

PAIN

Disambiguating pharmacological mechanisms from placebo in neuropathic pain using functional neuroimaging

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Abstract

Background: A lack of objective outcome measures and overreliance on subjective pain reports in early proof-of-concept studies contribute to the high attrition of potentially effective new analgesics. We studied the utility of neuroimaging in providing objective evidence of neural activity related to drug modulation or a placebo effect in a double-blind, randomized, placebo-controlled, three-way crossover trial.

Methods: We chronically administered pregabalin or tramadol (first-line and second-line analgesics, respectively), recommended for neuropathic pain, in 16 post-traumatic neuropathic pain patients. We measured subjective pain reports, allodynia-evoked neural activity, and brain resting state functional connectivity from patients during the three sessions and resting state data at baseline from patients after washout of their current medication. All data were collected using a 3 T MRI scanner.

Results: When compared with placebo only, pregabalin significantly suppressed allodynia-evoked neural activity in several nociceptive and pain-processing areas of the brain, despite the absence of behavioural analgesia. Furthermore, placebo significantly increased functional connectivity between the rostral anterior cingulate and the brainstem, a core component of the placebo neural network.

Conclusions: Functional neuroimaging provided objective evidence of pharmacodynamic efficacy in a proof-of-concept study setting where subjective pain outcome measures are often unreliable. Additionally, we provide evidence confirming the neural mechanism underpinning placebo analgesia as identified in acute experimental imaging studies in

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patients during the placebo arm of a clinical trial. We explore how brain penetrant active drugs potentially interact with this mechanism.

Clinical trial registration: NCT0061015

Keywords: analgesics; magnetic resonance imaging; neuralgia; placebos; randomized controlled trial

Editor's key points

- A lack of objective outcome measures contributes to the high attrition of potentially effective new analgesics.
- Non-invasive functional magnetic resonance imaging was used to measure brain activity and connectivity in chronic pain patients treated with pregabalin, tramadol or placebo.
- This proof-of-concept study demonstrates the utility of this noninvasive neuroimaging approach to provide objective evidence of analgesic efficacy and placebo effect on patients with neuropathic pain.

Existing analgesics provide 50% pain relief in only a third of patients, with significant individual and societal costs.^{1,2} Many promising new compounds fail to reach the market as effective analgesics in patients because potentially effective compounds are discarded in early drug development due to a lack of statistically significant reductions in pain reports in randomized placebo-controlled trials (RPCTs).² Pain relief in the placebo treatment arm—which is often large—can confound potentially valuable, mechanistic and pharmacodynamically produced analgesic effects of the study drug.³ Further, subjective pain reports during drug-induced analgesia are significantly influenced (negatively and positively) by the expectation of treatment outcome.⁴ Therefore there is a clear need for additional objective outcome measures of pharmacodynamic efficacy that can demonstrate target engagement and analgesic drug modulation of relevant neural activity in early patient studies so that effective analgesics reach chronic pain patients.

Non-invasive functional magnetic resonance imaging (fMRI) is a useful method for characterizing central nervous system (CNS) activity in chronic pain and for objectively demonstrating analgesic drug modulation of such activity.^{5,6} Using a double-blind RPCT design and a healthy volunteer model of central sensitization, fMRI was used to demonstrate that gabapentin suppressed neural activity in relevant brain areas irrespective of behavioural pain reports.^{7,8} We aimed to establish this principle in post-traumatic neuropathic pain patients using pregabalin and tramadol as the study drugs, chronically administered at the proposed clinical dose, as these are recommended first- and second-line therapies, respectively, for neuropathic pain.⁹

The assumption that in RPCTs expectation-driven placebo analgesic effects are non-specific, and therefore equal in both the drug and placebo arms, is now being questioned¹⁰ because placebo analgesic responses have distinct neural mechanisms that CNS-acting drugs can interact with.¹¹ Therefore we hypothesized that brain networks underpinning placebo analgesia would be measurable in the placebo arm (to date, not shown in a patient study with chronic dosing) but not during active treatment.

Methods

Participants

The study was approved by the Oxford Research Ethics Committee C (08/H0606/5) and registered with [ClinicalTrials.gov](https://www.clinicaltrials.gov) (NCT00610155). Subjects were enrolled from three study centres in the UK after obtaining written informed consent. Neuroimaging was performed at the Oxford Centre for Functional Magnetic Resonance Imaging of the Brain (FMRIB). Patients between the ages of 18 and 75 years with a confirmed diagnosis of post-traumatic neuropathic pain associated with brush allodynia that has persisted for at least 3 months with at least moderate-intensity daily and allodynic pain were included in the study. Patients with other neuropathic pain conditions; a history of failure to respond to gabapentin, pregabalin or tramadol; contraindications for MRI scanning; and patients with any medical, psychological or social condition that would interfere with study participation were excluded (inclusion and exclusion criteria details are in the Supplementary Material).

