Dynamic Brain Imaging Response to SCS Differential Frequencies: (DIFY SCS-PET) a pilot single blind clinical trial

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Introduction

Various neuroimaging studies over the past decade have attempted to bridge the gap between subjective experiences and objective brain activity pertinent to noxious stimuli and therapeutic management [1,2,3]. Existing evidence suggests role of different pain matrix areas in the brain in relation to varied spectrum of pain perception i.e. sensory to affect and cognition, based on their differential neuronal activity [4,5]. Through experimental neuroimaging, six areas of the brain, namely thalamus, the insular cortex (IC), the primary and secondary somatosensory cortices (SSCI and SSCII), the anterior cingulate cortex (ACC), and the prefrontal cortex (PFC) have consistently been shown to respond to pain. They are believed to play an important role in the sensory- discriminative, cognitive, and affective aspects of pain processing [5,6,7].

Spinal cord stimulation (SCS) is an established, FDA and NICE approved, reversible and cost-effective treatment for the management of several chronic pain conditions with robust evidence in the form of randomised controlled trials (RCT's) for failed back surgery syndrome [8, 9], complex regional pain syndrome (CRPS) [10], refractory angina (RA) [11,12], chronic pains of trunk and limbs and critical limb ischemia [13,14].

Frequency focussed paraesthesia mapping regimes have shown their significance in various clinical trials. Frequency as high as 10,000 Hz has been successfully used in managing patients with Failed Back Surgery Syndrome (FBSS) [9]. Recent studies employing sub-perception frequencies such as 1000 Hz (PROCO trial) [15] and 10Hz (The HALO study) [16] demonstrated significant clinical improvement for chronic pain patients.

Although the clinical benefit and spinal mechanism of conventional and new frequency paradigms is fairly well reported, their effect and the neuronal response of brain pain matrices to the delivered charge remains largely inconclusive. While some work has been done to assess the brain responses following SCS [17], the trajectory of novel therapy ascension has left a large gap between the central mechanism of action and clinical efficacy.

Functional neuroimaging can be used to quantify these effects by measuring change in neuronal activity in terms of alterations in metabolism (i.e., blood flow, volume, oxygen, or glucose metabolism) or neurochemistry (i.e., neurotransmitter precursor uptake or receptor binding)[18]. Of these, functional Magnetic Resonance Imaging (fMRI) has been widely used albeit it remains an indirect method of measurement.

Additionally, majority of newer SCS systems are only compatible to 1.5 Tesla Magnetic Resonance Imaging (MRI), hence not suitable for the high field (3-10T) fMRI [19]. Positron emission tomography-computed tomography, PET-CT allows use of labelled radiotracers to detect change in metabolic activity. The most commonly used radiotracer is F^{18} fluorodeoxyglucose (F^{18} FDG), a molecule similar to glucose. This is a common and highly sensitive diagnostic tool used in detection of early cancer. Metabolically active cells may absorb glucose at a higher or lower rate in relation to increased/decreased rate of metabolism or chemical events at receptor and neurotransmitter reuptake sites [20].
Whilst reasonable evidence is present regarding fMRI and electrophysiological brain responses following SCS therapy with comprehensive reviews [17,21-28], the results have been

largely inconclusive for PET scan brain mapping and SCS therapy. Further a recent systematic review based on human, animal and computational studies, highlights predominant modulation of the descending nociceptive inhibitory pathways and the modulation of the ascending medial and lateral pathways, as the basis of supraspinal mechanism of action of SCS [26].

fMRI studies have demonstrated, activation of the primary motor cortex, insula, and secondary somatosensory cortex during SCS which could be attributed to direct interference with the processing of neuropathic pain [21,22]. A recent regions-of-interest, voxel-based morphometry has further demonstrated reduction in grey matter volumetric changes in hippocampus at three months following high frequency SCS with a positive correlation of back pain intensity [24,25]. An increased connectivity over time between the anterior insula (affective network) and regions of the frontoparietal network has been demonstrated using a resting state functional magnetic resonance imaging (rsfMRI) protocol at three months following 10K Hz SCS [23]. There was a positive correlation of increased strength of connectivity with sleep quality index suggesting possible role of 10KHz in emotional awareness of pain. Although differential frequencies may have a unique individual activation pattern, in general suprathreshold stimulation has been reported in fMRI studies to result in greater activity of the frontal brain regions; the sensory, limbic, and motor cortices; and the diencephalon in comparison with subthreshold stimulation.

Previously, $H_2{}^{15}O$ PET studies and SPECT studies have shown variation in regional blood flow in pain matrices with the use of SCS in chronic pain conditions dictating the role of supraspinal pathways [29,30]. In the first published FDG-PET study with SCS in 2004, Nihashi et al. reported variable uptake in different pain matrices and suggested that thalamus uptake of FDG might be related to whether or not SCS was effective [1].
Sufianov et al. reported normalisation of previously increased metabolism (comparative to control group) in certain pain matrix areas; post central gyrus, orbitofrontal cortex, thalamus, and anterior cingulate cortex (ACC), 3 months following SCS therapy [2]. Further, a comparison of a small cohort of patients (n=7) in a single centre subset analysis of SUNBURST study using 18F-FDG PET-CT scan reported increase in metabolic rate in the premotor cortex with both burst and tonic stimulation with a differential increased activity seen at ACC and prefrontal cortex (PFC) with burst, as compared to tonic stimulation, suggesting predominant involvement of medial pathway with the burst stimulation [3].

