Published in final edited form as:

Anesthesiology. 2016 January ; 124(1): 159–168. doi:10.1097/ALN.0000000000000924.

Disambiguating pharmacodynamic efficacy from behaviour with neuroimaging: implications for analgesic drug development

Vishvarani Wanigasekera, DPhil^{*}, Melvin Mezue, DPhil^{*}, Jesper Andersson, PhD^{*}, Yazhuo Kong, PhD^{*}, and Irene Tracey, DPhil^{*}

^{*}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. Telephone +441865 222736

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—We assessed the utility of functional magnetic resonance imaging with capsaicin-induced central sensitisation, 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. We used a double-blind randomised 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 (NCF)], and left (contralateral) posterior insular and secondary somatosensory cortex (SII). Similarly, only gabapentin suppressed resting state functional connectivity during central sensitisation between the thalamus and SII that was plasma gabapentin level dependent.

A power analysis showed that with 12 datasets, when using neural activity from the left posterior insula and right NCF, a statistically significant difference between placebo and gabapentin was detected with a probability 0.8. When using subjective pain ratings this reduced to 0.6.

Conclusion—Functional imaging with central sensitisation 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 as it requires no task or pain provocation.

Corresponding author: Vishvarani Wanigasekera vishvarani.wanigasekera@ndcn.ox.ac.uk.

Author contributions: V.W. and I.T. designed research; V.W. and M.M. performed research; V.W., M.M., J.A., and Y.K. analyzed data; and V.W., M.M., J.A., Y.K. and I.T. interpreted data and wrote the paper.

Conflict of interest: The authors declare no conflict of interest

Meetings at which the work has been presented:

14th World congress on Pain, 27th – 31st August, Milan, Italy.

15th World Congress on Pain, 7th – 11th October 2014, Buenos Aires, Argentina

Keywords

Brain imaging; analgesics; neuropathic pain; central sensitisation; brainstem and resting state

Introduction

Chronic pain affects 20% of the European population¹ yet there is a dearth of effective analgesics². Many potential compounds identified in preclinical research fail to reach the market as effective analgesics³, 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 efficacy⁷. However, these have considerable within subject variability and are highly context dependent⁸. 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. 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, by detecting both truly effective and truly ineffective compounds in early drug development⁹.

Neuropathic pain is a chronic pain condition¹⁰ characterised by spontaneous pain, hyperalgesia and allodynia of the affected area causing significant impairment in quality of life¹¹. Several mechanisms are involved in neuropathic pain and central sensitisation is a potentially key one¹². Topical capsaicin in humans induces central sensitisation which has similar behavioural manifestations to that of neuropathic pain¹³. Therefore, it is considered a suitable human model to study central sensitisation¹⁴ and assess therapeutic efficacy of analgesics¹⁵.

Gabapentin provides clinically relevant pain relief in painful polyneuropathies¹⁶. Yet evidence of analgesic efficacy from behavioral outcome measures in small scale human studies is variable and inconsistent¹⁷. Furthermore, behavioural 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 expectation⁸.

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 brain¹⁸. Using fMRI, we have highlighted the critical involvement of the brainstem in central sensitisation induced by capsaicin^{19,20} and shown that gabapentin attenuates brainstem activity²¹. Indeed, the brainstem plays a crucial role in maintaining central sensitisation in chronic pain states^{12,22}.

This evidence suggest that fMRI in conjunction with capsaicin induced central sensitisation 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 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 cross-over study in healthy volunteers with central sensitisation.

Materials and Methods

The study (reference 08/H0606/50) was approved by Oxfordshire Research Ethics Committee, UK on 30th July 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 24 ± 4.2 years [mean $\pm$ SD (standard deviation)]; 13 females]. One subject was withdrawn during the 1st 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 hours prior to attending the study centre. Subjects received either placebo, gabapentin 1200mg or ibuprofen 600mg orally (order randomised) followed by food. Capsaicin cream 1% was applied 90 minutes after dosing with study medication on an area (4×4 cm) of skin on the antero-medial aspect of the right lower leg at least 14 cm above the medial malleolus. An area (4×2 cm) 2cm 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 minutes after dosing. Scans were obtained sequentially in blocks (Figure 1A) while eliciting allodynia and hyperalgesia, followed by an arterial spin labelling 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 ~200 min after drug administration from 22 subjects (two subjects declined venipuncture).

