

Disambiguating Pharmacodynamic Efficacy from Behavior with Neuroimaging

Implications for Analgesic Drug Development

Vishvarani Wanigasekera, D.Phil., Melvin Mezue, D.Phil., Jesper Andersson, Ph.D., Yazhuo Kong, Ph.D., Irene Tracey, D.Phil.

ABSTRACT

Background: Attrition rates of new analgesics during drug development are high; poor assay sensitivity with reliance on subjective outcome measures being a crucial factor.

Methods: The authors assessed the utility of functional magnetic resonance imaging with capsaicin-induced central sensitization, a mechanism relevant in neuropathic pain, for obtaining mechanism-based objective outcome measures that can differentiate an effective analgesic (gabapentin) from an ineffective analgesic (ibuprofen) and both from placebo. The authors used a double-blind, randomized phase I study design (N = 24) with single oral doses.

Results: Only gabapentin suppressed the secondary mechanical hyperalgesia-evoked neural response in a region of the brainstem's descending pain modulatory system (right nucleus cuneiformis) and left (contralateral) posterior insular cortex and secondary somatosensory cortex. Similarly, only gabapentin suppressed the resting-state functional connectivity during central sensitization between the thalamus and secondary somatosensory cortex, which was plasma gabapentin level dependent. A power analysis showed that with 12 data sets, when using neural activity from the left posterior insula and right nucleus cuneiformis, a statistically significant difference between placebo and gabapentin was detected with probability ≥ 0.8 . When using subjective pain ratings, this reduced to less than or equal to 0.6.

Conclusions: Functional imaging with central sensitization can be used as a sensitive mechanism-based assay to guide go/no-go decisions on selecting analgesics effective in neuropathic pain in early human drug development. We also show analgesic modulation of neural activity by using resting-state functional connectivity, a less challenging paradigm that is ideally suited for patient studies because it requires no task or pain provocation. (ANESTHESIOLOGY 2016; 124:159-68)

CHRONIC pain affects 20% of the European population,¹ yet there is a dearth of effective analgesics.² Many potential compounds identified in preclinical research fail to reach the market as effective analgesics,³ key reasons being the discarding of potentially effective compounds in randomized controlled trials⁴ and poor translation of analgesic efficacy in animals to patients.^{5,6} It is important to identify ineffective compounds in early drug development to stop their progression to large-scale patient studies, thereby limiting cost and unnecessary patient exposure to ineffective compounds.

Tools reliant on subjective reports are integral to clinical assessment of analgesic efficacy.⁷ However, these have considerable within-subject variability and are highly context dependent.⁸ Therefore, subjective pain reports when used as sole outcome measures in early drug development studies, where sample sizes are small, can both miss efficacious target engagement and fail to detect ineffective compounds.

What We Already Know about This Topic

- Human neurophysiologic measures of analgesic response are largely lacking
- Functional magnetic resonance imaging can be used to provide such measures in experimental volunteer models and patients

What This Article Tells Us That Is New

- After development of experimental central sensitization, gabapentin reduces activation of pain-related brain areas as well as functional connectivity between the thalamus and secondary somatosensory cortex, whereas ibuprofen does not when compared with placebo
- Functional imaging may be a viable tool for evaluating analgesic efficacy during early stages of drug development

Objective outcome measures of drug modulation of neural activity in human mechanism-based models of pain can provide valuable evidence to guide clear go/no-go decisions,

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by detecting both truly effective and truly ineffective compounds in early drug development.⁹

Neuropathic pain is a chronic pain condition¹⁰ characterized by spontaneous pain, hyperalgesia, and allodynia of the affected area causing significant impairment in quality of life.¹¹ Several mechanisms are involved in neuropathic pain, and central sensitization is a potentially key one.¹² Topical capsaicin in humans induces central sensitization, which has similar behavioral manifestations to that of neuropathic pain.¹³ Therefore, it is considered a suitable human model to study central sensitization¹⁴ and assess therapeutic efficacy of analgesics.¹⁵

Gabapentin provides clinically relevant pain relief in painful polyneuropathies.¹⁶ Yet evidence of analgesic efficacy from behavioral outcome measures in small-scale human studies is variable and inconsistent.¹⁷ Furthermore, behavioral outcome measures alone do not provide evidence that the compound is actually modulating the neural activity involved in maintaining the chronic pain state and its concomitant pain experience, as they can be dramatically influenced by other factors such as expectation.⁸

Functional magnetic resonance imaging (fMRI) is a non-invasive neuroimaging technique that allows us to both map and quantify nociceptive and pain processing areas within the human brain.¹⁸ By using an fMRI, we have highlighted the critical involvement of the brainstem in central sensitization induced by capsaicin^{19,20} and shown that gabapentin attenuates brainstem activity.²¹ Indeed, the brainstem plays a crucial role in maintaining central sensitization in chronic pain states.^{12,22}

This evidence suggests that an fMRI in conjunction with capsaicin-induced central sensitization could be a useful mechanism-based technique to demonstrate drug modulation of relevant neural activity; as such, it would support the drug being a potentially clinically effective analgesic compound in neuropathic pain. Here, we aim to demonstrate the utility of the fMRI in disambiguating a drug, gabapentin, that is effective and a drug, ibuprofen, that is ineffective in neuropathic pain irrespective of drug-induced pain reports in a phase I setting using a double-blind, randomized, placebo-controlled three-way crossover study in healthy volunteers with central sensitization.