Evidently, current evidence is inconsistent and limited with small size of study cohorts and without any clear pattern of stimulation or inhibition of pain matrices. Hence, it becomes challenging to provide a conclusive relationship of direct brain metabolic activity to pain responses following SCS. Our study with sequential 18F-FDG PET-CT scanning was designed to investigate whether there was any objective measurable effect of differential frequency stimulation (40Hz, 4000Hz and 10,000Hz frequency) and in turn neural dosing on the brain metabolism with further correlation of brain activity with pain improvement (≥50% reduction in NRS). We also aimed to establish specific pain matrix areas response in line with the receptive and cognitive component of chronic pain in the patients undergoing SCS for intractable lumbar neuropathic pain due to failed back surgery syndrome.

Methods

This is a single-blind, randomised, cross-over trial performed at Bart's Neuromodulation Centre and Department of Nuclear Medicine at St Bartholomew's Hospital, London, UK. The study received national research ethical approval: 17/LO/0655 and was registered with trial registry Clinical Trial Gov: NCT03716557. 22 patients undergoing spinal cord stimulation for intractable lumbar neuropathic pain as standard of their care were recruited and underwent 18F-FDG-PET scan of the brain at baseline before the implant, and 4 weeks after receiving each differential frequency 40Hz, 4000Hz, 10,000Hz stimulation post implant. The stimulation parameters were "on" during the PET scan.

Patients meeting the inclusion (Box 1) and exclusion criteria (Box 2) were recruited in the study. Written informed consent was obtained from each patient prior to participation in this study following adequate explanation of the aims, methods, anticipated benefits and potential hazards of the study.

Box 1: Inclusion Criteria
Patients with intractable lumbar neuropathic pain due to failed back surgery syndrome, and who were deemed suitable to receive Percutaneous Spinal Cord Stimulation as part of their standard treatment.
Patients between 18 and 75 years of age.
Patients who have given their written informed consent.
Female patients of childbearing potential must be using adequate contraception (i.e. using oral or IM contraception or an IUCD) and must have a negative urine pregnancy test.

Box 2: Exclusion Criteria
Patient's refusal to consent
Patients with diabetes or any underlying neurological condition
Patients known to have a condition that in the investigator's judgement precludes participation in the study
Patients with ongoing psychiatric conditions like severe anxiety, depression and fear of movement, that could interfere with study procedures
Patients who have received an investigational drug or have used an investigational device in the 30 days preceding study entry
Patients unable to comply with the study assessments and to complete the questionnaires
Patients with morbid obesity (body mass index of 35 or greater)
Patients involved in legal actions or benefit tribunals related to their low back pain
Pregnant or breast-feeding patients (verbal confirmation obtained during screening) and urine sample confirmation before each interventional and radiological procedure
Any patient unable to undergo PET scans

Dorsal column stimulator trial and placement

Percutaneous fluoroscopic guided SCS trial at 40 Hz for 1 week (Boston Scientific 8 contact standard leads) and, if successful (NRS>50% improvement), permanent implantable pulse generator, (Boston Scientific Precision Plus^{R} IPG) procedure was performed with midline anatomical parallel placement of thoracic dual leads to cover T8 and T9.

Randomisation & masking

After a period of 40Hz tonic stimulation for 4 weeks, patients were randomised 1:1 to receive 4000Hz or 10000Hz frequency stimulation, using sequentially numbered, opaque, sealed envelopes containing a previously generated allocation sequence. The envelopes were securely stored and dispensed by independent third parties who had no further contribution to the clinical trial. The patients, investigators and neuro-physicist were blinded to the allocation and the chief investigator (VM) always had access to sealed envelopes. After a period of 4 weeks of receiving randomised frequency (4000Hz or 10,000Hz), patients were crossed-over to the other frequency (10,000Hz or 4,000Hz) for another 4 weeks before receiving a final scan. The patients underwent programming with the clinical nurse specialist as per standard clinical practice for these patients.
All medication usage was recorded for each patient as per standard of care. However, to eliminate the confounding factor of medication variability in brain mapping, the patients continued with the same medications during the study period.

Outcomes

Primary outcomes: The primary outcome of the clinical trial was to evaluate clinical response and metabolic changes in the brain as observed by PET scans from baseline following the differential frequencies.
Secondary outcomes: The secondary outcomes were to establish any relationship between the neural dose at different frequencies and PET changes in the brain, and correlation between pain scores and PET changes at differential frequencies. Data pertaining to pain scores and quality of life following differential frequencies was also collected.
The following questionnaires (paper copies) were filled at the time of study visits by the patients and data collected at each visit:

1. Numerical Rating Scale (NRS): NRS score would be used to measure the pain intensity, enabling the patient to express the severity of pain by giving it a numerical value from 0 to 10 on an 11-point scale
2. Oswestry Disability Index (ODI): A commonly used scale for back pain patients with a neuropathic pain component. The test is considered the 'gold standard' of low back functional outcome tools.
3. Quality of life Index (EQ5D5L): This comprises a battery of questions used to assess quality of life index.

Box 3: Study visits for the study
Visit 1: Baseline visit and informed consent obtained prior to SCS implant
Visit 2: Baseline PET scan (1st scan) performed. Questionnaires administered.
Visit 3: Patients underwent first stage SCS followed by 40Hz stimulation for trial.
Visit 4: After successful trial patients received permanent IPG and tonic stimulation 40 Hz.
Visit 5: 4 weeks following tonic stimulation (40Hz) 2nd PET scan performed. Questionnaires administered.
Visit 6: (0-7 days after 2nd PET scan) Patients randomised to receive either 4000 Hz or 10,000 Hz frequency parameters.
Visit 7: 4 weeks following high frequency programming (4000 Hz or 10,000 Hz) 3rd PET scan performed. Questionnaires administered.
Visit 8: (0-7 days after 3rd PET scan) Patients crossed-over to receive either 4000 Hz or 10,000 Hz frequency parameters.
Visit 9: 4 weeks following cross-over high frequency programming (4000 Hz or 10,000 Hz) 4th PET scan performed. Questionnaires administered.
Study visits completed.