The doses of the study drugs and the timing of the functional scans were based on previous studies testing analgesic efficacy of the study drugs in human experimental models^{21,23,24}.

Sensory testing

Allodynia was elicited with a soft standardised brush (Somedic, Sweden) over 10 min by delivering 15 identical 6s stimuli to the target area with an inter-stimulus interval jittered between 28-46s. Each stimulus had three ~2s strokes in the medio-lateral direction. Average pain intensity and unpleasantness elicited by stimuli were recorded 12s and 18s after the last

stimulus using a visual analogue scale (VAS) with anchors ‘not painful and extremely painful’ and ‘not unpleasant and extremely unpleasant’. Hyperalgesia was elicited by delivering 18 identical 1s punctate stimuli with a punctate probe that delivers a force of 512mN over 10 min to the target area with an inter-stimulus interval jittered between 28-32s¹⁹. Stimulus intensity was recorded 12s after each stimulus using a VAS with anchors ‘not intense and extremely intense’. Average unpleasantness of stimuli was recorded 12s 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 Spielberger state anxiety scale²⁵ and sedation using the Bond-Lader mood scale²⁶ immediately before dosing, 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: 30ms echo-time, field of view 192×192 mm, matrix 64×64, 3mm-thick axial slices. Functional scans had 200 volumes and resting scan had 128 volumes. The repetition time for functional scans was 3s and for resting scan it was 2.514s. Fieldmaps were acquired (field of view 192×192 mm, matrix 64×64) after the resting state scan to correct for 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 stereo-tactic space (Montreal Neurological Institute – 152 template). Pulse and respiratory waveforms were recorded during scanning for physiological noise correction in data analysis²⁷. Arterial spin labeling data acquisition is not described here, as this data will not be presented in this paper.

Analysis and statistical methods

Analysis of behavioural data—Twenty-four data sets were analysed. 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’s correlation coefficient was used for correlational analyses. For data that were non-normally distributed we used the Wilcoxon Signed Rank Test and Spearman’s rho. We used SPSS Inc. version 21 for analysis of psychophysical data.

Analysis of imaging data—All images were analyzed using tools in the FMRIB Software Library (FSL) Version 5.0 (<http://fsl.fmrib.ox.ac.uk>). 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 sensitised 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 pre-processed to remove noise and artefacts using tools provided within FSL²⁷⁻³⁰. A Gaussian kernel of FWHM 5mm was used for spatial smoothing. Boundary-based³¹, linear and non-linear 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 this data between treatments were identified using whole brain analyses with mixed effects³³ and a cluster based family wise error correction for multiple comparisons (Z score >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 while 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 NCF and is known to be involved in central sensitisation^{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 values³⁵ yielding a family wise 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's brainstem atlas³⁶ as an automated detailed brainstem atlas is not available.

To see whether resting state BOLD data is useful in detecting drug modulation of functional connectivity between key nociceptive processing brain areas, similar to task data, we generated statistical maps which identified 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 as it is a main relay station for ascending nociceptive dorsal horn neurons before reaching the cortical nociceptive processing areas³⁷. 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 to 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 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 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 to 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 relevant brain regions¹⁹⁻²¹, 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 sub-samples of the full sample to see if 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, using each outcome measure we performed the group comparison between placebo and gabapentin visits (paired t test) 1000 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.

