) duloxetine and milnacipran also received this indication. Milnacipran is distinguished from other SNRIs by its preferential noradrenergic action, compared to, e.g. duloxetine, which is more selective for serotonin reuptake inhibition [3,4]. Modulation of noradrenergic and serotonergic neurotransmission has been implicated in the control of descending pain-inhibitory pathways. In addition to SNRIs tricyclic antidepressants (TCA) have been shown to reduce pain intensity in FM. Thus increased availability of these transmitters in spinal and supraspinal structures may increase inhibitory control of nociceptive neurons [5]. Yet, the exact mechanisms by which milnacipran can reduce FM symptoms are still unknown.

There is vast evidence for central dysfunction of pain regulation in FM [6–9]. For example, there is evidence for greater temporal summation of pain [9], stronger pain intensities and larger referred areas [10], suggesting that central processing of pain is facilitated. In 2002, functional magnetic resonance imaging (fMRI) was used to investigate the brain responses to evoked pain in FM patients and healthy controls, using a pressure-pain paradigm [11]. FM patients reported higher pain intensities than healthy controls in response to standardized pressure stimuli and displayed a concomitant augmentation of brain activity in pain-related brain regions. In addition, two of our previous fMRI-studies demonstrated that patients with FM display attenuated activation [12] and connectivity [13] of the brain's pain inhibitory network in response to pressure-pain stimuli.

The aim of this 13-week mechanistic study was to investigate the effect of treatment with milnacipran on pressure pain sensitivity and cerebral correlates of pressure evoked pain in patients with FM ($n = 92$) using a double-blind and placebo-controlled clinical trial design. Psychophysical measures and fMRI assessments were performed before and after double-blind administration of milnacipran 200 mg/day or placebo. The primary objective was to assess the effect of milnacipran on sensitivity to pressure-evoked pain in FM patients. Secondary objectives were to (a) measure the effect of milnacipran on cerebral processing of pressure-evoked pain using fMRI and (b) to assess the tolerability and safety of milnacipran 200 mg/day in FM. We hypothesized that patients treated with milnacipran would report less sensitivity to pressure pain and also display attenuated brain responses to pressure-evoked pain, compared to patients treated with placebo.

2. Materials and methods

2.1. Overview

This was a 13-week, multicenter, randomized, double-blind, placebo-controlled, 2-arm parallel group study conducted at 3 outpatient clinical centers in England, Sweden, and Germany, respectively from October 21 2005 to April 27 2007. The study protocol (EudraCT # 2004-004249-16) was reviewed and approved by the Independent Ethics Committees at each participating site, and the trial was conducted in accordance with the ethical principles of the Declaration of Helsinki and consistent with Good Clinical Practice Guidelines. All patients gave written informed consent.

The study was sponsored and performed in collaboration with Pierre Fabre. The design and the statistical analysis plan were based on the scientific expertise of the authors at the three centers. Monitoring and storage of data was performed by Pierre Fabre. Before unblinding, data was critically reviewed in a structured process by the authors during two data validation meetings. Stimulus–Response-Curve Responders were categorized at this point (see below) and the different analysis sets were determined. All imaging analysis were performed at the Karolinska Institute, Pierre Fabre was responsible for storing copies of the original data and analysis of the non-imaging data. This manuscript was written predominantly by the two first authors who had access to all the original data. An abstract of the present study was previously published by one of the co-authors as a supplement in Human Psychopharmacology [14].

2.2. Entry criteria

Patients were eligible for the study if they were right-handed females, 18–55 years of age, who met the 1990 American College

of Rheumatology (ACR) diagnostic criteria for FM [15] and had a baseline mean pain intensity score of ≥40 on a 0–100 mm visual analog scale (VAS) anchored by 0 (no pain) and 100 (strongest possible pain). Prior to randomization, women of childbearing potential were required to have a negative urine pregnancy test and to be using study approved contraception for at least 2 months prior to randomization. Patients had to be willing to withdraw from all central nervous system acting therapies commonly used to treat FM, including antidepressants, anticonvulsants, mood stabilizers, opioids, narcotic patches, and to discontinue treatment with transcutaneous electrical nerve stimulation, biofeedback, tender and trigger point injections, acupuncture, and anesthetics. All analgesics were prohibited during the study, except for paracetamol, dipyrone and nonsteroidal anti-inflammatory agents (NSAIDs), which were used as rescue medications. The rescue medications had to be prescribed at the lowest available dose and for the shortest period of time necessary to manage the patient's acute pain. Moreover, treatment with rescue medications was not to exceed a total of 5 days per month. Zolpidem was allowed for treatment of insomnia and likewise limited to 5 days a month. Use of any rescue analgesic or hypnotic drug had to be discontinued 48 h prior to the assessments of pain sensitivity. Exclusion criteria included the following: severe psychiatric illness (including severe melancholic depressive episode); serious suicide risk; history or behavior that would prohibit study compliance; history of substance, drug, or alcohol abuse; heavy cigarette smoking (>25 cigarettes/day); presentation of an intracranial anomaly; significant cardiovascular, pulmonary, gastrointestinal, hepatic, or renal disease; history of autoimmune disease; current systemic infection; active cancer (except basal cell carcinoma) or current cancer therapy; unstable endocrine disease; severe sleep apnea; pregnancy or breastfeeding.