Study design and procedures

This was a double-blind (patients and investigators), third-party open (sponsor), three-way crossover RPCT. Patients were randomized to receive 7 days of dosing with pregabalin [75 mg on day 1, 75 mg twice a day (BID) on day 2, 150 mg BID on days 3-7, and 150 mg on the morning of the fMRI visit], tramadol sustained-release tablets (50 mg on day 1 and morning of day 2, 100 mg on evening of day 2 and morning of day 3, 200 mg on evening of day 3, and 200 mg BID on days 4-7 and 200 mg on the morning of the MRI visit), or placebo. Paracetamol and codeine were permitted as rescue medication. An overview of the study procedures is shown in [Figure 1](#) (study procedure and randomization details are in the [Supplementary material](#)).

Data collection

During visits 4-6, we collected averaged pain scores (DPS) from daily pain diaries, including the morning of the scanning day summarized as the past 1 (DPS1), 3 (DPS3) and 7 days (DPS7), and scores from several validated questionnaires (listed in [Table 1](#)).

DPS7 and the Neuropathic Pain Symptom Inventory (NPSI)¹³ were also collected during visit 3. During visits 3-6, we collected the following measures: present pain intensity (PPI) of the ongoing background pain at the beginning of the scanning session and pain intensity when brushing the affected site (DMAa) and the unaffected control site (DMAc). For all pain ratings we used an 11-point numeric rating scale, with 0=no pain and 10=worst pain possible.

Allodynia was elicited outside the scanner during visit 3 and inside the scanner during visits 4-6 while obtaining