Results

Psychophysics

Hyperalgesia pain intensity and unpleasantness were significantly reduced ($p < 0.05$) only by gabapentin but not by ibuprofen when compared to placebo. The differences in these two measures between gabapentin and ibuprofen visits did not survive Bonferroni correction for multiple comparisons. (Figure 1B). Similarly the differences in ongoing pain scores (Figure 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 minutes after dosing where gabapentin significantly increased mental sedation when compared to both placebo and ibuprofen (Figure 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 to placebo and by gabapentin when compared to ibuprofen (Figure 2). The BOLD response evoked by hyperalgesia in the left insula and SII was also suppressed by gabapentin but not by ibuprofen when compared to placebo and by gabapentin when compared to ibuprofen (Figure 3). There was no significant evidence of ibuprofen or placebo related suppression of evoked neural activity in 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 to placebo and by gabapentin when compared to ibuprofen (Figure 4). Furthermore, connectivity between the left thalamus and left SII during the gabapentin visit showed a significant negative correlation with 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 behavioural reports and the neural activity—There were no statistically significant relationships between gabapentin induced suppression of hyperalgesia evoked BOLD response 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 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 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 < 0.6 when using subjective pain scores or the %BOLD response evoked by hyperalgesia from the anatomically defined left SII (Figure 5). The effect size expressed as mean difference between placebo and gabapentin with 95% confidence interval (CI) for ongoing pain was 5.86, 95% CI (0.49 to 11.22), for punctate intensity was 8.76, 95% CI (3.50-14.03), 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). Standardised mean difference (Cohen's d) for on-going pain was 0.44, for punctate intensity was 0.57, for neural activity from NCF was 1.16, from posterior insula 0.74 and from SII was 0.75.

Discussion

Previous studies have shown the utility of imaging techniques in demonstrating analgesic drug modulation of brain nociceptive processing areas^{21,40} and differentiating an analgesic from a non-analgesic⁴¹ drug.

We have taken this a step further and demonstrated in a small cohort of healthy volunteers the utility of imaging combined with a mechanism-based model of central sensitisation, 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 (Figure 2); an area with increased activity

in capsaicin induced central sensitisation^{19,20}. In contrast, the analgesic ibuprofen failed to suppress this activity in line with clinical evidence that ibuprofen has doubtful efficacy in neuropathic pain⁴³. The NCF, a part of the MRF, is a component of the descending pain modulatory system that receives direct projections from the spinal nociceptive neurons⁴⁴. It sends dense neural projections to the rostral ventromedial medulla (RVM)⁴⁵ and has facilitatory on-cells and inhibitory-off cells⁴⁶ similar to those found in the periaqueductal grey⁴⁷ and RVM nuclei⁴⁸. Increased NCF activity has been observed in animal models of neuropathic pain^{49,50}, human models of experimentally induced states of central sensitisation^{19,20,51}, migraine patients^{52,53} and expectation induced pain modulation⁵⁴. Descending brainstem facilitation is increasingly recognised as a key component maintaining sensitisation of spinal dorsal horn nociceptive neurons in chronic pain conditions⁵⁵. Gabapentin has been shown to reduce dorsal horn nociceptive neuron excitability in the presence of central sensitisation in nerve injury models^{56,57}. Therefore, as participants were dosed pre-injury in our study, activity in the spino-bulbar 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 while ibuprofen did not (Figure 3). Posterior insula and SII are key nociceptive processing regions in humans³⁸. They are consistently activated in imaging studies in chronic neuropathic pain patients³⁷ indicating their involvement in processing nociceptive inputs from the sensitised 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, as these areas are rich in gabapentin binding sites⁵⁸. 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 sites⁵⁸ and nociceptive spinal dorsal horn neurons involved in central sensitisation relay to the thalamus before reaching the posterior opercula-insular region³⁷. Connectivity analysis revealed that gabapentin but not ibuprofen or placebo suppressed the connectivity between left thalamus and left SII during central sensitisation in a gabapentin plasma level dependent manner (Figure 4) suggesting that suppression of thalamic-SII connectivity might contribute towards 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 effects of gabapentin from placebo and ibuprofen while behavioural 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 (placebo) and negative (nocebo) treatment outcome⁸, 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 randomised controlled trial can be as much as 30%⁹, while 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 efficacy⁵⁹. 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 (Figure 5). The neural activity evoked by hyperalgesia from the right NCF and the left posterior insula is more sensitive than the behavioural 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 > 0.8 probability. 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 to that between gabapentin and placebo. The posterior insula is the more transferable of the two regions, as it has clear landmarks and can be defined objectively using automated cortical atlases. The NCF area on the otherhand is more difficult to define anatomically. That is why in the present 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 the present 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 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 which is subsequently reported using scales and measures devised externally. FMRI 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 evidence⁶⁰.