2.3. Study design

After an initial screening for eligibility criteria and completion of a 1–4-week washout phase (length based on the class of medications to be washed out by a predefined protocol), patients returned for a baseline visit (V) on two consecutive days where safety and efficacy data were recorded (V2 and V3, week 0, Fig. 1). Pressure pain testing was performed on V2 and an fMRI examination on the next day (V3). Patients who met the eligibility criteria at V3 were randomized to receive placebo or milnacipran 200 mg/day (100 mg BID). Clinical staff, investigators, patients, and study sponsors were blinded to treatment allocation. Randomization was performed with three independent randomization lists for each of the centers. Treatment allocation was done under the responsibility of the investigator in the chronological order of treatment unit classification. Patients who did not tolerate the stable dose of milnacipran 200 mg/day were discontinued from the study. For blinding purposes, placebo patients underwent "dose escalation" along with patients receiving active medication; identical appearing capsules were used by all patients during all phases of the study. After completing a 9-week stable-dose phase at milnacipran 200 mg/day (100 mg BID), patients returned again for two visits on two consecutive days (V6 and V7). Finally, patients entered into a 9-day down-titration phase at which time dosage of study drug was reduced to milnacipran 100 mg (50 mg BID) for 3 days; milnacipran 50 mg (25 mg BID) for 3 days; and no treatment for 3 days.

2.4. Pressure pain stimuli

Responses to pressure-pain were assessed by pressures of 2.5 s in duration at 30 s intervals, using an automated, pneumatic, computer-controlled stimulator with a plastic piston that applies pressure to the thumbnail via a 1 cm^2 hard rubber probe [16]. The thumb was inserted into a cylindrical opening and positioned such that the probe applied pressure to the nail bed. Each patient's individually calibrated pressure-pain threshold (VAS > 0 mm) and maximum pain (VAS > 60 mm) was determined by an ascending series of pressure stimuli presented in steps of 50 kPa of increased pressure. Patients rated the pain intensity evoked by each stimulus using a paper-and-pencil 0–100 mm VAS. Ratings from the ascending series were used to calculate the pressures for a subsequent series of random pressures. Five different pressure intensities between each patient's threshold and maximum pain were chosen, e.g. if the pain threshold was 200 kPa and the maximum pain rating was reached at 600 kPa, the random series would consist of pressures of 200 kPa, 300 kPa, 400 kPa, 500 kPa and 600 kPa. In the random series, 15 stimuli were delivered in a random order at 30 s intervals. A Stimulus–Response (S–R) curve was calculated constructed from patients' VAS responses and a third-order polynomial regression function was used to determine each individual's representation of VAS 50 mm (P50).

2.5. Functional imaging

To investigate the effect of milnacipran on pain-evoked brain activity, all participants underwent 2 fMRI sessions, once prior to treatment (V3) and once after treatment (V7) (Fig. 1). Images were collected using 3 different 1.5 Tesla scanners: in London, a General Electric HDx scanner was used; in Stockholm, a General Electric Twinspeed Signa Horizon was employed; and in Cologne, a PHILIPS scanner was used. T2*-weighted single-shot gradient Echo Planar Imaging sequences were used to acquire blood oxygen level dependent (BOLD) contrast images. The following parameters were used: repetition time, 3000 ms (35 slices acquired); time to echo (TE), 40 ms; flip angle, 90°; field of view, 24 cm × 24 cm, 64 × 64 pixel matrix, 4 mm slice thickness with a gap of 0.4 mm and sequential image acquisition order, voxel size 4 mm × 2 mm × 2 mm. These parameters allowed coverage of the entire brain. Cushions were used to reduce head movement. The patients wore MRI-compatible

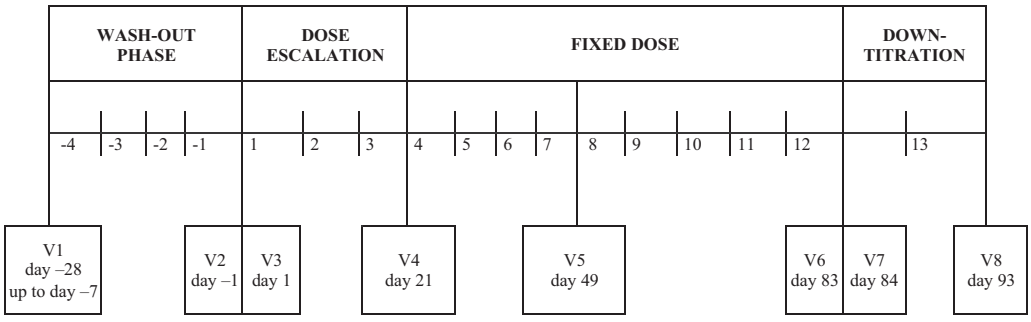

Fig. 1. Study design. At baseline (day −1) and at the end of the fixed dose period (day 83) the stimulus–response assessment was performed, 1 day before the fMRI scan at baseline (day 1) and at study end (day 84). The abbreviation "V" represents "Study Visit".

ear-cuffs for reducing scanner noise and head movement. Functional images were acquired over 4 separate scans for a total duration of approximately 30 min. Two different pressures were used during fMRI scanning: non-painful (perceived only as light touch; 0 mm VAS) and painful pressures representing patient's calibrated P50. Each pressure lasted for 2.5 s and was delivered randomly at intervals that varied between 10 and 20 s (mean Stimulus Onset Asynchrony was 15 s) preventing the patients from predicting the time of onset and stimulus type. In addition to the functional scans, high-resolution T1-weighted structural images were acquired in coronal orientation for anatomical reference purposes and screening for cerebral anomalies. Parameters were: Spoiled Gradient Recalled 3D sequence, repetition time, 24 ms; echo time, 6 ms; flip angle, 35°, 124 contiguous 1.5 mm coronal slices (image resolution 256 mm × 256 mm × 186 mm, voxel size 0.9 mm × 0.9 mm × 1.5 mm). Two different analyses were performed in order to determine if there was any variance in pain-evoked brain activity that could be explained by the site-factor. Firstly, an ANOVA was performed within SPM5, including the factor SITE (Stockholm, London, Cologne), and the factor TIMEPOINT (before or after treatment). Secondly, the pain-evoked brain activity in a commonly activated anatomical location (secondary sensory cortex coordinate) was extracted for each individual and grouped by site. A univariate ANOVA within the statistical software SPSS 16.0 for Windows was performed, using SITE as the between-subject factor (Stockholm, London, Cologne).