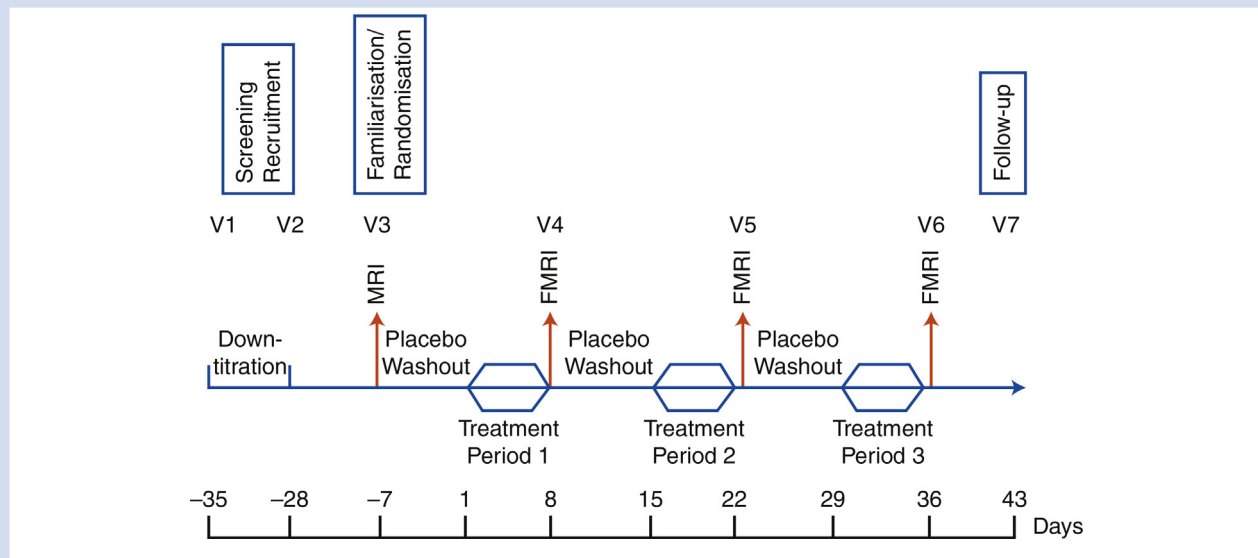


Fig 1. Overview of study procedures and illustrative time-line showing maximum possible time boundaries in the period before V3. At visit 1 (V1) after obtaining informed consent, patients were evaluated for study criteria. If deemed appropriate, then down-titration of existing medication was started. These patients attended visit 2 (V2) to confirm their eligibility and completion of down titration. The earliest V2 can occur is at -28 (at least 1 week after V1). However, V2 might occur much later but must not be more than 11 days and no less than 1 day before V3. The latest V3 can occur is 28 days from V1. After V3 the timing becomes rigid regarding placebo washout and treatment periods of seven days each, respectively. At V3, patients were familiarized with the scanning procedure, and a baseline resting-state scan and the structural scan were acquired after which the patients were randomized to the treatment sequence. During this visit and visits 4 and 5, patients received a study medication pack for 14 days that consisted of placebo for the first 7 days (washout period) followed by the medication appropriate for the treatment period. Patients were advised to take the medication at approximately the same time in the morning and evening on each day. They were informed in the subject information sheet that they were dosed with placebo, tramadol, or pregabalin at various times during the study, but they were not informed when they were transferred from one treatment to another. During these visits the pain and drug diaries were reviewed, patients completed pain-related questionnaires, and during visits 4, 5, and 6, functional MRI (fMRI) scans were obtained. The first, second, and seventh (follow-up) visit took place at the recruiting site. Visits 3-6 took place at the neuroimaging site in Oxford.

Table 1 Treatment effect on patient-reported scores. Mean and SD of patient-reported scores during treatment periods with pregabalin, tramadol, or placebo are given in columns 2, 3, and 4 from the left. P-values of two-tailed tests for between-group comparisons (paired t-test for normally distributed data and Wilcoxon signed-rank test for non-normally distributed data) are shown in the remaining columns. ^aSignificant after Bonferroni correction for the three comparisons. BDI, Beck Depression Inventory II¹²; DMAa, dynamic mechanical allodynia from the affected site; DMAc, pain intensity evoked by brushing of the unaffected control site; DPS1, Daily Pain Score on the morning of the scan; DPS3, Daily Pain Score during the past 3 days; DPS7, Daily Pain Score during the past 7 days; NPSI, Neuropathic Pain Symptom Inventory¹³; PPI, Present Pain Intensity; PCS, Pain Catastrophizing Scale¹⁴; SD, standard deviation; STAI-T and -S, Spielberger's State and Trait Anxiety Inventory scores¹⁵ for Trait (T) and State (S).

	Pregabalin		Tramadol		Placebo		Pregabalin us. tramadol	Pregabalin us. placebo	Tramadol us. placebo
	Mean	SD	Mean	SD	Mean	SD	P-value two-tailed	P-value two-tailed	P-value two-tailed
DMAc	0.44	0.89	0.63	1.02	0.94	2.17	0.18	0.52	1.00
DMAa	6.44	2.34	5.69	2.21	6.00	1.97	0.23	0.34	0.57
PPI	5.50	2.50	4.19	2.26	5.75	2.11	0.04	0.56	0.01 ^a
NPSI	47.94	21.98	38.44	21.11	47.63	19.35	0.08	0.91	0.04
DPS1	5.79	1.53	5.33	1.88	6.13	1.64	0.88	0.42	0.10
DPS3	5.63	1.66	5.13	1.79	6.23	1.98	0.58	0.24	0.03
DPS7	5.60	1.34	5.27	1.35	6.31	1.73	0.40	0.07	0.01 ^a
BDI	14.75	14.97	16.47	12.46	15.38	15.00	0.10	0.48	0.34
PCS	20.31	18.49	21.25	17.27	22.00	15.70	0.36	0.32	0.59
STAI-T	46.81	5.32	45.06	6.82	46.44	4.40	0.18	0.84	0.25
STAI-S	46.06	3.75	45.07	4.33	45.81	4.64	0.66	0.83	0.62

functional scans. Body sites were brushed using a Samedic brush for 10 min on each side. Subjects rated the average pain of all 15 stimuli. This was followed by a control visual stimulation task for 5 min to assess any global haemodynamic

influences of study drugs (stimulus delivery details are in the [Supplementary material](#)).

Functional, resting state and structural scans were acquired using a 3 T MR system (Siemens, Munich, Germany)

Table 2 BOLD responses in pain-responsive regions shown in group mean maps during each treatment period (Fig. 2) and in group mean contrast maps between treatment periods (Fig. 3). Cluster size (in voxels); z-scores and MNI coordinates (mm) of the peak voxel. ACC, anterior cingulate cortex; aIN, anterior insula; PFC, pre-frontal cortex; pIN, posterior insula; SI, primary somatosensory cortex; SII, secondary somatosensory cortex; SMA, supplemental motor area