Materials and Methods

The study (reference 08/H0606/50) was approved by Oxfordshire Research Ethics Committee, United Kingdom on July 30, 2010. After obtaining written informed consent, we recruited 25 healthy subjects from July 2011 to March 2012 after an initial screening visit. During the screening visit, all these subjects developed capsaicin-induced secondary mechanical punctate hyperalgesia. The intensity of the punctate stimuli they perceived was significantly higher (paired *t* test; *P* < 0.05) after application of topical capsaicin than before. We used the same method of capsaicin

application as during the study visits. Twenty-four subjects completed the study (age [mean ± SD], 24 ± 4.2 yr; 13 women). One subject was withdrawn during the first study visit due to their inability to lie still in the scanner.

Study Overview

There were three study visits at least 1 week apart during which the subjects fasted for 6 h before attending the study centre. Subjects received placebo, 1,200 mg gabapentin, or 600 mg ibuprofen orally (order randomized) followed by food. Capsaicin cream 1% was applied 90 min after dosing with study medication on an area (4 × 4 cm) of skin on the anteromedial aspect of the right lower leg at least 14 cm above the medial malleolus. An area (4 × 2 cm) 2 cm directly below and parallel to the lower border of capsaicin application was selected as the target area for eliciting dynamic mechanical allodynia and hyperalgesia. Subjects were scanned in a 3T magnetic resonance imaging scanner 150 min after dosing. Scans were obtained sequentially in blocks (fig. 1A) while eliciting allodynia and hyperalgesia, followed by an arterial spin labeling sequence and an echo planar imaging resting-state sequence. Capsaicin was removed at the end of scanning, and a venous blood sample was obtained for assay of drug levels approximately 200 min after drug administration from 22 subjects (2 subjects declined venipuncture). The doses of the study drugs and the timing of the functional scans were based on the previous studies testing analgesic efficacy of the study drugs in human experimental models.^{21,23,24}

Sensory Testing

Allodynia was elicited with a soft standardized brush (Somedic, Sweden) over 10 min by delivering 15 identical 6-s stimuli to the target area with an interstimulus interval jittered between 28 and 46 s. Each stimulus had three approximately 2-s strokes in the mediolateral direction. Average pain intensity and unpleasantness elicited by stimuli were recorded 12 and 18 s after the last stimulus using a visual analog scale (VAS) with anchors “not painful and extremely painful” and “not unpleasant and extremely unpleasant.” Hyperalgesia was elicited by delivering 18 identical 1-s punctate stimuli with a punctate probe that delivers a force of 512 mN over 10 min to the target area with an interstimulus interval jittered between 28 and 32 s.¹⁹ Stimulus intensity was recorded 12 s after each stimulus using a VAS with anchors “not intense and extremely intense.” Average unpleasantness of stimuli was recorded 12 s after the last stimulus with anchors “not unpleasant” and “extremely unpleasant.” The unprovoked ongoing pain induced by the capsaicin cream was recorded at the start and the end of scanning using a VAS with anchors “no pain and severe pain.” The average of these two values was used for assessing the unprovoked pain.

Gabapentin is known to cause sedation, and the related compound pregabalin is an anxiolytic. Therefore, we obtained self-reported measures of anxiety using the