2.6. Primary outcome measure

The primary outcome was defined as the shift of the S–R curve from baseline to study end, between the 2 treatment groups. The S–R curve is a representation of patients' sensitivity to repeated pressure pain and was used in this study as a quantification of a core feature of FM pathology.

2.7. Tolerability and safety assessments

Adverse events (AEs) spontaneously reported by patient self-report and investigator-observed treatment-emergent adverse events (TEAEs) were recorded at each study visit along with the dates and onset and resolution. AEs were coded using the Medical Dictionary for Regulatory Activities Version 9.1. Clinical laboratory tests (hematology, serum chemistries, and urinalysis) were performed by the investigator at the screening visit and at the end of the dose escalation phase and at study end. Vital signs (standing and supine heart rate, blood pressure) and weight were measured by the investigator at the screening visit and at all subsequent clinic visits.

2.8. Statistical analyses

2.8.1. S–R

The safety population consisted of all randomized patients who received at least 1 dose of double-blind study medication. In general, efficacy analyses were performed on the "full analysis set" (FAS), defined as patients in the safety data set who had at least 1 baseline and 1 post baseline evaluation of an efficacy criterion. The primary outcome, i.e. pressure pain sensitivity, was evaluated on all complete data sets calculating the shift of the mean profile of S–R curves before and after treatment (including patients with premature withdrawal if the dose escalation was completed), which were estimated using a polynomial regression model with pressure, treatment, and center as fixed factors, interaction terms between treatment and pressure, baseline P50 as covariate, and patient as a random factor. S–R responders were defined for this data as any patient who met the following criteria: (1) either no overlap between S–R curves and rightward shift of study end curve compared with baseline curve; or (2) if curves overlap, rightward shift of study end curve as compared with baseline curve at the 2 highest pressures, *and* no baseline VAS score lower than any study end VAS score in the interval of the 2 highest study end pressures, *and* study end P50 equal or higher than baseline P50. Between-treatment group S–R responders were analyzed using a Cochran–Mantel–Haenszel test stratified by center at study end. Any P50 changes from baseline to study end were assessed by using an ANCOVA model, with treatment and center as main effects and baseline P50 as covariate. Statistical tests were 2-tailed hypothesis tests performed at the 5% level of significance unless otherwise specified. All confidence intervals were 2-tailed 95% confidence intervals. All statistical analyses were performed using SAS Version 8.2.

2.8.2. fMRI

Functional imaging data was preprocessed using the Statistical Parametric Mapping 5 (SPM5) software (http://www.fil.ion.ucl.ac.uk/spm/) implemented in the Matlab 7.1 package (Mathworks Inc., MA, USA). Realignment of brain volumes was performed in order to correct for head movements during scans. The reconstructed images were automatically realigned within SPM. Normalization of all images was performed in order to represent data in the standardized anatomical space of Montreal Neurological Institute (MNI space). Finally, all functional images were smoothed using an 8 mm Gaussian kernel. The fMRI data was evaluated using the General Linear Model (GLM) approach. A statistical model was created for every subject where the experimental conditions were expressed as regressors and convolved with a canonical hemodynamic response function (HRF). The statistical model was then used to individually calculate the signal change relating to the defined regressors, voxel by voxel over the time series.

To assess pain-specific cerebral activity, brain activation during the non-painful pressures was individually subtracted from activity during the calibrated P50 pressures. This was done in order to extract pain components from sensory-discriminative aspects of brain response as well as control for individual differences in cerebral responsiveness. For the purposes of the main analysis, data from all the 4 scans was used (4× 8 min). However, the potential difference in cerebral response after repeated pressure stimulation was analyzed by directly comparing the first and the last scan (first vs. last 8 min scan) as a representation of temporal summation.

According to our hypothesis about altered cerebral pain processing in response to milnacipran, and according to previous studies of pain modulation, pre-defined regions of interest for fMRI analyses were determined using Montreal Neurological Institute (MNI) coordinates from previously published neuroimaging studies on pain. Descending pain inhibitory regions: the rACC and brainstem [17], amygdala [18], caudate nucleus [8], and anterior insula [19]. Ascending regions: S1, S2, ACC and the posterior insula [11].

For pre-defined anatomical regions, the statistical threshold was set at voxel-wise $p < 0.05$, uncorrected for multiple comparisons. For all other brain regions, a threshold of voxel-wise $p < 0.05$, corrected for multiple comparisons, was used.