Treatment	Region	Cluster size	z-score	x	y	z
Pregabalin	SII, left	34	3.46	-62	-26	22
	Thalamus, right	356	4.94	18	-18	16
	Thalamus, left	301	5.04	-12	-8	8
	aIN, right	53	3.57	40	14	-2
Tramadol	SII, left	23	2.92	-60	-22	20
	SMA/premotor	157	4.71	4	8	60
	ACC	69	4.03	2	18	36
	Thalamus, right	355	5.04	14	-10	6
	Thalamus, left	418	5.5	-12	-6	6
	aIN, right	151	4.3	44	12	-4
	aIN, left	144	4.49	-42	2	0
	PFC, right	1645	5.93	38	46	18
	SII, right	13	3.68	66	-18	20
	SII, left	97	4.18	-60	-28	20
Placebo	SMA/premotor	64	4.45	6	16	50
	ACC	96	3.72	-6	22	30
	Thalamus, right	358	4.04	16	-10	8
	Thalamus, left	294	3.77	-16	-12	6
	aIN, right	269	4.48	38	10	2
	aIN, left	159	5.23	-42	2	-2
	PFC, right	858	5.08	40	42	20
Contrast map	Region	Cluster size	z-score	x	y	z
Placebo > pregabalin	SI, right	283	4.29	48	-12	32
	SI, left	226	3.97	-52	-12	34
	SII, right	157	4.06	58	-8	8
	SII, left	48	3.37	-56	-12	10
	aIN, left	20	3.24	-42	2	-2
	pIN, left	15	2.72	-38	-10	8
Tramadol > pregabalin	SI, right	118	3.39	48	-10	50
	SII, right	35	4.65	42	-12	22
	ACC	289	3.59	-2	22	32
	SMA/premotor	99	4	-4	6	48

(technical and data management details are in the [Supplementary material](#)).

Behavioural data analysis

The Shapiro-Wilk normality test was used to examine the distribution of psychophysical data. For normally distributed data, a paired two-tailed t-test was used for comparison of data between two treatments, with Bonferroni correction to account for the three comparisons. For data that were non-normally distributed, the Wilcoxon signed-rank test was used. Behavioural placebo analgesia, hypothesized as having lower pain reports during placebo than during baseline, was tested by using a one-tailed test. For consistency, we used the same to compare pain reports between the baseline and active-treatment periods. We used SPSS version 21 (IBM, Armonk, NY, USA) for analysis of psychophysical data.

Imaging data analysis

Imaging data were pre-processed and analysed using the FMRIB Software Library (FSL), version 4.1.7 (<http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/>; accessed 8 September 2017). Parameter estimates for the regressors that described blood oxygen level-dependent (BOLD) neural activity evoked by the sensory stimuli for

individual subjects for each treatment period were generated using a general linear model and images were registered to structural and standard structural space (MNI152).

Group analyses were performed using whole-brain, mixed-effects analysis (FLAME1) and outlier inference.^{16,17} To identify regions showing statistically significant activation accounting for multiple comparisons, statistical maps were thresholded using cluster-based thresholding with a cluster-forming threshold $Z > 2.3$. Clusters with corrected significance of $P=0.05$ were retained in the thresholded maps.¹⁸

We used resting-state data obtained during baseline (after washout of their current medication), placebo and the two active treatments to study connectivity between the rostral anterior cingulate (rACC), periaqueductal-grey (PAG), amygdala and brainstem; regions chosen a priori based on previously published data on placebo networks.¹⁹⁻²¹ These studies show involvement of various brainstem areas, such as the periven-tricular grey, pons and rostral ventromedial medulla, in exper-imentally induced placebo analgesia in healthy volunteers. However, the placebo response in our cohort of chronic pain patients receiving chronic analgesic dosing in a double-blind RPCT setting might not necessarily be limited to these same brainstem areas. Therefore we used a more inclusive whole brainstem mask to explore placebo analgesia in our subjects. Correlations between the BOLD time series from each region of

interest were calculated, yielding six sets of connectivities. Statistical differences in connectivity between the baseline and the three treatment periods were assessed using a mixed model including regressors accounting for any effects due to scan order. We used a two-tailed test, as directionality of the neural connectivity changes in chronic pain patients during placebo in an RPCT setting is unknown, although in healthy volunteers we know it increases.²⁰ Bonferroni correction was used to account for multiple comparisons in this analysis (image analysis technical details in the [Supplementary material](#)).

Results

Twenty-one subjects who satisfied the inclusion/exclusion criteria attended visit 3 (see [Supplementary Fig. S1](#)). Of these, two were unsuitable for MRI and three withdrew from the study. This left 16 subjects (5 males, 11 females) who completed all visits, and their data were included in all analyses. The use of rescue medication was similar in the three treatment groups (subject characteristics and rescue medication details are in the [Supplementary material](#)). The baseline group mean for NPSI was 49.0 (SD 18.6), DPS7 was 6.6 (SD 1.2), DMAa was 7.0 (SD 1.4) and PPI was 5.7 (SD 1.2).

There were no statistically significant differences between treatments in psychophysical scores, except for tramadol, that significantly reduced PPI and DPS7 compared with placebo ([Table 1](#)). Compared with baseline, placebo significantly reduced DMAa ($P=0.04$), pregabalin significantly reduced DPS7 ($P=0.002$) and tramadol significantly reduced PPI ($P=0.01$), DMAa ($P=0.01$), DPS7 ($P=0.001$) and NPSI ($P=0.02$).