18F-FDG-PET scan of the brain

18F-FDG-PET CT Brain scans acquired on GE-Discovery 710 PET system with a 128 slice CT were analysed using PMOD software (PMOD Technologies Ltd, Sumatrastrasse 25, 8006 Zurich, Switzerland). Patients received a dose of 250 MBq 18F-FDG intravenously with an uptake time of 30 minutes. The exposure factors of the scanner were set at 120kV (tube voltage) and 50 mAs (tube current). The tube rotation time was set at 0.5s (the emission time per frame 15 frames for 1 minute each). The 15 dynamic frames were viewed for quality control. The total research protocol dose in this study was therefore 22 mSv for patients receiving 4 administrations. The radiation dose from this study therefore falls into risk category III, as defined in ICRP 62 (effective dose > 10 mSv) and is considered as a moderate level of risk.

Region based analysis

Automated processing of the 18F-FDG Brain PET CT images was done by leveraging the localization of brain areas, as encoded in the validated built in PMOD software atlas. This allowed adjustment of the atlas to individual brain anatomy using spatial normalization procedure obtained directly from the PET images. Volume of interest statistics were obtained from template matched original PET images to make the results from each follow up scan comparable with the baseline scan using a total uptake calculation. SUVmax was the figure of merit used in subsequent analysis due to its extensive use in clinical routine and research studies [31,32]. 18 brain areas that represent pain regions namely: right and left prefrontal cortex, right and left insula, right and left anterior cingulate cortex, right and left hippocampus, right and left amygdala, right and left primary somatosensory cortices, right and left secondary somatosensory cortices, right and left thalami, parabranchial and periaqueductal grey were analysed based on previous studies [4,5]. In addition to the absolute value of the PET uptake (SUVmax), change and % change in PET uptake (SUVmax) at each frequency (40Hz, 4000Hz and 10,000Hz) from baseline were quantified for each patient (at each of the pain matrix regions as well as the average of all the regions). This further provided normalisation to the PET uptake (SUVmax) intrinsic to individual patient characteristics.

Statistical Analysis

All statistical analyses were performed using SPSS 25.0 (IBM Corp, Released 2017. IBM SPSS Statistics for Mac, Armonk, NY, USA: IBM Corp). Differences and percentage change in the PET uptake at each frequency (40 Hz, 4000 Hz and 10000 Hz) from baseline was assessed using non-parametric Wilcoxon paired test which was applied to pooling of PET uptake from all pain matrix regions (18 areas across 57 scans). A hierarchical linear mixed (fixed-random) effect model assessed the overall change in the PET uptake (intercept) at each frequency (40 Hz, 4000 Hz and 10000 Hz) from baseline by taking into consideration the contribution of each pain matrix region (fixed-effect) within individual patients (random-effect). Least squares means plot was used to visualise the changes (trend) in PET uptake at each frequency (40Hz, 4000Hz, 10000Hz) from baseline across the 18 pain matrix regions. Absolute change (decrease/increase) plots for all the patients were drawn to visualise the PET uptake at each frequency from baseline across the 18 pain matrix regions.
Proportion of regions having a reduction in the PET uptake at each frequency (40 Hz, 4000 Hz and 10000 Hz) from baseline was assessed using a two-tailed 1-sample Proportions test with continuity correction.

Differences in the patients clinical outcome metrics (NRS back, NRS leg, ODI) at each frequency (40 Hz, 4000 Hz and 10000 Hz) from baseline were assessed using non-parametric Wilcoxon paired test on pooled data. At each frequency, association between NRS (back and leg) and PET uptake, neural dose and PET uptake, and neural dose and NRS (back and leg) for the 18 pain matrix regions was analysed using non-parametric Spearman's rank correlation. For the most significant associations, the ability of PET uptake at each pain matrix region to differentiate between patients with lower pain-score and patients with higher pain score was assessed using non-parametric Mann Whitney test. Box plots for the most significant differentiators are presented. A significant difference/correlation was demonstrated with p-value of less than 0.05.

Results

18 patients underwent baseline PET scan before the procedure (four withdrew after consenting). 15 patients were implanted with the IPG as three patients were trial failure and did not undergo IPG implantation. 14 patients received 40 Hz for 4 weeks followed by 2nd PET-CT. They underwent 1:1 randomization (4000Hz/ 10,000 Hz) for further 4 weeks followed by 3rd PET-CT. 14 patients crossed over to (10,000 Hz/ 4000Hz) for further 4 weeks followed by 4th PET-CT. One patient did not respond to 40 Hz but responded to 10,000 Hz. The patient received 2nd scan post 10,000 followed by post randomisation 3rd scan and cross over 4th scan.

57 PET-CT scans (15 for baseline and 14 each for 40 Hz, 4000 Hz and 10,000 Hz) were analysed for Standardized Uptake Value (SUVmax) difference and % change in SUVmax from baseline (Figure 1).