The other significant pharmacodynamic effect of gabapentin on behaviour 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 sensitisation 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 sensitised state. We found no significant relationship between any of the gabapentin modulated pain reports and neural activity in the nociceptive and pain processing areas. While 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 behavioural 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 sensitisation. 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 many decision-making measures that are increasingly used in drug development to provide objective evidence of target binding and modulation of target function⁴. 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 studies⁶¹. 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}.

Acknowledgments

Funding disclosure: This work was supported by Grants from National Institute for Health Research Oxford Biomedical Research Centre, Medical Research Council of Great Britain and Northern Ireland, the Wellcome Trust (London, UK) and the Innovative Medicines Initiative Joint Undertaking (Brussels, Belgium), under grant agreement no 115007 resources of which are composed of financial contribution from the European Union's Seventh Framework Programme (FP7/2007–2013) and EFPIA companies' in kind contribution.

Reference

1. van Hecke O, Torrance N, Smith BH. Chronic pain epidemiology and its clinical relevance. *Br J Anaesth.* 2013; 111:13–8. [PubMed: 23794640]
2. Relieving Pain in America: A Blueprint for Transforming Prevention, Care, Education, and Research. Washington (DC): 2011.
3. Kissin I. The development of new analgesics over the past 50 years: a lack of real breakthrough drugs. *Anesth Analg.* 2010; 110:780–9. [PubMed: 20185657]
4. Hewitt DJ, Hargreaves RJ, Curtis SP, Michelson D. Challenges in analgesic drug development. *Clin Pharmacol Ther.* 2009; 86:447–50. [PubMed: 19675542]
5. Mao J. Current challenges in translational pain research. *Trends Pharmacol Sci.* 2012; 33:568–73. [PubMed: 22959652]
6. Hill R. NK1 (substance P) receptor antagonists--why are they not analgesic in humans? *Trends Pharmacol Sci.* 2000; 21:244–6. [PubMed: 10871891]