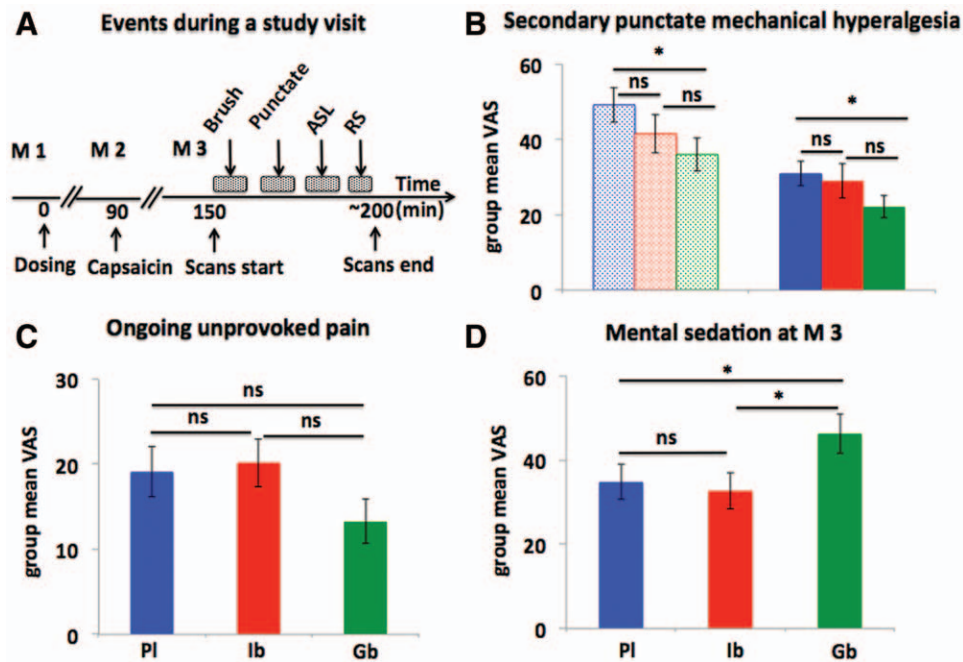


Fig. 1. Study events and drug-induced effects on psychophysics. (A) Events during a study visit. In the scanner, functional scans were obtained while delivering blocks of brush stimuli over approximately 10 min to elicit dynamic mechanical allodynia followed by punctate stimuli over approximately 10 min to elicit hyperalgesia. This was followed by two task-free scans: an arterial spin-labeling sequence (ASL) lasting approximately 10 min and a resting-state sequence (RS) lasting for approximately 5.5 min. At the end of the scan (approximately 200 min from dosing), capsaicin cream was removed, and a venous blood sample was obtained for drug assay. Mood measurements were obtained before dosing (M1), 90 min after dosing (M2) just before capsaicin application, and at 150 min after dosing (M3) just before commencement of scanning. Group mean scores of secondary punctate mechanical hyperalgesia intensity (*solid bars*) and unpleasantness (B), ongoing unprovoked pain (C), and mental sedation 150 min after dosing (M3; D) during placebo (PI), ibuprofen (Ib), and gabapentin (Gb) visits are shown. Y-axis shows the group mean visual analog scale (VAS) scores. Error bars indicate SEM. * $P < 0.05$ after Bonferroni correction. ns = statistically not significant.

Spielberger state anxiety scale²⁵ and sedation using the Bond–Lader mood scale²⁶ immediately before dosing and 90 and 150 min after dosing.

FMRI Data Acquisition

These were acquired using a 3T scanner fitted with a 32-channel head-only radiofrequency coil. Functional scans and resting-state scans were acquired with a whole brain gradient echo-planar imaging sequence with the following parameters: 30-ms echo time, field of view 192×192 mm, matrix 64×64 , and 3-mm thick axial slices. Functional scans had 200 volumes, and resting scan had 128 volumes. The repetition time was 3 s for functional scans and 2.514 s for resting scan. Fieldmaps were acquired (field of view 192×192 mm, matrix 64×64) after the resting-state scan to correct for the regions of field inhomogeneity. A T1-weighted structural (1-mm³ voxel) image was acquired during the screening visit for the registration of statistical activation maps to the standard stereotactic space (Montreal Neurological Institute, Montreal, Quebec, Canada; 152 template). Pulse and respiratory waveforms were recorded during scanning for physiologic noise correction in data analysis.²⁷ Arterial spin labeling data acquisition is not described here, because these data will not be presented in this article.

Analysis and Statistical Methods

Analysis of Behavioral Data. Twenty-four data sets were analyzed. 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 visits with Bonferroni correction to account for the three comparisons. Pearson correlation coefficient was used for correlational analyses. For data that were nonnormally distributed, we used the Wilcoxon signed-rank test and Spearman rho. We used SPSS software, version 21 (SPSS, Inc., USA) for analysis of psychophysical data.

Analysis of Imaging Data. All images were analyzed using tools in the Functional Magnetic Resonance Imaging of the Brain (FMRIB) Software Library (FSL) version 5.0 (Analysis Group, FMRIB, United Kingdom; <http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/>, accessed October 9, 2015). Automated atlases as implemented in FSL were used for anatomically defining cortical and subcortical areas.

To establish blood oxygen level-dependent (BOLD) imaging as an objective outcome measure for detecting drug modulation of neural response to nociceptive stimuli in a centrally sensitized state, we generated statistical maps with parameter estimates for the regressors that described the task-evoked BOLD response for each task-based scan.

First, data were preprocessed to remove noise and artefacts using tools provided within FSL.^{27–30} A Gaussian kernel of 5-mm full width half maximum was used for spatial smoothing. Boundary-based³¹ linear and nonlinear tools³² were used to register functional data to structural and standard space. Statistical maps were generated using the general linear model as implemented in FSL. Then group differences in these data between treatments were identified using whole brain analyses with mixed effects³³ and a cluster-based familywise error correction for multiple comparisons (Z score more than 2.3; $P < 0.05$). The contrasts used for identifying evidence of analgesic effects of gabapentin were placebo > gabapentin and ibuprofen > gabapentin. Evidence for potential analgesic superiority of ibuprofen was investigated in the placebo > ibuprofen and gabapentin > ibuprofen contrasts, whereas that of placebo was investigated in the gabapentin > placebo and ibuprofen > placebo contrasts. The whole brain analysis has inherently poor sensitivity due to the nature of multiple comparison correction. Therefore, for the task data, we additionally performed a directed search in the mesencephalic reticular formation (MRF), which contains the nucleus cuneiformis (NCF) and is known to be involved in central sensitization.^{19,20} Specifically we performed a small-volume correction on a mask defining the MRF using nonparametric permutation testing (5,000 permutations)³⁴ on threshold-free cluster-enhanced values,³⁵ yielding a familywise error correction rate of 0.05. The MRF mask that included the area of the mesencephalon excluding the substantia nigra and the crus cerebri was defined using the Duvernoy brainstem atlas³⁶ as an automated detailed brainstem atlas is not available.