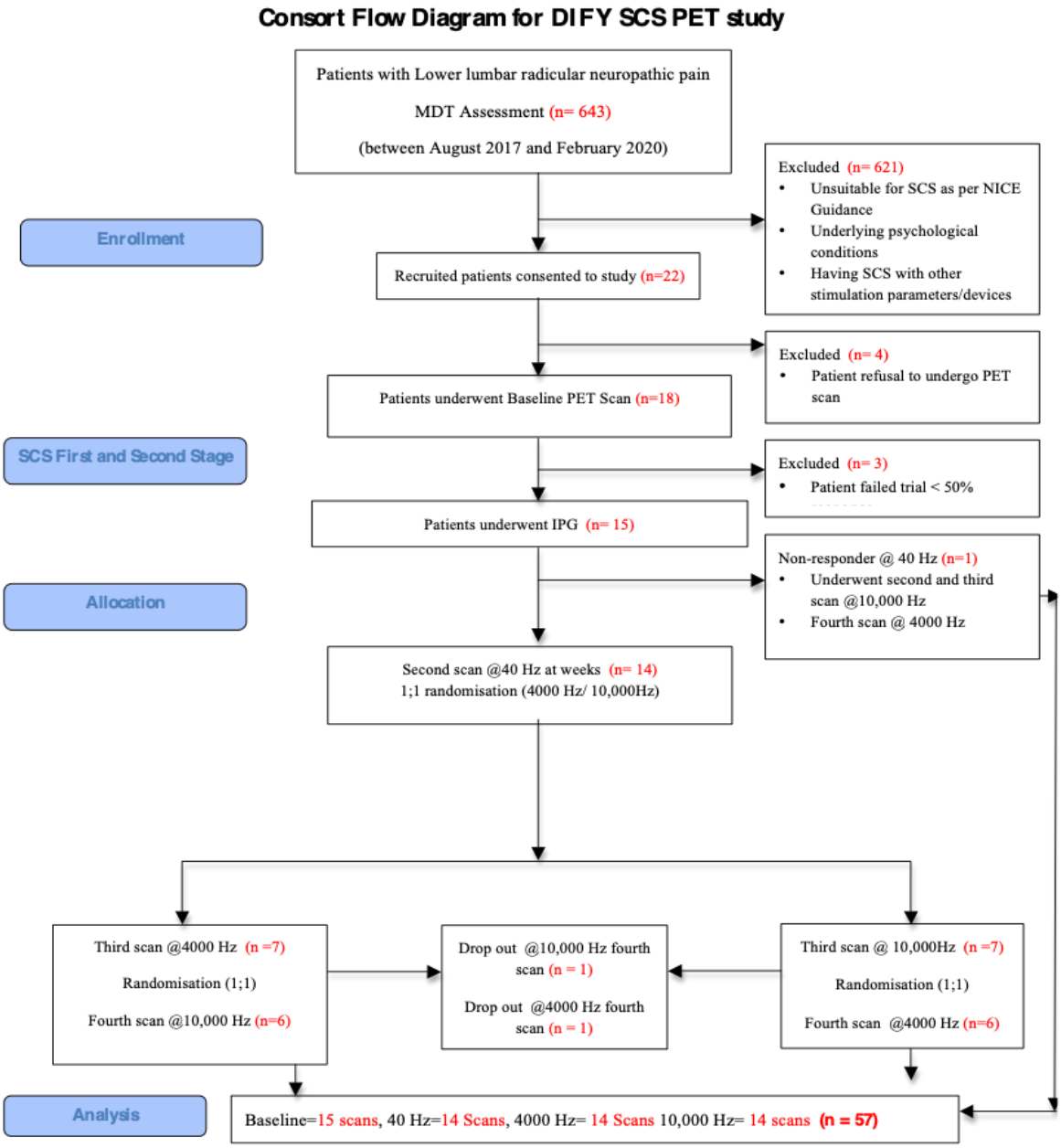

Figure 1: Trial profile: SCS= spinal cord stimulation, IPG= implantable pulse generator, PET= positron emission tomography

1. **Male: Female**	04:11
2. **Age years** (Mean±SD)	51.8±11.16

3. **NRS Back** (Mean±SD)	9.0 ± 0.75
4. **NRS Leg** (Mean±SD)	8.1 ± 2.06
5. **ODI** (Mean±SD)	57.88 ±11.62
6. **EQ5D5L** (Mean±SD)	0.52 ± 0.15

Table 1: Baseline demographics and characteristics for all randomly assigned patients.

There were statistically significant reductions in SUVmax at 40 Hz (median = 8.24, p=0.002) and 4000Hz (median = 8.6, p=0.001) compared to baseline (median = 9.03), when pooled across all of the 18 pain matrices. Similarly, a reduction in SUVmax at 10,000 Hz (median = 8.64) compared to baseline (median = 9.03) was also observed, which however did not reach statistical significance (p=0.573). Further, hierarchical linear mixed (fixed-random) effect model identified that the overall SUVmax was lowest for 4000 Hz from baseline (intercept: -0.257, p=0.562)) with 15 out of 18 pain matrix regions having a lower SUVmax (intercept ranged from -0.384 to -0.024). Amongst 15 pain matrix regions, left secondary somatosensory cortex had the lowest (most significant) SUVmax at 4000 Hz compared to baseline (intercept: -0.384, p=0.0259), followed by right secondary somatosensory cortex (intercept: -0.310, p=0.0716), left insula (intercept: -0.295, p=0.0864), left prefrontal cortex (intercept: -0.287, p=0.0953) and right prefrontal cortex (intercept: -0.285, p=0.0975) which were close to significance (at p<0.1 level).

Least squares means plot demonstrated change in PET uptake (SUVmax) at each frequency from baseline across the 18 pain matrix regions with highest reduction in SUVmax was at 4000 Hz from baseline across the 18 pain matrix regions (Figure 2).