There were significant DMAa-evoked activations of nociceptive and pain-related brain areas during placebo, tramadol and to a lesser extent pregabalin ([Fig. 2](#), [Supplementary Figs. S2-S4](#) for all axial slices, and [Table 2](#) for BOLD activity data). Group mean comparisons of DMAa-evoked neural activity between treatments revealed that, relative to placebo, only pregabalin and not tramadol significantly suppressed DMAa-evoked neural activity in several brain areas, including the left anterior (aIN) and posterior insula (pIN), bilateral primary (SI) and secondary somatosensory cortices (SII) ([Fig. 3](#) and [Table 2](#) for BOLD activity data). Significant suppression of DMAa-evoked neural activity by pregabalin was also observed relative to tramadol in the dorsal mid-ACC (anterior cingulate cortex) and right SI and SII.

There were no significant differences in the neural activity in response to the control visual stimulus during treatments,

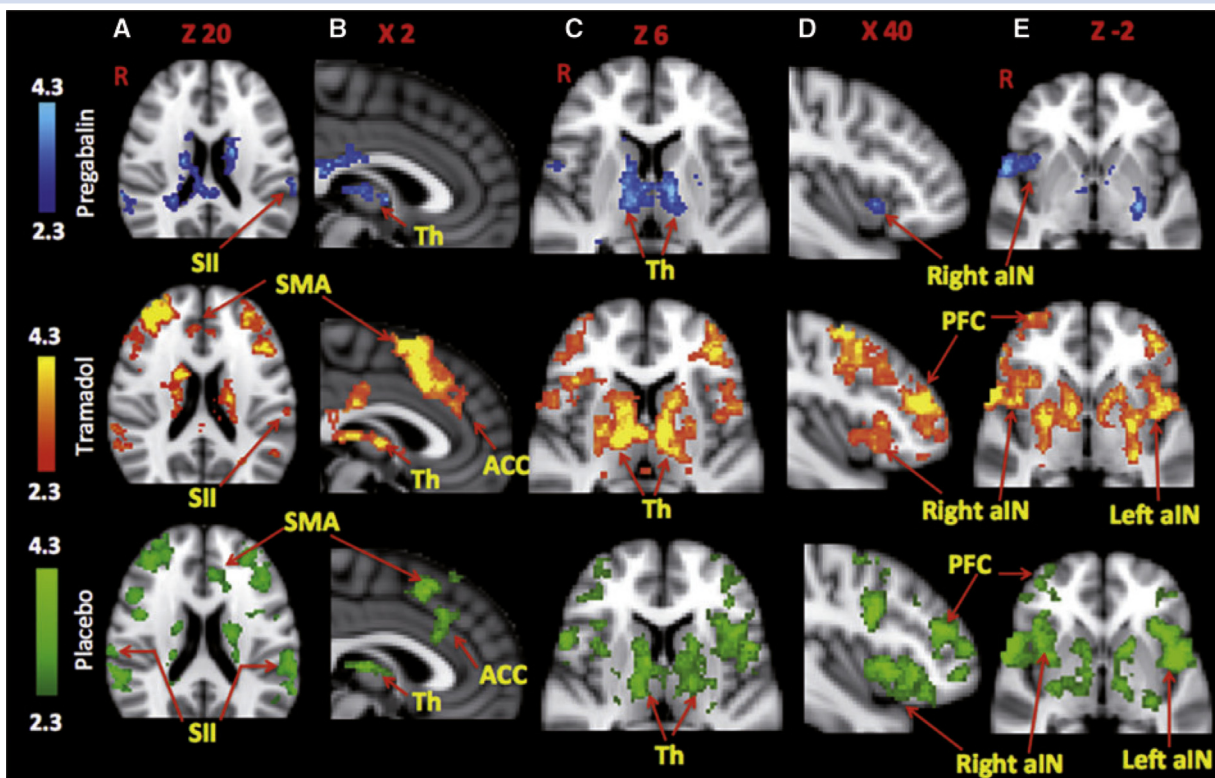


Fig 2. Group mean activation maps. Significant group mean activations in response to dynamic mechanical allodynia on the affected site were observed in the supplementary motor cortex (SMA) and anterior cingulate cortex (ACC), bilateral thalami (Th), bilateral anterior insula (aIN), bilateral secondary somatosensory cortex (SII), and bilateral prefrontal cortices (PFC) during placebo (bottom row in green) and tramadol treatment (middle row in red). During pregabalin treatment (top row in blue), significant activations were observed in the bilateral thalami, right aIN, and right SII. A whole-brain mixed-effects analysis with cluster-based thresholding (Z threshold at $Z>2.3$) and a significance level of $P=0.05$ were used for detecting these group mean activations. Image slices from MNI coordinate Z20 are in column A, from X2 are in column B, from Z6 are in column C, from X40 are in column D, and from Z-2 are in column E.