3. Results

3.1. Patient population

Of the 157 patients screened, a total of 92 patients (58.6%) were randomized to receive milnacipran 200 mg/day ($n = 46$) or placebo ($n = 46$) (Fig. 2), and 90 of these patients were in the full analysis set (FAS), defined as those patients who received at

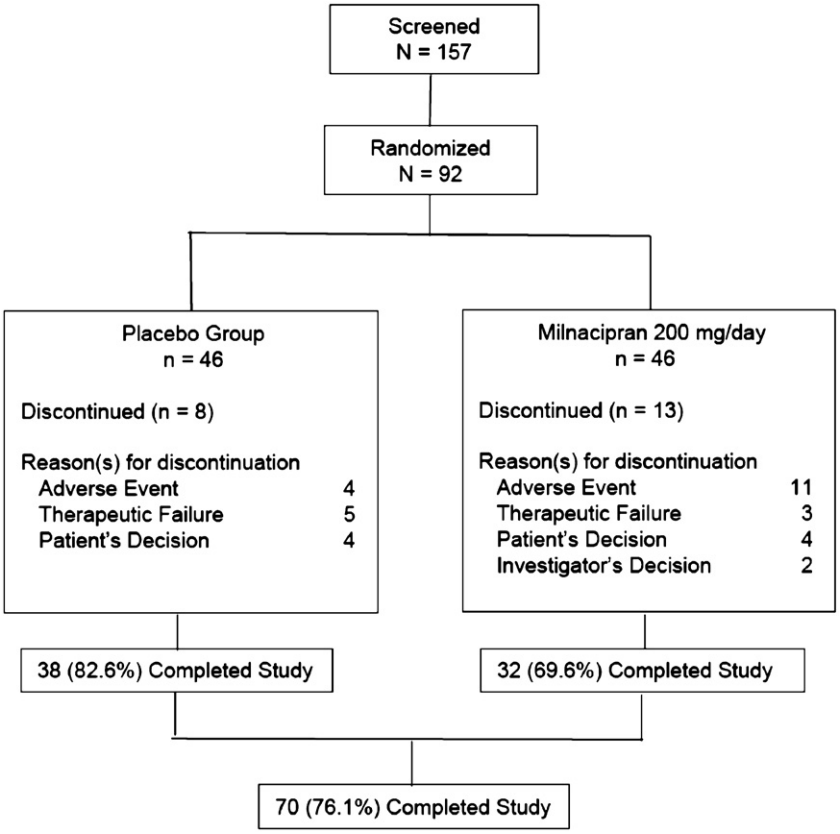

Fig. 2. Patient disposition.

Table 1
Patient demographics and baseline characteristics. All variables are reported as means and standard deviations (SD), given in parentheses.

Variable	Placebo ($n = 45$)	Milnacipran ($n = 45$)
Age [years]	45.6 (8.5)	42.8 (7.8)
Weight [kg]	72.5 (14.6)	70.5 (11.6)
Body Mass Index [kg/m^2]	26.7 (5.1)	25.9 (4.0)
FM duration [years]	11.4 (7.8)	10.7 (8.0)
Beck Depression Inventory (BDI) total score [0–63]	17.8 (9.9)	17.8 (10.3)
Weekly average pain, visual analog scale [0–100]	66.9 (16.7)	59.1 (15.5)
Current pain, visual analog scale [0–100]	53.1 (23.2)	52.0 (21.5)
Pressure pain thresholds [kPa]	173.4 (10.7)	169.2 (124.8)
Short Form McGill Pain Questionnaire (SF-MPQ) total score [0–45]	23.3 (7.8)	23.3 (8.7)
Fibromyalgia Impact Questionnaire (FIQ) total score [0–100]	64.8 (15.3)	62.0 (16.9)
FIQ physical function (PF) [0–3]	1.45 (0.76)	1.39 (0.65)
Chalder Fatigue Scale (CFS) total score [0–33]	25.8 (5.7)	24.3 (5.8)

least 1 dose of study medication and had at least 1 postbaseline assessment. A total of 70 patients (76.1%) completed the study (Fig. 2); rates of completion were 82.6% (38/46) for placebo and 69.6% (32/46) for milnacipran. In both groups, the most frequent reason for discontinuation was adverse events (placebo, 8.7% [4/46]; milnacipran 200 mg/day, 23.9% [11/46], $p < 0.001$). Therapeutic failure was the second most frequent reason for discontinuation in both treatment groups (placebo, 10.9% [5/46]; milnacipran 200 mg/day, 6.5% [3/46]). There were no notable differences between treatment groups in key demographic or baseline characteristics (Table 1).

3.2. Primary outcome – S–R analysis

Of the 90 FAS patients, 38 patients in the placebo group and 36 in the milnacipran group had pre- and post-treatment data for the primary analysis of VAS pain sensitivity. At baseline, pain sensitivity was similar across the entire range of pressure stimuli in the milnacipran and placebo treatment groups, with superimposed mean stimulus–response (S–R) curves (Fig. 3). There were no significant differences at baseline in S–R curves with respect to slope ($p = 0.15$), curvature ($p = 0.09$), or profile ($p = 0.81$). This allowed straightforward analysis of the primary outcome, which was the shift between the mean S–R curves of the 2 treatment groups after treatment.

After treatment, mean S–R curves of the placebo and milnacipran groups were similar in shape, with no significant differences in slope and curvature ($p = 0.57$ and $p = 0.86$, respectively), allowing for reliable interpretation of the primary criterion. After treatment, there was a 5.2 mm (SE: 3.2 mm) downward shift of the milnacipran mean S–R curve from the placebo curve over the entire panel of applied pressures (i.e. from pain threshold to maximum pain) (Fig. 3). This shift indicates that milnacipran-treated patients required more pressure to generate subjectively calibrated pain, compared to placebo. This observed downward shift of the S–R curve in the milnacipran group did not reach

Table 2
Regions with increased BOLD fMRI signal in response to calibrated pressure pain to the right thumb. All results are derived from the subtraction of painful pressures minus non-painful pressures. After-treatment results represent a subtraction of after-treatment minus baseline activations in the milnacipran and the placebo group respectively. Coordinates (*x*, *y*, *z*) correspond to the anatomical space as defined in the MNI standard brain atlas. The baseline result represents one large cluster of more than 38,000 voxles, containing significant activations of 10 different brain regions (sub-clusters); indicated by italics in the "Cluster size" column.