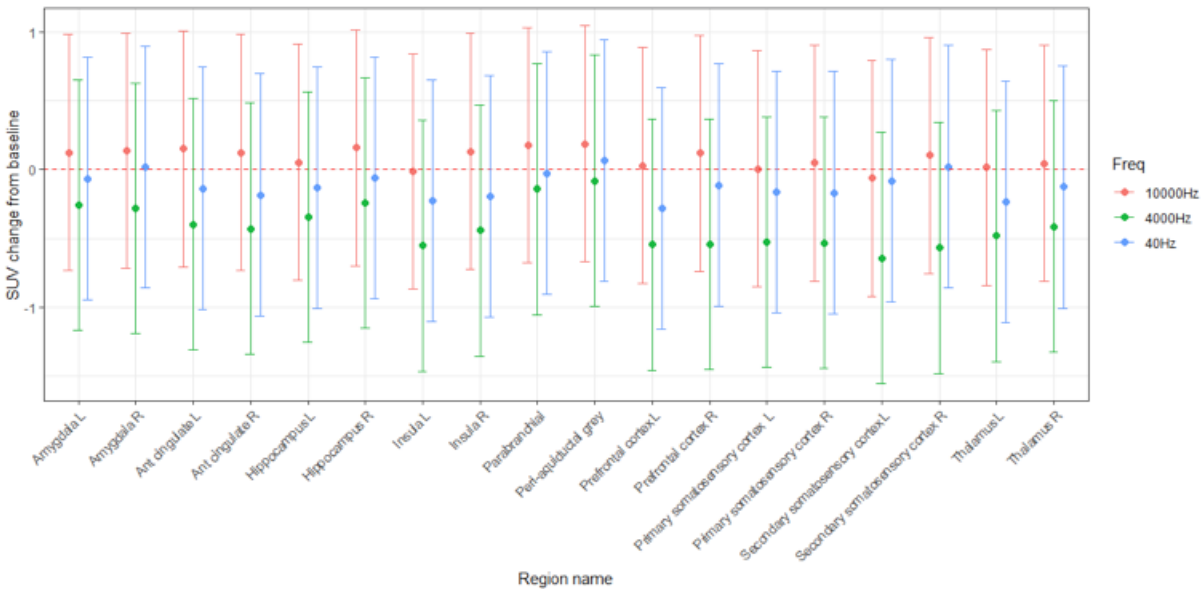

Figure 2: Least squares means plot demonstrates change in PET uptake (SUVmax) at each frequency from baseline across the 18 pain matrix regions.

Out of the pooled data from 252 pain matrix regions, 156 (61.9%), 134 (53.1%) and 115 (45.6%) regions showed reduction in SUVmax at 40 Hz, 4000 Hz and 10,000 Hz respectively. The 1-sample proportion test with continuity correction demonstrated significant proportion of regions with reduction in SUVmax from baseline (p<0.001) at 40Hz only. At each frequency

40Hz, 4000Hz and 10,000 Hz, average PET uptake was lower than baseline in total 9,7 and 5 patients respectively. Key pain matrix regions i.e. thalamus, prefrontal cortex, insula, ACC, primary SSC, secondary SSC and PAG showed proportionately higher number of patients (9,10,9,9,9,8,9) with reduction in SUV max at 40 Hz comparative to 4000Hz (8,7,7,7,9,8,6) and 10,000Hz (8,5,5,6,7,6,6) respectively (Figure 3a, 3b, 3c).

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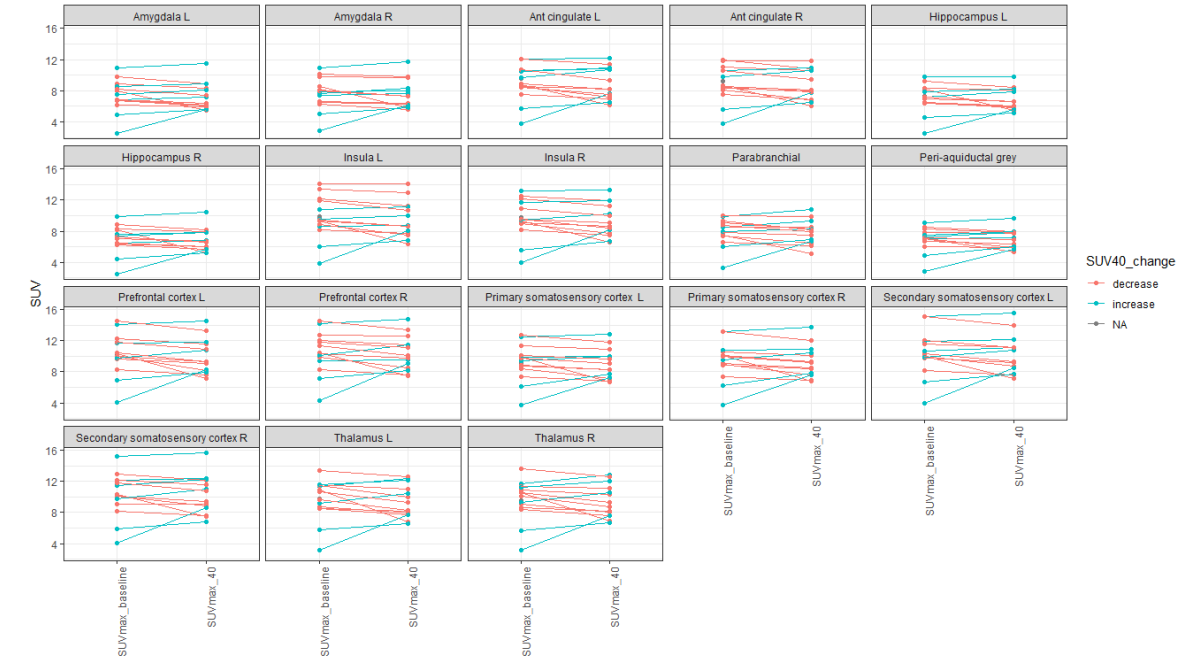