7. Dworkin RH, Turk DC, Peirce-Sandner S, Burke LB, Farrar JT, Gilron I, Jensen MP, Katz NP, Raja SN, Rappaport BA, Rowbotham MC, Backonja MM, Baron R, Bellamy N, Bhagwagar Z, Costello A, Cowan P, Fang WC, Hertz S, Jay GW, Junor R, Kerns RD, Kerwin R, Kopecky EA, Lissin D, Malamut R, Markman JD, McDermott MP, Munera C, Porter L, Rauschkolb C, Rice AS, Sampaio C, Skljarevski V, Sommerville K, Stacey BR, Steigerwald I, Tobias J, Trentacosti AM, Wasan AD, Wells GA, Williams J, Witter J, Ziegler D. Considerations for improving assay sensitivity in chronic pain clinical trials: IMMPACT recommendations. *Pain*. 2012; 153:1148–58. [PubMed: 22494920]
8. Bingel U, Wanigasekera V, Wiech K, Ni Mhuircheartaigh R, Lee MC, Ploner M, Tracey I. The effect of treatment expectation on drug efficacy: imaging the analgesic benefit of the opioid remifentanyl. *Science translational medicine*. 2011; 3 70ra14.
9. Chizh BA, Priestley T, Rowbotham M, Schaffler K. Predicting therapeutic efficacy - experimental pain in human subjects. *Brain Res Rev*. 2009; 60:243–54. [PubMed: 19168094]
10. Bouhassira D, Lanteri-Minet M, Attal N, Laurent B, Touboul C. Prevalence of chronic pain with neuropathic characteristics in the general population. *Pain*. 2008; 136:380–7. [PubMed: 17888574]
11. Attal N, Lanteri-Minet M, Laurent B, Fermanian J, Bouhassira D. The specific disease burden of neuropathic pain: results of a French nationwide survey. *Pain*. 2011; 152:2836–43. [PubMed: 22019149]
12. Woolf CJ. Central sensitization: implications for the diagnosis and treatment of pain. *Pain*. 2011; 152:S2–15. [PubMed: 20961685]
13. LaMotte RH, Shain CN, Simone DA, Tsai EF. Neurogenic hyperalgesia: psychophysical studies of underlying mechanisms. *J Neurophysiol*. 1991; 66:190–211. [PubMed: 1919666]
14. Klein T, Magerl W, Rolke R, Treede RD. Human surrogate models of neuropathic pain. *Pain*. 2005; 115:227–33. [PubMed: 15876495]
15. Olesen AE, Andresen T, Staahl C, Drewes AM. Human experimental pain models for assessing the therapeutic efficacy of analgesic drugs. *Pharmacol Rev*. 2012; 64:722–79. [PubMed: 22722894]
16. Finnerup NB, Sindrup SH, Jensen TS. The evidence for pharmacological treatment of neuropathic pain. *Pain*. 2010; 150:573–81. [PubMed: 20705215]
17. Wallace MS, Schulteis G. Effect of chronic oral gabapentin on capsaicin-induced pain and hyperalgesia: a double-blind, placebo-controlled, crossover study. *Clin J Pain*. 2008; 24:544–9. [PubMed: 18574364]
18. Denk F, McMahon SB, Tracey I. Pain vulnerability: a neurobiological perspective. *Nat Neurosci*. 2014; 17:192–200. [PubMed: 24473267]
19. Lee MC, Zambreau L, Menon DK, Tracey I. Identifying brain activity specifically related to the maintenance and perceptual consequence of central sensitization in humans. *J Neurosci*. 2008; 28:11642–9. [PubMed: 18987200]
20. Zambreau L, Wise RG, Brooks JC, Iannetti GD, Tracey I. A role for the brainstem in central sensitization in humans. Evidence from functional magnetic resonance imaging. *Pain*. 2005; 114:397–407. [PubMed: 15777865]
21. Iannetti GD, Zambreau L, Wise RG, Buchanan TJ, Huggins JP, Smart TS, Vennart W, Tracey I. Pharmacological modulation of pain-related brain activity during normal and central sensitization states in humans. *Proc Natl Acad Sci U S A*. 2005; 102:18195–200. [PubMed: 16330766]
22. Porreca F, Ossipov MH, Gebhart GF. Chronic pain and medullary descending facilitation. *Trends Neurosci*. 2002; 25:319–25. [PubMed: 12086751]
23. Bickel A, Dorfs S, Schmelz M, Forster C, Uhl W, Handwerker HO. Effects of antihyperalgesic drugs on experimentally induced hyperalgesia in man. *Pain*. 1998; 76:317–25. [PubMed: 9718250]
24. Dirks J, Petersen KL, Rowbotham MC, Dahl JB. Gabapentin suppresses cutaneous hyperalgesia following heat-capsaicin sensitization. *Anesthesiology*. 2002; 97:102–7. [PubMed: 12131110]
25. Spielberger, C.; Gorsuch, R.; Lushene, R.; Vagg, P.; Jacobs, G. *Manual for the State-Trait Anxiety Inventory (form Y)*. Consulting Psychologists Press; Palo Alto (CA): 1983. 1983
26. Bond AJ, Lader MH. The use of analogous scales in rating subjective feelings. *Br J Med Psychol*. 1974; 47:211–218.
27. Brooks JC, Beckmann CF, Miller KL, Wise RG, Porro CA, Tracey I, Jenkinson M. Physiological noise modelling for spinal functional magnetic resonance imaging studies. *Neuroimage*. 2008; 39:680–92. [PubMed: 17950627]