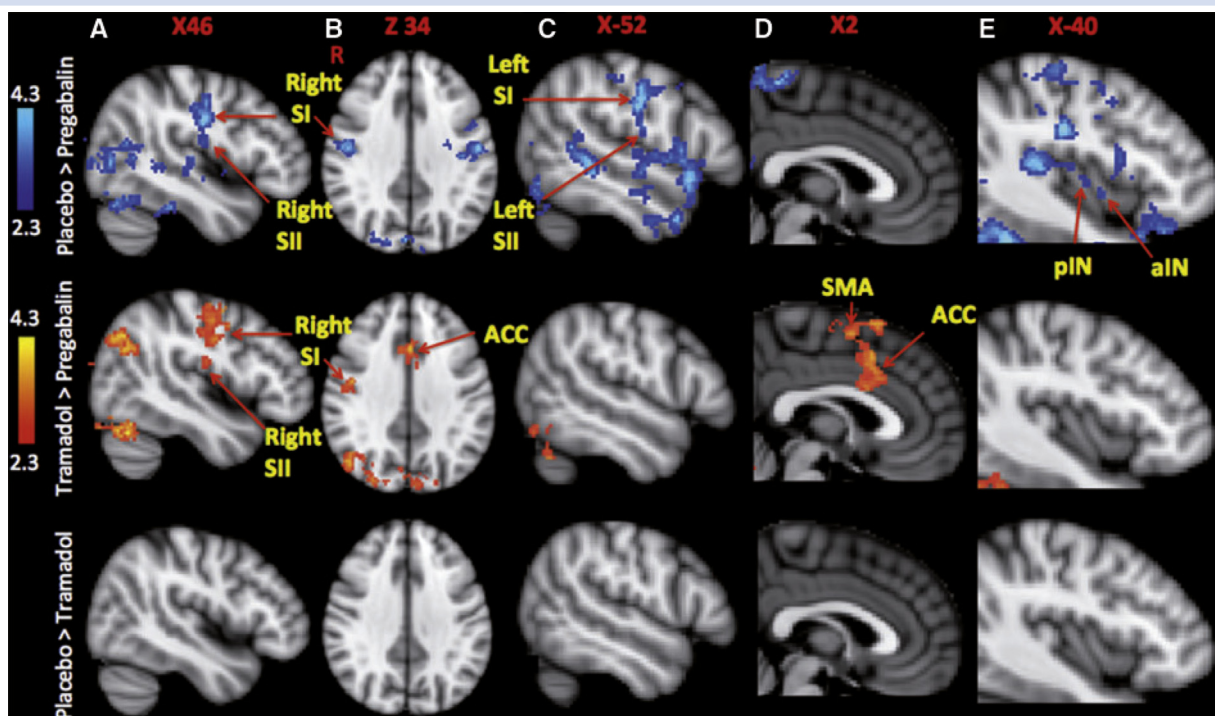


Fig 3. Contrast maps—whole-brain analysis. Significant suppression of neural activity evoked by dynamic mechanical allodynia on the affected site was observed bilaterally in the primary and secondary somatosensory cortices (SI and SII) and in the left anterior and posterior insula (aIN and pIN) during pregabalin when compared with placebo (top row in blue). When compared with tramadol, pregabalin significantly suppressed neural activity in the right SI, right SII, anterior cingulate cortex (ACC), and supplementary motor cortex (SMA) (middle row in red). There were no areas with suppressed neural activity during tramadol when compared with placebo (bottom row) that reached statistical significance. A whole-brain mixed-effects analysis with cluster-based thresholding (Z threshold at $Z > 2.3$) and a significance level of $P = 0.05$ were used for these paired comparisons. Image slices from MNI coordinate X46 are in column A, from Z34 are in column B, from X-52 are in column C, from X2 are in column D, and from X-40 are in column E.

indicating a lack of global treatment effects on neurovascular coupling.

Resting-state functional connectivity analysis between the four brain regions tested revealed significantly higher connectivity between the brainstem and rACC during placebo treatment compared with baseline (the latter had no expectation of treatment outcome). However during the two active treatments and despite the presence of treatment expectation, the brainstem-rACC connectivity was not significantly different compared with baseline (Fig. 4). The effect size expressed as standardized effect size (Cohen's d) and 95% confidence interval (CI) between baseline and tramadol was 0.59 (95% CI -0.13 to 1.28), between baseline and pregabalin was 0.64 (95% CI -0.09 to 1.33), and between baseline and placebo was 1.04 (95% CI 0.27 to 1.75).