	MNI *x*	MNI *y*	MNI *z*	Cluster size (voxels)	Peak *T*-score
Before treatment – all patients					
Predefined regions of interest					
R. PAG	10	−24	−12	*38,003*	5.31
L. Amygdala	−24	0	−8	*38,003*	6.56
L. S1	−28	−24	70	*38,003*	7.41
L. S2	−40	−18	16	*38,003*	6.92
R. ACC	2	20	32	*38,003*	6.88
L. Posterior insula	−54	0	0	*38,003*	8.14
R. Posterior insula	56	−16	8	*38,003*	6.82
Whole brain					
R. Cerebellum	26	−56	−24	*38,003*	10.08
R. Mid insula	38	6	2	*38,003*	7.24
R. Thalamus	10	−4	2	*38,003*	6.44
After treatment (week 12) – milnacipran					
Predefined regions of interest					
R. Caudatus nucleus	16	4	6	561	4.25
L. Anterior Insula	−42	18	4	210	4.54
R. Anterior Insula	40	14	−4	337	4.20
L. Amygdala	−26	−4	−16	165	4.66
L. S1	−40	−22	52	375	4.52
L. ACC	−4	12	42	1797	5.31
Whole brain					
L. Posterior cingulum	−2	−32	44	369	4.00
R. Temporal	56	−2	2	175	4.43
R. Cerebellum	34	−56	−34	244	4.18
R. Thalamus	12	−4	10	199	4.16
After treatment (week 12) – placebo					
Whole brain					
L. Parietal	−24	−56	42	1959	6.01
L. Mid insula	−34	4	4	770	5.55
R. Mid insula	36	12	8	55	4.41

statistical significance ($p = 0.055$, one-tailed). On an individual analysis level, the proportion of patients who were classified as S–R curve responders for the milnacipran group (57.1% [20/35]) was not significantly different from the S–R curve responders on placebo (42.1% [16/38]); $p = 0.24$.

The milnacipran and placebo groups displayed comparable P50 values (SD) at baseline with mean values of 399 (182) kPa and 401 (159) kPa, respectively. After treatment, an ANOVA measuring the P50 change from baseline pointed toward an improvement for milnacipran (+96 kPa) compared to placebo (+54 kPa); however the results did not reach statistical significance ($p = 0.37$).

3.3. Secondary outcome – fMRI analyses

3.3.1. Whole-group analysis at baseline

Of the 90 FAS patients, 64 (32 in each treatment group) were eligible for fMRI data analyses. The 26 missing data sets were due to dropouts with a missing post-treatment scan in 20 subjects and to imaging artifacts in 6 subjects. Four patients who withdrew prematurely had a second pressure pain testing but no second fMRI. An estimation of P50 change using the same ANOVA procedure on the patients who also had a second fMRI, revealed similar results (+101 kPa for milnacipran treated patients

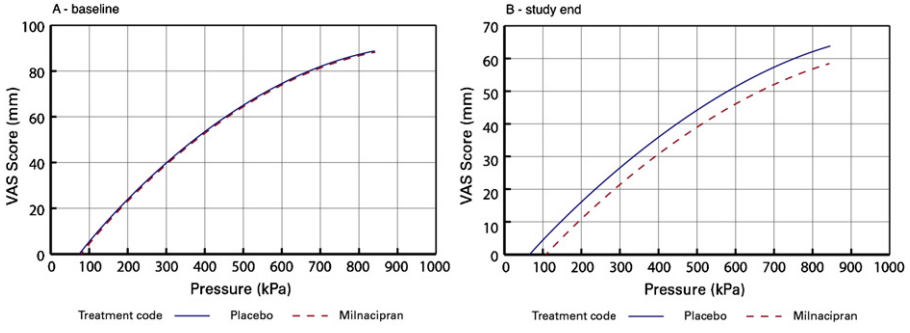

Fig. 3. Stimulus–response outcomes. Mean stimulus–response (S–R) curves of pain sensitivity at (A) baseline (day −1) and (B) study end (day 83) in placebo (solid blue line) and milnacipran-treated patients (dotted red line). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

and +48 kPa for placebo, $p = 0.31$). In all patients at baseline (milnacipran and placebo patients), pain-evoked brain activity was detected in 10 regions (Table 2). These included regions within the predefined descending pain inhibitory network (PAG and amygdala), ascending pain regions (S1, S2, ACC, and posterior insula) and the cerebellum, mid insula, and thalamus; reproducing the pain-matrix previously described in the literature [20,21].

3.3.2. Between-site analysis

Results from the analysis of site-related differences in pain-evoked brain activity revealed no brain regions with significant variance that could be attributed to any of the sites (Stockholm, London, Cologne) at any of the two timepoints (baseline and after treatment). Results from the SPSS analysis, where measures of pain-evoked signal intensities was extracted from a pre-defined coordinate in secondary somatosensory cortex ($x = -42$, $y = -20$, $z = 20$), revealed no significant difference between the three sites (Stockholm, London, Cologne) $F(2,82) = 0.69$, $p = 0.51$, n.s.