To see whether resting-state BOLD data are useful in detecting drug modulation of functional connectivity between key nociceptive-processing brain areas, similar to task data, we generated statistical maps that identified the brain areas that are functionally coherent with an *a priori* seed region for each resting scan. Then we used whole brain analyses similar to that used for task-evoked data to identify drug-modulated effects on functional connectivity between the *a priori* region and all other brain regions. We selected the left thalamus (contralateral to the site of capsaicin application) as the *a priori* seed region because it is a main relay station for ascending nociceptive dorsal horn neurons before reaching the cortical nociceptive-processing areas.³⁷ We used the anatomically defined whole left thalamus as the resolution, and contrast of these particular 3T functional images are inadequate to identify specific sensory thalamic nuclei robustly.

To estimate the sensitivity of task-evoked BOLD imaging as a tool for assessing drug efficacy and to compare it with subjective self-reports, we performed a power analysis using two psychophysical outcome measures (hyperalgesia intensity and ongoing pain) and three imaging outcome measures. The imaging outcome measures were the parameter estimates from three regions of interest, most likely to be the optimal brain regions for highlighting drug modulation

due to their previously identified roles in nociception and central sensitization^{19–21,37–39} (NCF, the contralateral posterior insula, and secondary somatosensory cortex [SII]). The posterior insula and SII were anatomically defined, and the masks thresholded at $P > 0.5$. The NCF is difficult to define anatomically because of imaging-related contrast issues and the lack of any image analysis atlases in this region. Therefore, it was defined functionally. We used Featquery in FSL to extract %BOLD response evoked by hyperalgesia from these three regions.

To assess the sample sizes that would be needed for future studies given an effect size comparable with that between gabapentin and placebo, we performed a power analysis. Examining cohorts of 12 healthy volunteers and using a similar experimental paradigm, previous studies from our group were able to detect statistically significant activation evoked by hyperalgesia from the relevant brain regions,^{19–21} alongside gabapentin-induced suppression of evoked neural activity within these brain regions. Therefore, to facilitate the power analysis, we doubled the sample size to 24, so that we can draw meaningful subsamples. Power analysis was performed by taking subsamples of the full sample to see whether the statistical effects from the full sample was still present. It also yields the differences in statistical power between the different outcome measures, *i.e.*, the imaging and the psychophysical measures.

For each predetermined sample size, by using each outcome measure, we performed the group comparison between placebo and gabapentin visits (paired *t* test) 1,000 times using different permutations of subjects drawn from the pool of 24 data sets. The probability of detecting a difference between gabapentin and placebo for each sample size was calculated for each outcome measure. Probabilities were computed using MATLAB (Mathwork, Inc., USA).

Results

Psychophysics

Hyperalgesia pain intensity and unpleasantness were significantly reduced ($P < 0.05$) only by gabapentin but not by ibuprofen when compared with placebo. The differences in these two measures between gabapentin and ibuprofen visits did not survive Bonferroni correction for multiple comparisons (fig. 1B). Similarly the differences in ongoing pain scores (fig. 1C) and pain and unpleasantness of allodynia between visits were not statistically significant (not shown in figure).

There were no significant differences between the visits in any of the mood or state anxiety measures at any of the time points except at 150 min after dosing where gabapentin significantly increased mental sedation when compared with both placebo and ibuprofen (fig. 1D). However, there was no significant correlation ($P > 0.6$) between mental sedation and pain scores during the gabapentin visit.

Imaging

Task-evoked Data. The BOLD response evoked by hyperalgesia in an area of the MRF that is known to contain the right NCF was significantly suppressed by gabapentin but not by ibuprofen when compared with placebo and by gabapentin when compared with ibuprofen (fig. 2). The BOLD response evoked by hyperalgesia in the left insula and SII was also suppressed by gabapentin but not by ibuprofen when compared with placebo and by gabapentin when compared with ibuprofen (fig. 3). There was no significant evidence of ibuprofen or placebo-related suppression of evoked neural activity in the brain areas that are implicated in processing nociceptive stimuli or pain perception. There were no significant differences in BOLD response activation induced by allodynia between the any of the treatment visits.

Functional Connectivity Data. The resting-state functional connectivity between the left thalamus and left SII was suppressed by gabapentin but not by ibuprofen when compared with placebo and by gabapentin when compared with ibuprofen (fig. 4). Furthermore, connectivity between the left thalamus and the left SII during the gabapentin visit showed a significant negative correlation with the gabapentin plasma levels. The group mean (\pm SD) plasma gabapentin level was 7.1 mg/l (\pm 1.5). Similar to task-evoked imaging data, there was no evidence of analgesic effects of ibuprofen or placebo in the thalamic connectivity data.

Correlation between Gabapentin-induced Behavioral Reports and the Neural Activity. There were no statistically significant relationships between gabapentin-induced suppression of hyperalgesia-evoked BOLD responses from the right NCF, left anterior and posterior insula, and left SII and pain reports, or between left thalamus-SII connectivity and ongoing pain.