There were no significant differences that survived correction for multiple comparisons between baseline and the three study visits in any of the other connectivity networks tested.

Discussion

We administered treatment doses of pregabalin and tramadol to 16 post-traumatic neuropathic pain patients for 1 week per drug using a double-blind, three-way crossover, RPCT design.

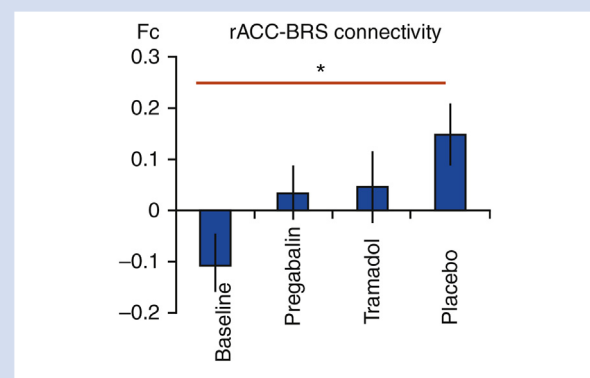


Fig 4. Resting-state connectivity. Group mean resting-state functional connectivity (Fc) between the brainstem (BRS) and the rostral anterior cingulate cortex (rACC) during baseline, pregabalin, tramadol, and placebo are shown in the bar chart. Error bars indicate standard error of the mean. * $P < 0.05$ after Bonferroni correction. There is no statistically significant difference in BRS-rACC Fc between the baseline and tramadol and between baseline and pregabalin.

Treatment effects were studied using behavioural and neuroimaging outcome measures.

We observed significant allodynia-evoked neural activity that was most prominent and widespread during the placebo treatment in nociceptive and pain-processing brain areas, corroborating previously reported activation patterns in patients with neuropathic pain.^{22 23}

Pregabalin significantly suppressed allodynia-evoked neural activity in pain-processing brain regions, in keeping with its well-known efficacy in neuropathic pain. Further, placebo treatment increased connectivity between two key regions of the placebo neural network; an effect limited to placebo and indicative of a chronic change in network coupling. This probably explains, in part, the behavioural placebo analgesia seen during that treatment arm, in addition to other as yet unidentified networks.

In preclinical neuropathic pain models, gabapentinoids produce analgesia by reducing spinal dorsal horn excitability, diminishing ascending nociceptive inputs.^{24 25} Areas such as the pIN and SII receive these ascending nociceptive-specific inputs.^{26 27} The pIN, in particular, plays a fundamental role in nociceptive-induced ongoing pain²⁸ and its utility in revealing pregabalin pharmacodynamic effects in chronic pain conditions has been recently shown.²⁹ Therefore we believe that suppression of allodynic neural activity by pregabalin within the pain-processing brain areas, including the left pIN and bilateral SII, represents a pharmacodynamic effect of pregabalin.

In contrast, pregabalin did not demonstrate behavioural analgesia. The pain report of an individual is modified by many factors, including treatment outcome expectation, that can enhance or diminish the behavioural pain relief from an analgesic.⁴ Analgesia is defined as a statistically significant reduction in pain report during drug compared with placebo in an RPCT. Here, a positive treatment outcome expectation will drive a reduction in pain reports in the placebo arm, such that a large sample size is required to demonstrate additional pharmacodynamic analgesic efficacy.³ Clinical trials demonstrating behavioural analgesic efficacy of pregabalin have sample sizes that range from 100 to 300.^{30 31} Therefore, lack of behavioural analgesia in our cohort of 16 patients is most likely due to the small sample size. Another reason could be the short dosing period. In clinical practice, patients are advised it might take a month to experience meaningful analgesia (https://www.britishpainsociety.org/static/uploads/resources/files/FPM-Pregablin_2.pdf, accessed 8 September 2017). However, we have shown that neuroimaging can detect drug-induced suppression of neural activity to a clinically relevant painful experience in a small cohort of neuropathic pain patients after 1 week of exposure to pregabalin. Indeed, previous studies have shown that pharmacodynamic effects of an analgesic can be detected at a neural level independent of the effects of treatment outcome expectation on behavioural pain reports.^{4 32} However, we acknowledge that the regions we report as suppressed, while likely relevant to some features of what constitutes the pain report, cannot account for a patient's pain or analgesia in its entirety. There will always be additional regions involved in the expression of a pain report beyond what is changed by the stimulus input and suppressed by the drug action. Furthermore, there is very likely a complicated temporal relationship between the reduction in activity of pain-related neural processing circuits by a drug and the patient experiencing analgesia because of other factors, such as mood, context, expectation, and

learning, masking at a behavioural level the effects of any neural suppression.