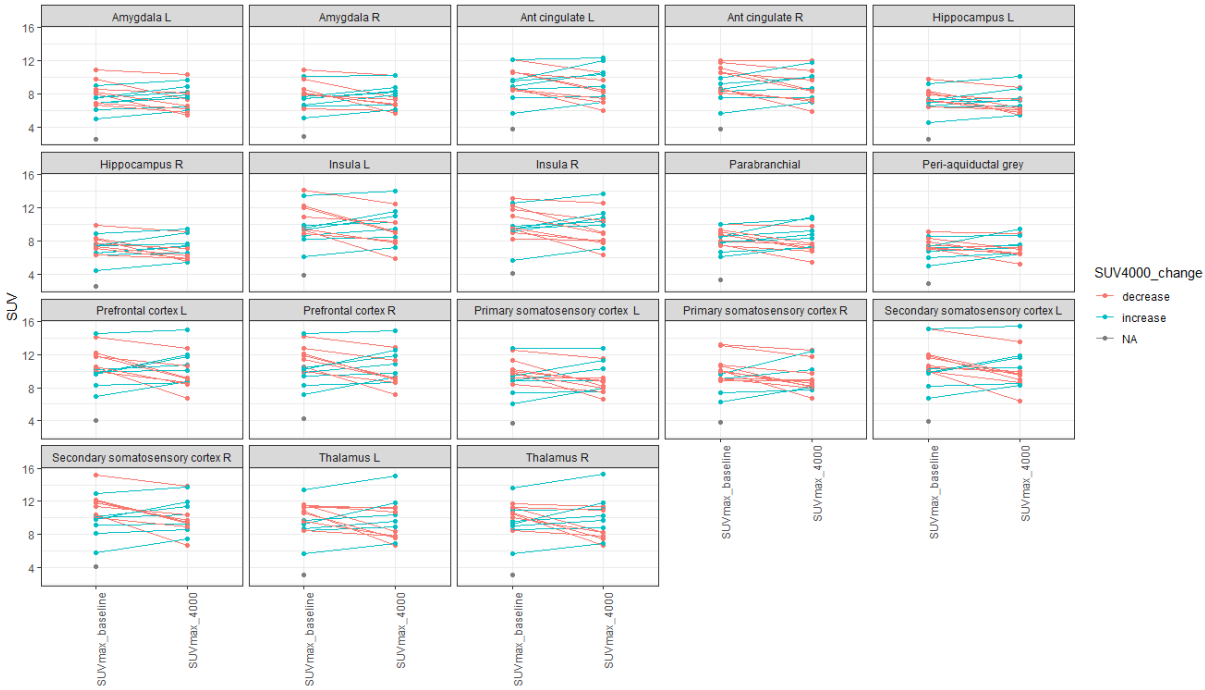

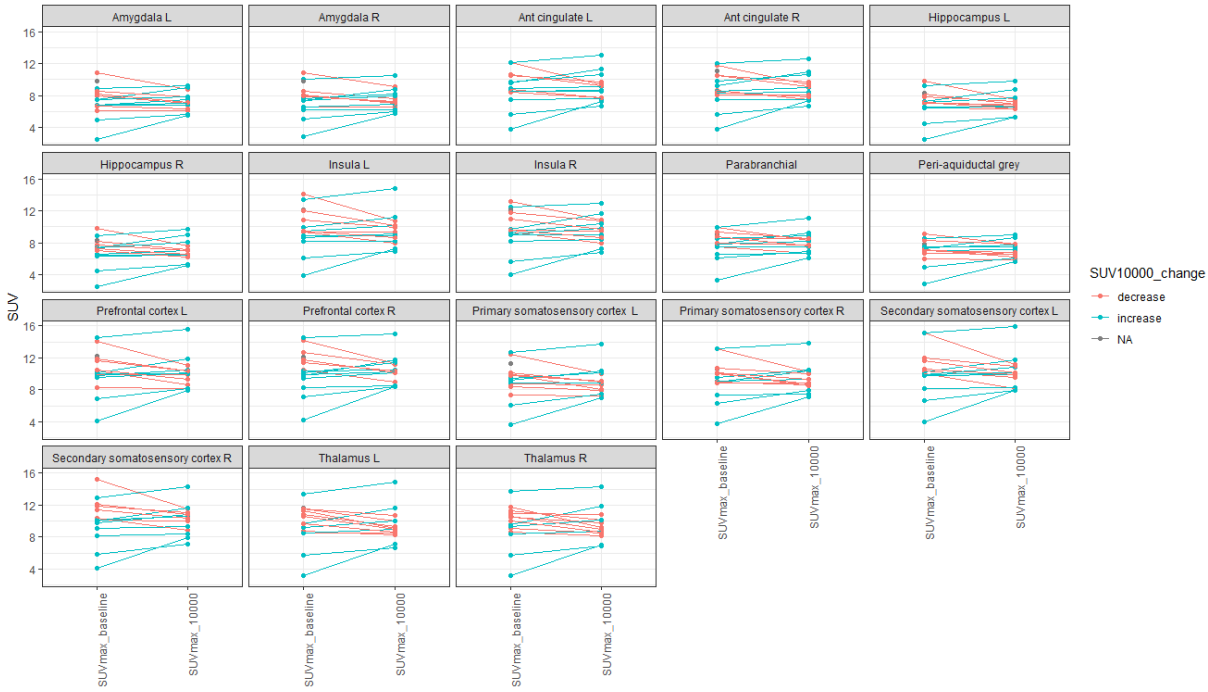

Figure 3a, 3b and 3c: Demonstration of change in SUVmax of 14 patients at 40Hz, 4000Hz and 10000Hz from baseline across the 18 pain matrix region.