3.3.3. Within-group analyses after treatment

After treatment, a paired-sample t-test for the milnacipran group [after treatment > before treatment] displayed increased pain-evoked brain activity in several of the predefined nodes of the descending pain inhibitory network, i.e. caudatus nucleus, anterior insula and the amygdala. Milnacipran-treated patients also showed increased pain-evoked brain activity in some of the predefined ascending pain regions (S1, ACC), and in the posterior cingulum, temporal region, cerebellum, and thalamus (Table 2). In contrast, a paired-sample t-test within the placebo group [after treatment > before treatment] did not reveal any differences in pain-evoked brain activity within the pre-defined regions of interest. The only difference in brain activity after treatment in the placebo group was detected in the parietal region and mid-insula (Table 2).

3.3.4. Between-group analyses after treatment

A statistical comparison of pre- to post-treatment differences in brain activity between milnacipran and placebo (2×2 ANOVA) revealed no significant changes within any of the pre-defined brain regions of interest. However, an exploratory whole-brain analysis revealed that patients treated with milnacipran displayed increased brain activity in the posterior cingulum and precuneus regions after treatment, compared to placebo ($p < 0.05$, uncorrected) (Fig. 4). The cluster consisted of 2334 voxels and was located at MNI coordinates $x = 14$, $y = -48$, $z = 22$, with a peak t-score of 3.73.

Effects of repeated painful pressure stimulation over time (or temporal summation) were assessed by comparing pain-evoked brain activations in the last 8 min of the experiment, compared to the first 8 min. After treatment, patients treated with milnacipran showed an increase of brain activity in the thalamus in response to accumulated painful stimuli, compared to placebo; an effect that approached statistical significance ($p = 0.057$); cluster size 1736 voxels, MNI coordinates $x = 6$, $y = 30$, $z = 12$, with a peak t-score of 2.83 (Fig. 5 Top panel). In the opposite contrast, however, the placebo group displayed increased activation in S2 ($p = 0.002$; cluster size 10,399 voxels, MNI coordinates $x = -54$, $y = -26$, $z = 28$, with a peak t-score of 3.50) and occipital regions ($p = 0.003$; cluster size 6314 voxels, MNI coordinates $x = -18$, $y = -96$, $z = -8$, with a peak t-score of 3.50) in response to accumulated painful stimuli (Fig. 5 Lower panel).

3.4. Secondary outcome – safety analysis

TEAEs were experienced by 87.0% (40/46) of placebo-treated patients and 97.8% (45/46) of milnacipran-treated patients. The most commonly reported AE was nausea, which occurred in 43.5% and 71.7% of placebo- and milnacipran-treated patients, respectively. AEs that occurred in ≥5% of patients in the milnacipran treatment group and at an incidence of at least twice that of placebo patients were vomiting (4.3% and 26.1%), abdominal pain upper (6.5% and 15.2%), constipation (0% and 8.7%), migraine (2.2% and 8.7%), blood pressure increased (6.5% and 32.6%), heart rate increased (2.2% and 8.7%), sinusitis (0% and 6.5%), FM (2.2% and 13.0%), pyrexia (4.3% and 8.7%), hyperhidrosis (2.2% and 17.4%), tachycardia (0% and 6.5%), pharyngolaryngeal pain (4.3% and 10.9%), and hot flush (0% and 6.5%). More patients discontinued treatment, at least in part due to adverse events of milnacipran, compared to placebo (Fig. 2: 24% vs. 9%, $p < 0.001$). A total of 8 serious AEs occurred in the study: 2 in 2 placebo-treated patients and 6 in 4 milnacipran-treated patients (1 patient presented with 3 serious AEs). There were no differences between groups in laboratory values. No clinically relevant supine systolic blood pressure (SBP) differences were found. Both supine diastolic blood pressure (DBP) and heart rate were elevated in patients treated with milnacipran compared with placebo. At the end of the fixed-dose phase (day 83), mean changes from baseline in supine SBP were −2.5 mm Hg and −0.6 mm Hg for placebo and milnacipran patients, respectively; mean changes from baseline in supine DBP were also small for both groups (placebo, −1.9 mm Hg; milnacipran, +6.1 mm Hg). Heart rate increased from baseline in the milnacipran group (+13.5 bpm) and decreased from baseline in the placebo group (−5.5 bpm); at the end of the down-titration phase, heart rate decreased in the milnacipran group but was still elevated compared with baseline (+6.1 bpm). In both treatment groups, supine SBP and DBP values approached baseline at the end of the down titration phase.

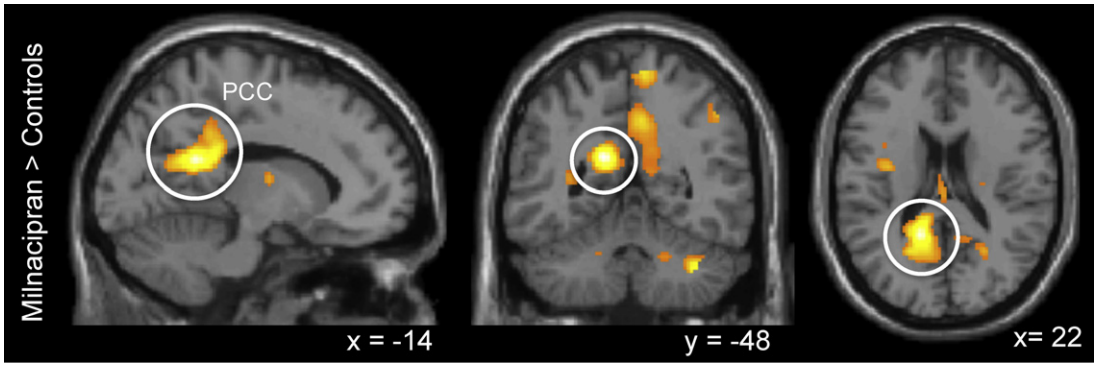

Fig. 4. Milnacipran vs. Placebo fMRI outcomes. Regions of the brain where milnacipran-treated patients display increased pain-evoked BOLD fMRI signal after treatment [after treatment > before treatment], compared to placebo-treated patients. The precuneus/posterior cingulate cortex region is indicated by white circles in the sagittal section (left), coronal section (center) and axial section (right) of the brain. The coordinate for each section is given in MNI coordinates (x, y, z).