28. Jenkinson M. Fast, automated, N-dimensional phase-unwrapping algorithm. *Magn Reson Med*. 2003; 49:193–7. [PubMed: 12509838]
29. Jenkinson M, Bannister P, Brady J, Smith S. Improved optimisation for the robust and accurate linear registration and motion correction of brain images. *Neuroimage*. 2002; 17:825–841. [PubMed: 12377157]
30. Smith SM. Fast robust automated brain extraction. *Hum Brain Mapp*. 2002; 17:143–55. [PubMed: 12391568]
31. Greve DN, Fischl B. Accurate and robust brain image alignment using boundary-based registration. *Neuroimage*. 2009; 48:63–72. [PubMed: 19573611]
32. Andersson, J.; Jenkinson, M.; Smith, S. FMRIB technical report TR07JA2. FMRIB Analysis Group of the University of Oxford; 2007. Non-linear registration, aka Spatial normalisation.
33. Woolrich MW, Behrens TE, Beckmann CF, Jenkinson M, Smith SM. Multilevel linear modelling for FMRI group analysis using Bayesian inference. *Neuroimage*. 2004; 21:1732–47. [PubMed: 15050594]
34. Nichols TE, Holmes AP. Nonparametric permutation tests for functional neuroimaging: a primer with examples. *Hum Brain Mapp*. 2002; 15:1–25. [PubMed: 11747097]
35. Smith SM, Nichols TE. Threshold-free cluster enhancement: addressing problems of smoothing, threshold dependence and localisation in cluster inference. *Neuroimage*. 2009; 44:83–98. [PubMed: 18501637]
36. Naidich, MD.; Duvernoy, H.; Delman, BN.; Sorensen, AG.; Kollias, SS.; Haacke, EM. Duvernoy's Atlas of the Human Brain Stem and Cerebellum. Third edition. SpringerWein; New York: 2009.
37. Garcia-Larrea L, Peyron R. Pain matrices and neuropathic pain matrices: a review. *Pain*. 2013; 154(Suppl 1):S29–43. [PubMed: 24021862]
38. Garcia-Larrea L. The posterior insular-opercular region and the search of a primary cortex for pain. *Neurophysiol Clin*. 2012; 42:299–313. [PubMed: 23040701]
39. Friebel U, Eickhoff SB, Lotze M. Coordinate-based meta-analysis of experimentally induced and chronic persistent neuropathic pain. *Neuroimage*. 2011; 58:1070–80. [PubMed: 21798355]
40. Harris RE, Napadow V, Huggins JP, Pauer L, Kim J, Hampson J, Sundgren PC, Foerster B, Petrou M, Schmidt-Wilcke T, Clauw DJ. Pregabalin rectifies aberrant brain chemistry, connectivity, and functional response in chronic pain patients. *Anesthesiology*. 2013; 119:1453–64. [PubMed: 24343290]
41. Upadhyay J, Anderson J, Schwarz AJ, Coimbra A, Baumgartner R, Pendse G, George E, Nutile L, Wallin D, Bishop J, Neni S, Maier G, Iyengar S, Evelhoch JL, Bleakman D, Hargreaves R, Becerra L, Borsook D. Imaging drugs with and without clinical analgesic efficacy. *Neuropsychopharmacology*. 2011; 36:2659–73. [PubMed: 21849979]
42. National Institute for Health and Care Excellence:. Neuropathic pain - pharmacological management, The pharmacological management of neuropathic pain in adults in non-specialist settings. National Institute for Health and Care Excellence; Manchester,UK: 2013.
43. Hempenstall K, Nurmikko TJ, Johnson RW, A'Hern RP, Rice AS. Analgesic therapy in postherpetic neuralgia: a quantitative systematic review. *PLoS Med*. 2005; 2:e164. [PubMed: 16013891]
44. McMahon SB, Wall PD. Electrophysiological mapping of brainstem projections of spinal cord lamina I cells in the rat. *Brain Res*. 1985; 333:19–26. [PubMed: 3995286]
45. Basbaum AI, Fields HL. Endogenous pain control systems: brainstem spinal pathways and endorphin circuitry. *Annu Rev Neurosci*. 1984; 7:309–38. [PubMed: 6143527]
46. Haws CM, Williamson AM, Fields HL. Putative nociceptive modulatory neurons in the dorsolateral pontomesencephalic reticular formation. *Brain Res*. 1989; 483:272–82. [PubMed: 2706520]
47. Heinricher MM, Cheng ZF, Fields HL. Evidence for two classes of nociceptive modulating neurons in the periaqueductal gray. *J Neurosci*. 1987; 7:271–8. [PubMed: 3806198]
48. Fields HL, Bry J, Hentall I, Zorman G. The activity of neurons in the rostral medulla of the rat during withdrawal from noxious heat. *J Neurosci*. 1983; 3:2545–52. [PubMed: 6317812]