Gabapentin-induced increases in mental sedation was not associated with the suppression of evoked neural activity from the right NCF ($P = 0.2$), left anterior insula ($P = 0.9$), left posterior insula ($P = 0.8$), and left SII ($P = 0.8$) or with suppression of left thalamus-SII connectivity ($P = 0.5$).

Power Analysis

With 12 subjects, there is more than or equal to 0.8 probability of detecting a statistically significant difference ($P < 0.05$) between placebo and gabapentin when using the %BOLD response evoked by hyperalgesia from the functionally defined NCF region or the anatomically defined left posterior insula. The corresponding probability value reduces to less than 0.6 when using subjective pain scores or the %BOLD response evoked by hyperalgesia from the anatomically defined left SII (fig. 5). The effect size expressed as mean difference between placebo and gabapentin for ongoing pain was 5.86 (95% CI, 0.49 to 11.22), for punctate intensity was 8.76 (95% CI, 3.50 to 14.03), for neural activity from NCF was 0.193 (95% CI, 0.11 to 0.27), from posterior insula was 0.112 (95% CI, 0.06 to 0.17), and from SII was 0.105 (95% CI, 0.04 to 0.17). Standardized mean

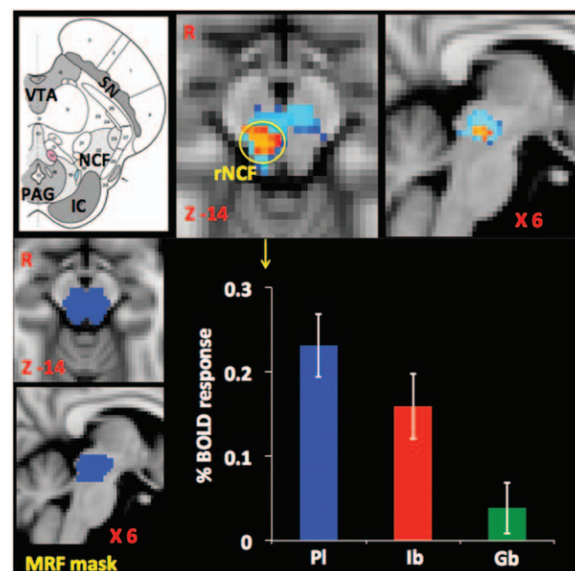


Fig. 2. Drug-induced effects on brainstem neural activity. Area of the brainstem mesencephalic reticular formation (MRF) that corresponds to the right nucleus cuneiformis (rNCF) showing a significant gabapentin (Gb)-induced suppression of blood oxygen level-dependent (BOLD) response evoked by secondary mechanical punctate hyperalgesia is shown. The axial and sagittal image slices in the top row show the area of BOLD activity suppressed by Gb when compared with placebo (PI) in blue and when compared with ibuprofen (Ib) in red. The Ib > Gb group contrast map (red) is overlaid on the PI > Gb group contrast map (blue). MNI coordinates of the voxel with peak effect of PI > Gb and Ib > Gb contrast are X, 6; Y, -28; and Z, -14. These group differences were identified with a hypothesis-driven small volume correction on a mask defining the MRF using nonparametric permutation testing on threshold-free cluster-enhanced values yielding a familywise error correction rate of 0.05. The annotated diagram of the mesencephalon in the top left is to aid visual localization of the area where Gb suppressed the hyperalgesia-evoked BOLD activity. The axial and sagittal slices below the annotated diagram show the MRF mask used for the hypothesis-driven search. The group average % BOLD response from the rNCF area (voxels with significant effect within a 3-mm sphere of the peak effect voxel) is shown in the bar chart for illustration purposes only, and in keeping with convention when illustrating imaging results, P values are not indicated. Error bars show the SEM. Montreal neurological institute-152 template coordinates are denoted (red) below each image slice. IC = inferior colliculi; NCF = nucleus cuneiformis; PAG = periaqueductal grey; SN = substantia nigra; VTA = ventral tegmental area.

difference (Cohen d) for ongoing pain was 0.44, for punctate intensity was 0.57, for neural activity from NCF was 1.16, from posterior insula was 0.74, and from SII was 0.75.

Discussion

Previous studies have shown the utility of imaging techniques in demonstrating analgesic drug modulation of the brain nociceptive-processing areas^{21,40} and differentiating an analgesic from a nonanalgesic⁴¹ drug.