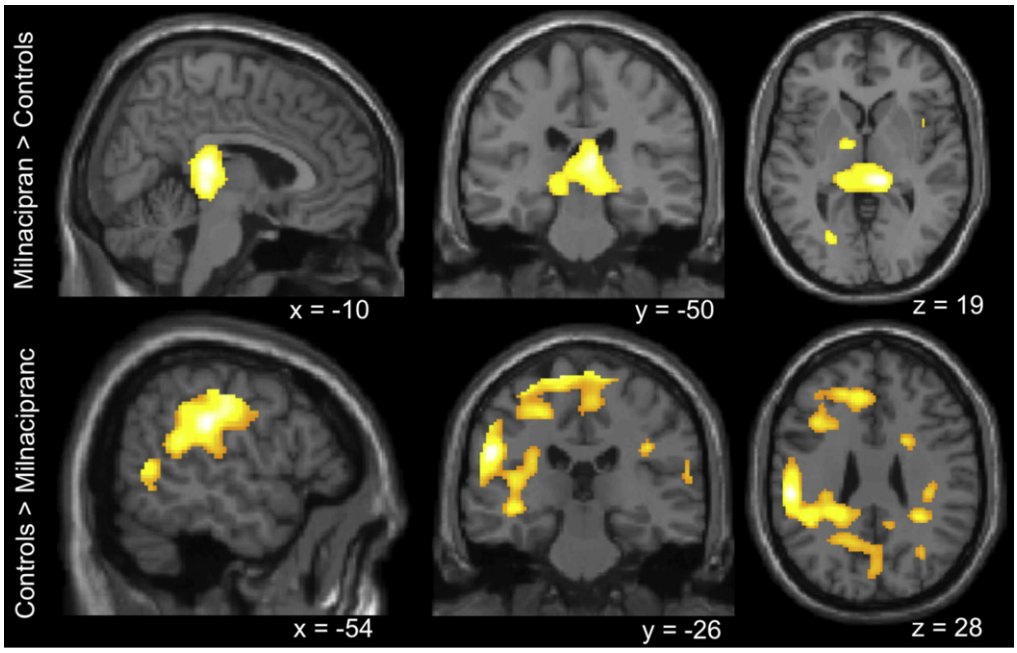

Fig. 5. Temporal summation fMRI outcomes. Region of the brain where milnacipran-treated patients displayed increased pain-evoked brain activity after temporal summation, compared to placebo (top panel). Temporal summation was modeled as the brain activity during the last 8 min of the pressure-pain paradigm compared to the first 8 min (total time of paradigm was 32 min). The top panel represents increased activation of the thalamus, seen from a sagittal (left), coronal (center), and axial view (right). Lower panel: Region where placebo-treated patients displayed increased pain-evoked brain activity after temporal summation, compared to milnacipran. The significant region is located in S2 and the peak of the activation is indicated by bright white color, viewed in a sagittal section (left), coronal section (center), and axial section (right). The coordinate for each section is given in MNI coordinates (x, y, z).

4. Discussion

4.1. General

This double-blind placebo controlled study provides preliminary evidence for changes in central processing of pain in FM patients treated with milnacipran, compared to placebo. Our findings indicate that treatment with milnacipran may lead to normalization of cerebral mechanisms that have recently been associated with FM pathology [12,22]. The relatively small sample size compared to the pivotal clinical trials [23] might have contributed to the modest statistical effects when comparing milnacipran and placebo outcomes. However, this is the first assessment of SNRI treatment mechanisms in FM and therefore provides insights for future mechanistic studies and directions for the development of novel treatment strategies.

4.2. S–R results

Subjective ratings of pressure pain were reduced after treatment with both milnacipran and placebo. However, treatment with milnacipran resulted in a relatively greater effect on pressure pain sensitivity, even if this effect only approached significance. The slope of the S–R curves for milnacipran and placebo revealed that the treatment effect on pressure pain sensitivity varied over the pain range, with a shift of approximately 40 kPa at pain threshold levels and a shift of approximately 100 kPa at higher subjective pain levels; indicating a trend for a specific pharmacological effect of milnacipran at higher pressure pain intensities. Previous clinical data indicates that only a subset of FM patients respond to pharmacological treatment with drugs such as milnacipran [24,25], creating large within-group variance, which could be one reason for the non-significant S–R result. A second factor is a qualitative difference in the patterns of interactions found in the two groups, i.e. the effect of milnacipran on pressure pain sensitivity was significantly greater for higher levels of pressure pain, compared to threshold pain. A third factor is that pressure pain sensitivity estimated with the P50 showed a considerable range of distribution, despite the formal inclusion criteria of a positive tender point count. This discrepancy has been previously shown for similar "objective" measures of pressure pain sensitivity and was interpreted as evidence for clinically relevant subgroups [26]. Despite having a similar sample size to the present study, our findings may have been affected by an unbalanced distribution of the proposed subgroups, which currently cannot be defined by clinical means. A fourth factor, which is present in most clinical trials, is the unknown contribution of an order effect. It is possible that the two groups experienced an unspecific reduction of pain sensitivity over time; an effect that could only have been accounted for by the inclusion of a no-treatment control group.