There was statistically significant improvement in NRS (back and leg) at all frequencies- 40 Hz (p=0.001), 4000 Hz (p= 0.001) and 10000 Hz (p = 0.001) from baseline (Figure 4, 5). Similarly, there was a statistical improvement in ODI, EQ5D index and EQ5D health VAS for 40Hz (p=0.002, 0.006, 0.033), 4000Hz (p=0.019, 0.001, 0.006) and 10,000 Hz (p=0.001, 0.001, 0.003) from baseline.

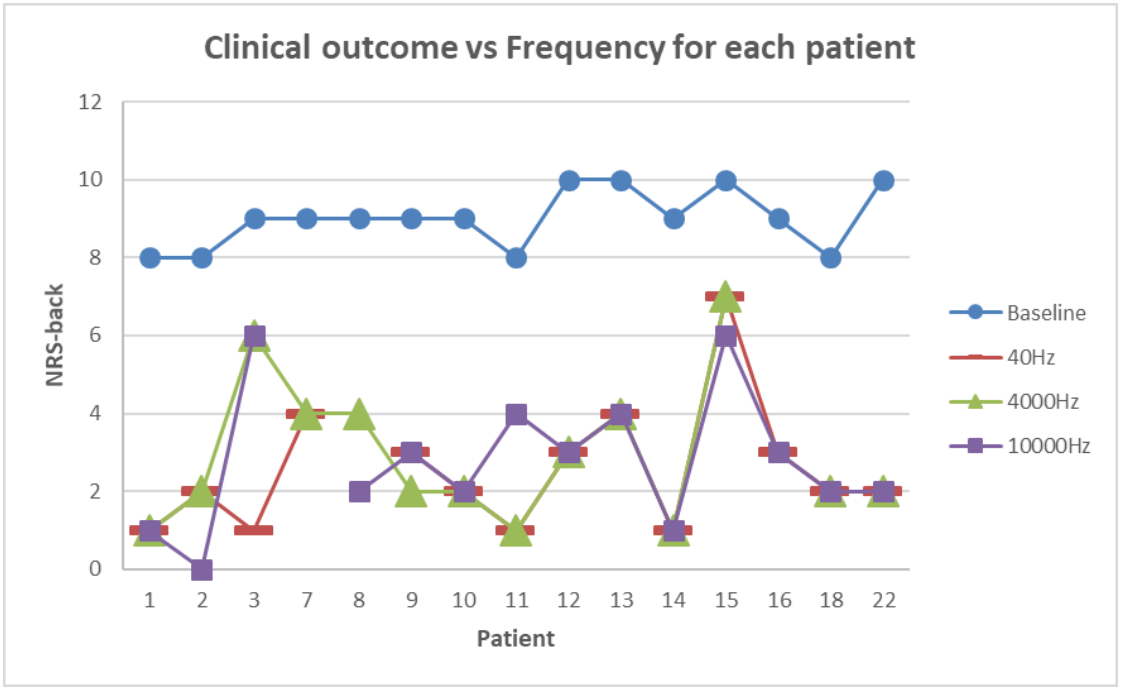

Figure 4: NRS score (back) for all 14 patients at baseline, 40 Hz, 4000Hz and 10,000Hz.

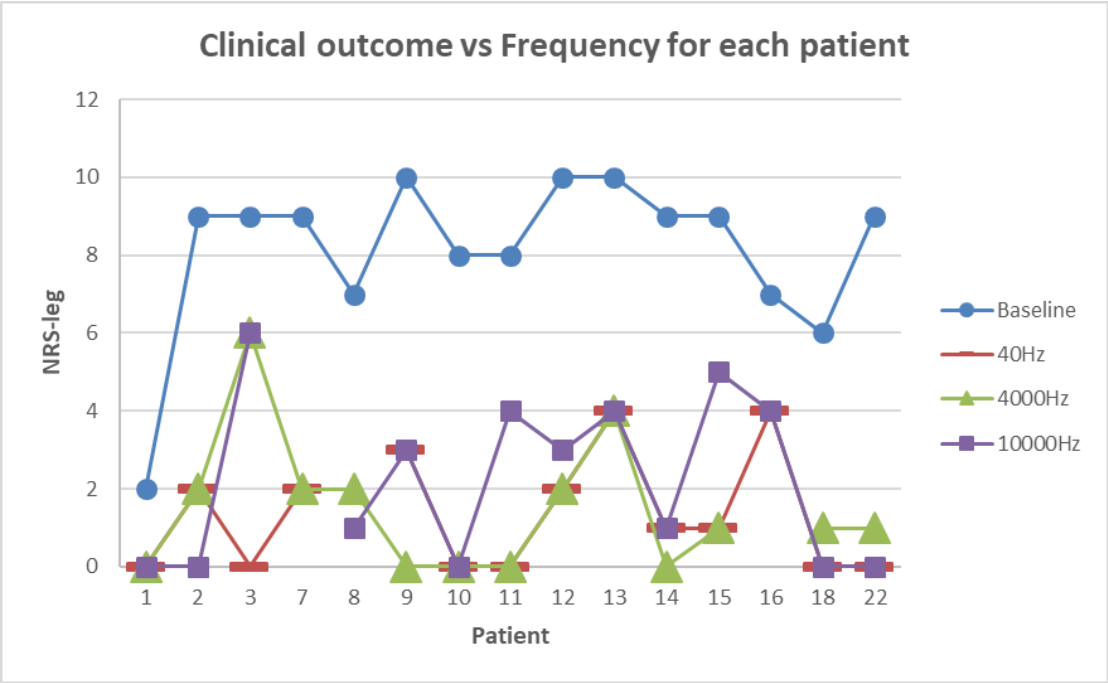

Figure 5: NRS score (leg) for all 14 patients at baseline, 40 Hz, 4000Hz and 10,000Hz.