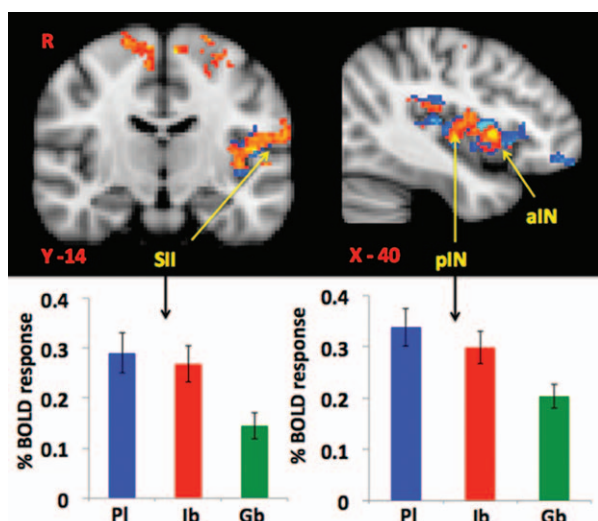


Fig. 3. Drug-induced effects on cortical neural activity. Cortical areas showing a significant gabapentin (Gb)-induced suppression of the blood oxygen level-dependent (BOLD) response evoked by secondary mechanical punctate hyperalgesia. The coronal and sagittal image slices in the *top row* show the area of BOLD activity suppressed by Gb when compared with placebo (PI) in *blue* and when compared with ibuprofen (Ib) in *red*. The Ib > Gb group contrast map (*red*) is overlaid on the PI > Gb group contrast map (*blue*). These group differences were identified using whole brain analysis with mixed effects and a cluster-based familywise error correction (Z score more than 2.3; $P < 0.05$). The bar charts below the image slices showing the group average %BOLD response from the left secondary somatosensory cortex (SII; *left*) and from the left posterior insula (pIN; *right*) are for illustration purposes only, and in keeping with convention when illustrating imaging results, P values are not indicated. Error bars show the SEM. Montreal Neurological Institute—152 template coordinates are denoted (*red*) below each image slice. aIN = anterior insular.

We have taken this step further and demonstrated in a small cohort of healthy volunteers the utility of imaging combined with a mechanism-based model of central sensitization, in differentiating an effective analgesic from an ineffective analgesic in neuropathic pain. Specifically gabapentin, one of the first-line therapies recommended for the treatment of neuropathic pain⁴² suppressed the neural activity evoked by capsaicin-induced hyperalgesia in an area of the brainstem that contains the NCF (fig. 2), an area with increased activity in capsaicin-induced central sensitization.^{19,20} In contrast, the analgesic ibuprofen failed to suppress this activity in line with clinical evidence that ibuprofen has doubtful efficacy in neuropathic pain.⁴³ The NCF, a part of the MRF, is a component of the descending pain modulatory system that receives direct projections from the spinal nociceptive neurons.⁴⁴ It sends dense neural projections to the rostral ventromedial medulla⁴⁵ and has facilitatory on cells and inhibitory off cells⁴⁶ similar to those found in the periaqueductal grey⁴⁷ and rostral ventromedial medulla nuclei.⁴⁸ The increased NCF activity has been observed in

animal models of neuropathic pain,^{49,50} human models of experimentally induced states of central sensitization,^{19,20,51} migraine patients,^{52,53} and expectation-induced pain modulation.⁵⁴ Descending brainstem facilitation is increasingly recognized as a key component maintaining sensitization of spinal dorsal horn nociceptive neurons in chronic pain conditions.⁵⁵ Gabapentin has been shown to reduce dorsal horn nociceptive neuron excitability in the presence of central sensitization in nerve injury models.^{56,57} Therefore, because participants were dosed preinjury in our study, activity in the spinobulbar facilitatory loop was likely influenced accounting for our results.

Gabapentin also suppressed the evoked BOLD response in the contralateral anterior and posterior insula and SII, whereas ibuprofen did not (fig. 3). Posterior insula and SII are key nociceptive-processing regions in humans.³⁸ They are consistently activated in imaging studies in chronic neuropathic pain patients,³⁷ indicating their involvement in processing nociceptive inputs from the sensitized spinal dorsal horn. Therefore, the reduced BOLD response in the posterior insula and SII in our study is further evidence of gabapentin effects on the spinal dorsal horn. It is also possible that gabapentin additionally directly suppresses the insula and SII responses to ascending nociceptive inputs, because these areas are rich in gabapentin-binding sites.⁵⁸ In contrast, ibuprofen, a well-established analgesic, in inflammatory pain conditions failed to suppress insula and SII activity to hyperalgesia in a centrally sensitized state.

The thalamus is rich in gabapentin-binding sites,⁵⁸ and nociceptive spinal dorsal horn neurons involved in central sensitization relay to the thalamus before reaching the posterior opercula-insular region.³⁷ Connectivity analysis revealed that gabapentin but not ibuprofen or placebo suppressed the connectivity between left thalamus and left SII during central sensitization in a gabapentin plasma level-dependent manner (fig. 4), suggesting that suppression of thalamic-SII connectivity might contribute toward the antihyperalgesic effects of gabapentin. Data that yield connectivity between different brain regions can be gathered over a few minutes during rest with little effort from the individual. This makes it potentially an ideal fMRI tool for demonstrating effects of analgesics on nociceptive neural mechanisms in patient studies where performance of complex tasks and lying still in the scanner for long periods of time become impractical.