4.3. fMRI within-group results

The baseline results for all subjects in this study showed robust activations of regions pertaining to regions in the CNS often referred to as "pain-matrix" [21]. The pressure-pain paradigm evoked brain activations in regions potentially contributing to pain inhibition such as the PAG and amygdala as well as regions involved in various aspects of afferent pain processing like S1, S2, ACC, posterior insula and cerebellum. The observed differences in brain activity from before treatment to after treatment within the milnacipran group might represent a drug-related change of central pain processing. There were 10 regions with altered pain processing in the milnacipran group, compared to 2 regions in the placebo group. Moreover, several of the regions with increased brain activity after treatment in the milnacipran group pertained to the descending pain inhibitory network, potentially indicating higher involvement of pain-evoked inhibition. Interestingly, there were no decreases of pain-evoked brain activity after milnacipran treatment. Based on previous findings reporting central augmentation

in patients with FM [11], we speculated that milnacipran would lead to less pain sensitivity and concomitant decreases of activity in afferent brain regions. Instead, our data suggests that milnacipran contributes to enhanced function of the brain's pain modulatory functions. Several previous studies found that FM pathology is associated with impaired function of the pain inhibitory system; a modulatory system that would normally suppress spontaneous pain and pressure-evoked pain. It is a possible explanation that the greater activations in regions associated with pain inhibition, found in the milnacipran group after treatment, reflect a partial normalization of impaired pain inhibition as previously described in FM [7,27]. In this scenario the pain modulatory system is fully functioning, at baseline, completely activated by ongoing FM pain [28] and partially restoring its responsiveness with pharmacological therapy. However, the presence of ongoing inhibition during FM pain is not supported by the activation of the PAG at baseline in this study. The numerous regions with significantly increased brain activations reported after treatment with milnacipran in this study might reflect a general normalizing effect in which brain responses in FM patients may more closely resemble responses from healthy control subjects. Recent data has challenged the concept of a pain-specific matrix, indicating that these regions are rather part of a more generalized system for the detection of the saliency of stimulus characteristics [29]. This thinking is especially interesting for patients with intractable pain, where the saliency of a painful stimulus is highly influenced by the perceived threat of the high intensity persistent pain [19,30].

4.4. Between group fMRI results

Despite the larger number of brain regions that changed from before to after treatment in the milnacipran group, none of them were statistically significant in a full interaction with placebo, controlling for multiple comparisons. However, in an exploratory whole-brain analysis, using less stringent statistical thresholds, the precuneus and posterior cingulum (PCC) were significantly more activated in milnacipran patients after treatment, compared to controls ($p < 0.05$, uncorrected). The precuneus and PCC region has repeatedly been implicated in response to non-opioidergic treatment in chronic pain patients [31,32] and healthy controls [33,34]. These regions are considered central for intrinsic brain activity [35] and there is first evidence for a connection between intrinsic brain connectivity and pain intensities in FM [22]. The present study thus may provide some preliminary evidence that the effect of milnacipran treatment could be reflected by the precuneus/PCC activation. However, future studies using the standard statistical criteria are needed to elucidate the role of the precuneus and PCC in the pathophysiology of FM and the response to FM treatment, since methodological factors like arbitrary differences in movement between the groups could potentially result in similar patterns.

4.5. Safety measures and clinical relevance

In the present study, no unexpected tolerability/safety concerns were reported for milnacipran. The most commonly reported AEs was nausea, which occurred in 43.5% of placebo- and 71.7% of milnacipran-treated patients probably contributing to the more frequent discontinuation of treatment. Moreover, there were no differences between the two treatment groups in laboratory or supine systolic blood pressure. In line with previous milnacipran studies, supine diastolic blood pressure and heart rate were elevated in patients treated with milnacipran, compared with placebo, but these effects approached baseline again at the end of the down titration phase.

These findings are in line with other clinical studies of milnacipran. Recent data from systematic reviews and meta-analysis [23,36] supports a role for milnacipran in FM, although the benefit in general was small to moderate, with substantial benefits only in a small number of subjects. A different perspective on clinical relevance probably resulted in the contrasting regulatory decisions in Europe and the US.

4.6. Pharmacological fMRI

There is growing interest in "pharmacological fMRI", for purposes such as individualizing medical treatments and drug discovery [37–39]. Most pharmacological fMRI studies have focused on acute effects of drugs such as the brain responses to alcohol, remifentanil, and ketamine [40–42], and only few studies have examined effects of drugs over periods of 6–8 weeks [43,44]. The current results provide preliminary information about the mechanism of action for treatment with milnacipran in FM. The present study has uncovered effects of long-term treatment for FM that will aid in the design of future studies that might ultimately lead to more effective treatment for patients with FM.

5. Conclusion

The results of the present study provide preliminary indications of changed psychophysical responses and increased pain inhibitory brain responses in milnacipran-treated FM patients, compared to placebo. The results may provide insights for future mechanistic studies of pharmacological treatment on central pain pathology.

Conflict of interest statement

This study (EudraCT # 2004-004249-16) was financed and performed in collaboration with the pharmaceutical company Pierre Fabre. There are no other conflicts of interest.

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