In a drug development setting, studies such as ours can provide objective evidence of potential analgesic benefits rather than establish the analgesic efficacy of a compound. Such results might provide a firmer rationale to progress a compound to the next stage and expose a larger number of patients to a potentially beneficial compound for a longer period of time. This larger cohort study should then obtain statistically significant reductions in pain reports, the ultimate gold-standard outcome measure of establishing the analgesic efficacy of a compound.³³

Tramadol failed to suppress allodynia-evoked neural activity when compared with placebo, most likely due to the small effect size of tramadol-induced suppression of neural activity, which failed to reach statistical significance. Furthermore, pregabalin suppressed evoked neural activity in areas of the ACC, right SI and SII when compared with tramadol. These findings perhaps suggest that in post-traumatic neuropathic pain tramadol is not as effective as pregabalin in suppressing allodynic pain. Interestingly, in recent neuropathic pain treatment guidelines, tramadol was not among the recommended treatments for post-traumatic neuropathic pain.³⁴

We also note that, when compared with placebo, tramadol significantly reduced spontaneously reported PPI and DPS7 scores but not evoked pain reports. DPSs were generated with data from paper diaries throughout the study. The accuracy of paper diary reports has been challenged,³⁵ yet subjective ratings remain the gold standard, which is appropriate. However, this debate, coupled with the fact that subjective ratings are multifactorial and readily influenced by factors including mood and context, lends support to the notion that ratings alone can be misleading in both directions when predicting the likely efficacy of a novel compound. This is particularly true for double-blind RPCTs in small patient cohorts at early stages of analgesic drug development. Furthermore, daily pain scores likely represent broader aspects of pain involving different sets of brain regions that are unlikely to be completely encapsulated by the allodynia-evoked neural activity. Future studies should include neural measures related to ongoing pain.

Emerging evidence suggests that placebo analgesia is underpinned by distinct neural mechanisms that CNS-acting drugs might interact with.⁴¹¹ We detected a significant placebo-induced connectivity between the brainstem and the rACC, key structures of the placebo network, indicating a possible expectation-induced effect. As such, this is an important observation in itself, suggestive of a sustained activation of this network in patients during chronic placebo dosing—to date only shown acutely in healthy volunteers. However, there was no significant difference in rACC-brainstem connectivity between the active treatments and baseline. The size of the placebo effect relative to baseline was not significantly different from the size of the tramadol or pregabalin effects relative to baseline. Yet the trend apparent in Fig. 4 suggests that the neural effects of expectation may not be the same in the presence of active treatment despite similar treatment outcome expectations in a double-blind study setting. This supports the mounting behavioural observations challenging the assumption of additivity in some instances.³⁶⁻³⁸

In conclusion, our results demonstrate the potential utility of functional imaging in early proof-of-concept patient studies in providing objective evidence of pharmacodynamic effects in relevant CNS areas when convincing and

consistent evidence of behavioural analgesia is lacking or noisy. We also present preliminary evidence that the placebo network is active in patients during a chronic placebo treatment arm and that CNS-active drugs can disrupt this network. This finding contributes to the growing body of evidence that challenges the validity of the 'additive' model in placebo-controlled trials.

Authors' contributions

Study design: J.P.H., W.V., P.R., B.H., and I.T.

Patient recruitment and data collection: V.W., K.W., P.R., and B.H.

Data analysis design and interpretation: V.W., K.W., J.P.H., E.P.D., W.V., M.W., N.M., and I.T.

Preparation of the manuscript: V.W., K.W., J.P.H., E.P.D., W.V., M.W., N.M., L.P., P.R., B.H., and I.T.

All authors contributed to the development of every draft of this manuscript and approved the final version.

Declaration of interests

M.W. and L.P. are employees of Pfizer and received stock or stock options in Pfizer. W.V., J.P.H., and N.M. were employees of Pfizer at the time of this study. I.T. has undertaken commercially funded work for research or lectures from Innovative Medicines Initiative European Consortium, Grunenthal, Pfizer, Abide Therapeutics, Lilly, Mundipharma, Amgen, Janssen, Celgene, Lundbeck, and Bayer. V.W.'s research work and salary have been funded by grants from Innovative Medicines Initiative European Consortium and Grunenthal. E.D., K.W., B.H. and P.R. declare no potential competing interests. Editorial support in the form of copy-editing and data checking was provided by Ray Beck, Jr, PhD of Engage Scientific Solutions and funded by Pfizer.

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Appendix A. Supplementary material

Supplementary material is available at British Journal of Anaesthesia online. <https://doi.org/10.1016/j.bja.2017.11.064>

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