In our study, imaging measures convincingly differentiated the effects of gabapentin from placebo and ibuprofen, whereas behavioral unprovoked ongoing pain reports did not. The hyperalgesia-evoked pain ratings were able to differentiate gabapentin from placebo but not from ibuprofen. Such inconsistency is most likely due to the high variability in subjective pain reports and the fact that they incorporate many features that are multifactorial by nature. Although subjective pain reports are integral to assessing analgesic clinical efficacy, they are context dependent and are powerfully influenced by individuals' expectation of both positive

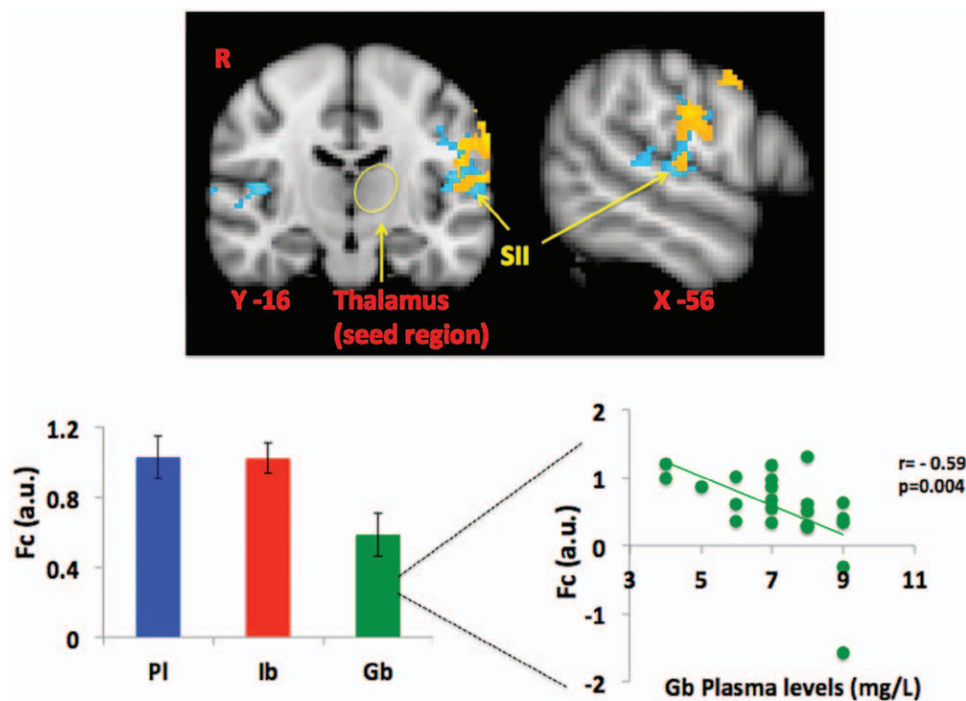


Fig. 4. Drug-induced effects on left thalamic functional connectivity (Fc). Gabapentin (Gb)-induced suppression of the Fc between the left thalamus seed region and the left secondary somatosensory cortex (SII) when compared with placebo (PI) in blue and when compared with ibuprofen (Ib) in yellow are shown in the coronal and sagittal image slices in the top row. The Ib > Gb group contrast map (yellow) is overlaid on the PI > Gb group contrast map (blue). These group differences were identified using whole brain analysis with mixed effects and a cluster-based familywise error correction (Z score more than 2.3; $P < 0.05$). The bar chart below the image slices showing the group average Fc between left thalamus and left SII is for illustration purposes only, and in keeping with convention when illustrating imaging results, P values are not indicated. Error bars show the SEM; Montreal Neurological Institute—152 template coordinates are denoted (red) below each image slice. The scatter plot shows the significant negative correlation ($r = 0.59$; $P < 0.05$ two tailed) between the left thalamus–SII Fc during the Gb visit and the Gb plasma levels obtained at approximately 200 min after dosing. Plasma Gb levels were available from 22 of the 24 participants who completed the study (2 declined venipuncture). a.u. = arbitrary units.

(placebo) and negative (nocebo) treatment outcome,⁸ mood as well as pathology. This is less so for the brain regions identified here that reflect basic nociceptive and hyperalgesic mechanisms. Pain relief in the placebo arm of a randomized controlled trial can be as much as 30%,⁹ whereas pain relief in the treatment arm could be reduced due to the nocebo effects resulting from the uncertainty of receiving an active treatment of unproven efficacy.⁵⁹ To prove analgesic efficacy, pain relief in the active treatment arm must exceed placebo values. Therefore, total reliance on subjective pain reports to identify potentially clinically effective analgesics in early drug development where sample sizes are often small can be very challenging.

We estimated also the sensitivity of selected imaging and psychophysical pain outcome measures in detecting a statistically significant ($P < 0.05$) difference between gabapentin and placebo (fig. 5). The neural activity evoked by hyperalgesia from the right NCF and the left posterior insula is more sensitive than the behavioral pain scores. Our findings are supported by the unconvincing results of gabapentin analgesic effects when using subjective outcome measures in small healthy volunteer studies, including multiple dosing

studies.^{17,21} When using neural activity evoked by hyperalgesia from the NCF or the posterior insula as outcome measures, we only need a sample of 12 to detect a statistically significant difference between gabapentin and placebo with $P > 0.8$. Therefore, with a sample size of 12 and the same outcome measures, future studies would have adequate statistical power to detect an effect size comparable with that between gabapentin and placebo. The posterior insula is the more transferable of the two regions, because it has clear landmarks and can be defined objectively using automated cortical atlases. Conversely, the NCF area is more difficult to define anatomically. That is why in this study we used the neural response from the functionally defined NCF. However, this makes the power analysis from the NCF circular and will need confirmation by an independent study that defines the NCF using the coordinates reported in this study.