49. Williams FG, Beitz AJ. Chronic pain increases brainstem proneurotensin/neuromedin-N mRNA expression: a hybridization-histochemical and immunohistochemical study using three different rat models for chronic nociception. *Brain Res.* 1993; 611:87–102. [PubMed: 8518953]
50. Haghighparast A, Naderi N, Khani A, Lashgari R, Motamedi F. Formalin-induced differential activation of nucleus cuneiformis neurons in the rat: an electrophysiological study. *J Pain.* 2010; 11:32–43. [PubMed: 19632161]
51. Wanigasekera V, Lee MC, Rogers R, Hu P, Tracey I. Neural correlates of an injury-free model of central sensitization induced by opioid withdrawal in humans. *The Journal of neuroscience : the official journal of the Society for Neuroscience.* 2011; 31:2835–42. [PubMed: 21414905]
52. Moulton EA, Burstein R, Tully S, Hargreaves R, Becerra L, Borsook D. Interictal dysfunction of a brainstem descending modulatory center in migraine patients. *PLoS One.* 2008; 3:e3799. [PubMed: 19030105]
53. Schwedt TJ, Larson-Prior L, Coalson RS, Nolan T, Mar S, Ances BM, Benzinger T, Schlaggar BL. Allodynia and descending pain modulation in migraine: a resting state functional connectivity analysis. *Pain Med.* 2014; 15:154–65. [PubMed: 24165094]
54. Keltner JR, Furst A, Fan C, Redfern R, Inglis B, Fields HL. Isolating the modulatory effect of expectation on pain transmission: a functional magnetic resonance imaging study. *J Neurosci.* 2006; 26:4437–43. [PubMed: 16624963]
55. Wang R, King T, De Felice M, Guo W, Ossipov MH, Porreca F. Descending facilitation maintains long-term spontaneous neuropathic pain. *J Pain.* 2013; 14:845–53. [PubMed: 23602267]
56. Tanabe M, Takasu K, Takeuchi Y, Ono H. Pain relief by gabapentin and pregabalin via supraspinal mechanisms after peripheral nerve injury. *J Neurosci Res.* 2008; 86:3258–64. [PubMed: 18655202]
57. Patel MK, Gonzalez MI, Bramwell S, Pinnock RD, Lee K. Gabapentin inhibits excitatory synaptic transmission in the hyperalgesic spinal cord. *Br J Pharmacol.* 2000; 130:1731–4. [PubMed: 10952660]
58. Taylor CP, Garrido R. Immunostaining of rat brain, spinal cord, sensory neurons and skeletal muscle for calcium channel alpha2-delta (alpha2-delta) type 1 protein. *Neuroscience.* 2008; 155:510–21. [PubMed: 18616987]
59. Colagiuri B. Participant expectancies in double-blind randomized placebo-controlled trials: potential limitations to trial validity. *Clin Trials.* 2010; 7:246–55. [PubMed: 20421243]
60. Tracey I. “Seeing” how our drugs work brings translational added value. *Anesthesiology.* 2013; 119:1247–8. [PubMed: 24343284]
61. Morgan P, Van Der Graaf PH, Arrowsmith J, Feltner DE, Drummond KS, Wegner CD, Street SD. Can the flow of medicines be improved? Fundamental pharmacokinetic and pharmacological principles toward improving Phase II survival. *Drug Discov Today.* 2012; 17:419–24. [PubMed: 22227532]
62. Duff EP, Vennart W, Wise RG, Howard MA, Harris RE, Lee M, Wartolowska K, Wanigasekera V, Wilson FJ, Whitlock M, Tracey I, Woolrich MW, Smith SM. Learning to identify CNS drug action and efficacy using multistudy fMRI data. *Sci Transl Med.* 2015; 7 274ra16.