There was also no significant correlation between gabapentin-induced suppression of subjective pain ratings and neural activity from the right NCF, left anterior and posterior insula, and left SII. Due to the highly variable and multidimensional nature of the subjective pain reports, an existing true correlation would need a larger sample for the

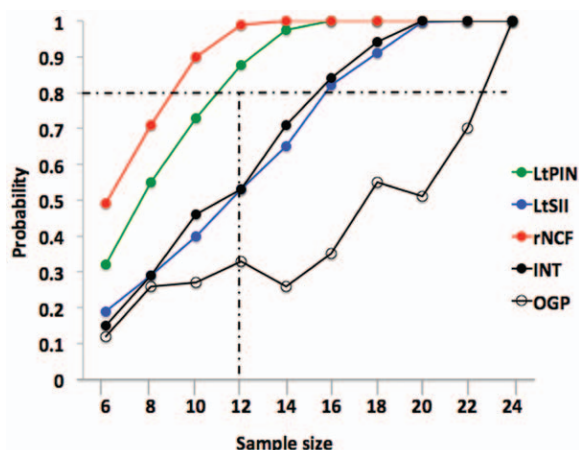


Fig. 5. Power analysis results. The probability of obtaining a statistically significant ($P < 0.05$) difference between placebo and gabapentin (Y-axis) at a given sample size (X-axis) for both objective and subjective outcome measures are shown. The objective outcome measures are the % blood oxygen level dependent response to secondary punctate mechanical hyperalgesia obtained from the left posterior insula (LtPIN; green), left secondary somatosensory cortex (LtSII; blue), and right nucleus cuneiformis (rNCF; red). The subjective outcome measures are the punctate hyperalgesia intensity (INT; black closed circles) and unprovoked ongoing pain (OGP).

effect to reach statistical significance. Many factors influence the final subjective pain report, and functional imaging should not be considered as a mere surrogate of pain intensity rating. Rather, it is a powerful measure of how the brain processes and modulates nociceptive inputs that eventually produce the subjective pain experience that is subsequently reported using scales and measures devised externally. Functional magnetic resonance imaging is able to detect target engagement and pharmacodynamic effects of the drug on nociceptive and hyperalgesic processing irrespective of the subsequent expression of convincing subjective evidence.⁶⁰

The other significant pharmacodynamic effect of gabapentin on behavior was increased mental sedation. However, this had no significant relationship with the gabapentin-induced suppression of neural activity and functional connectivity from the nociceptive-processing areas presented in this study.

We have shown in a small cohort of healthy subjects using the capsaicin-induced model of central sensitization that imaging can convincingly differentiate an analgesic that is effective in neuropathic pain from one that is not, as well as differentiate both from placebo. Although our model elicits some of the key features and mechanisms of neuropathic pain, there is no injury to the nervous system. Therefore, the mechanisms responsible for analgesic modulation of the neural response in neuropathic pain patients might not be precisely the same as those in our healthy volunteers—albeit in a centrally sensitized state. We found no significant relationship between any of the gabapentin-modulated pain reports and neural activity in the nociceptive- and pain-processing areas. Although this is most likely explained by the inherently large variability

of subjective pain reports from a small sample, it is possible that this gabapentin-induced modulation of neural activity might reflect additional central effects of gabapentin not measured by our behavioral measures. However, the strength in our data is that the neural modulation is found in more than one core nociceptive- and pain-processing region (*e.g.*, posterior insula and SII) together with an area of the brainstem's pain modulatory system (NCF) that is known to be involved in central sensitization. Therefore, the lack of a relationship is most likely due to the large variability of subjective pain reports. It should be noted that decisions in drug development are never solely based on surrogate efficacy models in healthy volunteers. Rather, they are one of the many decision-making measures that are increasingly used in drug development to provide objective evidence of target binding and modulation of target function.⁴ Such evidence is helpful during decision-making in early drug development and before progressing the compound to test for efficacy and target engagement in costly and time-consuming patient studies.⁶¹ Therefore, we believe that neuroimaging evidence from surrogate healthy volunteer models has the capacity to increase the confidence by which early go/no-go decisions are made in drug development.^{4,9,62}

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Competing Interests

The authors declare no competing interests.

Reproducible Science

Full protocol available from Prof. Tracey: irene.tracey@ndcn.ox.ac.uk. Raw data available from Prof. Tracey: irene.tracey@ndcn.ox.ac.uk.

Correspondence

Address correspondence to Dr. Wanigasekera: Centre for Functional Magnetic Resonance Imaging of the Brain (FMRIB) & Nuffield Division of Anaesthetics, Nuffield Department of Clinical Neurosciences, University of Oxford, John Radcliffe Hospital, Headington, Oxford, OX3 9DU, United Kingdom. vishvarani.wanigasekera@ndcn.ox.ac.uk. This article may be accessed for personal use at no charge through the Journal Web site, www.anesthesiology.org.

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