Summary statement

In this double-blind, placebo controlled, crossover study of healthy humans in a centrally sensitized state (n=24), neuroimaging detects pharmacodynamic efficacy of analgesics irrespective of pain behaviour. This highlights the potential role of neuroimaging combined with a mechanism based healthy volunteer model in decision making in early drug development.

Final Box Summary Statement

What we already know about this topic

- Human neurophysiological 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 sensitisation, gabapentin reduces activation of pain-related brain areas as well as functional connectivity between the thalamus and secondary somatosensory cortex while ibuprofen does not when compared to placebo.
- Functional imaging may be a viable tool for evaluating analgesic efficacy during early stages of drug development.

Figure 1.

Study events and drug-induced effects on psychophysics. Events during a study visit are shown in figure 1A. In the scanner, functional scans were obtained while delivering blocks of brush stimuli over ~ 10 min to elicit dynamic mechanical allodynia followed by punctate stimuli over ~ 10 min to elicit hyperalgesia. This was followed by two task free scans: an arterial spin labeling sequence (ASL) lasting ~10 min and a resting state sequence (RS) lasting ~5.5 min. At the end of the scan (~ 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 prior to commencement of scanning. Group mean scores of secondary punctate mechanical hyperalgesia intensity (solid bars) and unpleasantness (figure 1B), ongoing unprovoked pain (figure 1C) and mental sedation 150 min post dosing (M3) (figure 1D) during placebo (Pl), ibuprofen (Ib) and gabapentin (Gb) visits are shown. Y-axis shows the group mean visual analogue scale (VAS) scores. Error bars indicate SEM. (* $P < 0.05$ after Bonferroni correction; ns = statistically not significant).

Figure 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 to placebo (PI) in blue and when compared to ibuprofen (Ib) in red. The Ib>Gb group contrast map in red is overlaid on the PI>Gb group contrast map in blue. MNI coordinates of the voxel with peak effect of PI>Gb and Ib>Gb contrast are X 6, Y -28, 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 family wise error correction rate of 0.05. The annotated diagram of the mesencephalon in the top left hand corner is to aid visual localization of the area where Gb suppressed the hyperalgesia evoked BOLD activity. VTA, ventral tegmental area; SN, substantia nigra; PAG, periaqueductal grey; IC, inferior colliculi. 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 3mm 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 in red below each image slice.

Figure 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 to placebo (Pl) in blue and when compared to ibuprofen (Ib) in red. The Ib>Gb group contrast map in red is overlaid on the Pl>Gb group contrast map in blue. These group differences were identified using whole brain analysis with mixed effects and a cluster based family wise error correction (Z score >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) on the left] and from the left posterior insula [(pIN) on the 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. aIN (anterior insular). Montreal Neurological Institute – 152 template coordinates are denoted in red below each image slice.

Figure 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 to placebo (PI) in blue and when compared to ibuprofen (Ib) in yellow are shown in the coronal and sagittal image slices in the top row. The Ib>Gb group contrast map in yellow is overlaid on the PI>Gb group contrast map in blue. These group differences were identified using whole brain analysis with mixed effects and a cluster based family wise error correction (Z score >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 in red below each image slice. a.u. = arbitrary units. 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 ~200 min post dosing. Plasma gabapentin levels were available from 22 out of the 24 participants who completed the study (2 declined venipuncture).

Figure 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 % BOLD (blood oxygen level dependent) response to secondary punctate mechanical hyperalgesia obtained from the left posterior insula (LtPIN) in green, left secondary somatosensory cortex (LtSII) in blue and right nucleus cuneiformis (rNCF) in red. The subjective outcome measures are the punctate hyperalgesia intensity (INT) (black filled data points) and unprovoked ongoing pain (OGP).