The concept of neural dose, a combination of variables including frequency, pulse width, amplitude and impedance has been described as an overall charge (dose) delivered to the patient. This has been given by the formulae:

Charge per second (nano C/ sec) = Amplitude (m Amps) X Frequency (Hz) X Pulse Width (u Sec).

We compared the neural dose delivered at each frequency and correlated with PET uptake at that frequency. There was negative moderate to strong correlation between neural dose at 40 Hz and absolute SUVmax across all the 18 brain regions. (r_s=-0.66, p=0.025 to r_s =-0.80, p=0.003). There was no correlation between neural doses at 4000Hz and SUV max (r_s =0.04, p=0.870 to r_s =0.14, p=0.620) and 10000 Hz (r_s =0.02, p=0.920 to r_s =0.27, p=0.340).

There was no correlation between neural doses and NRS (back and leg) at 40 Hz, 4000Hz and 10,000Hz. We demonstrate significant positive correlations between PET uptake (% change in SUVmax from baseline) and NRS (back and leg) score at 10,000Hz across all 18 pain matrices (r_s = 0.56, p=0.030 to r_s =0.80, p=0.010).

We observed that for the most significant associations that is NRS leg at 10,000Hz, PET uptake at each pain matrix region was able to differentiate between patients with lower pain-score (NRS leg <= 2, median value as a cut off) with lower uptake and patients with higher pain score (NRS leg >2, median value as a cut off) with higher uptake comparative to baseline (p=0.011).(Figure 6) We were unable to establish such a relationship for NRS back and for any other frequencies.

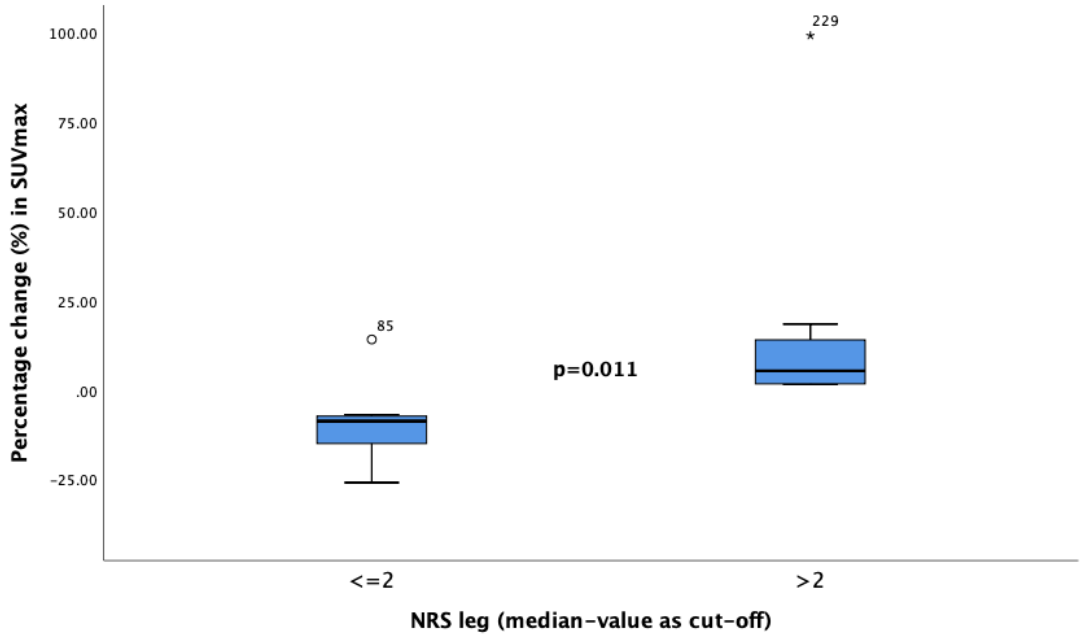

Figure 6: Box-plot illustrates the ability of SUVmax (%changes from baseline at 10,000 Hz to significantly (p=0.011) differentiate between patients with lower pain score (NRS leg <=2, median-value as cut-off) and patients with higher pain score (NRS>2, median-value as cut-off).

Discussion

This is the first in-human study which reports changes in brain metabolic activity (SUVmax) to differential frequencies as compared to baseline following spinal cord stimulation for intractable spinal neuropathic pain. We investigated three frequencies: 40 Hz, the traditional paraesthesia-based frequency, 10,000Hz, a well-established high frequency with clinically proven benefit in RCTs, and 4000Hz, an intermediate higher frequency between the two. To our knowledge this is the largest reported study of 252 pain matrix comparisons (18 regions for 14 scans in each arm) with sequential ^{18}F-FDG PET-CT scans (n=57). Our study provides significant reduction in pain matrix metabolic activity as measured by SUVmax of ^{18}F-FDG following SCS therapy.

This study reports that there is a decrease in brain metabolism/activity (SUVmax) in most of the pain matrix regions when pooled for all tested frequencies, with statistically significant difference for 40 Hz (p=0.002) and 4000Hz (p=0.001) from baseline. Whilst 10,000Hz showed reduction in SUVmax to baseline, it was not statistically significant. This was further supported by the hierarchical linear mixed (fixed-random) effect model, that identified that the overall pooled SUVmax was lowest for 4000 Hz from baseline (intercept: -0.257) with 15/18 pain matrix regions having a lower SUVmax (intercept ranged from -0.384 to -0.024). This is in accordance with neurophysiological studies that have demonstrated SCS related inhibition of somatosensory evoked potentials (SSEPs) and cortical excitability and in turn antalgic mechanism of SCS [33].

In the pooled data across all frequencies, we demonstrated that thalamic regions (59.5%) showed proportionately higher reduction in metabolic activity in comparison to other pain matrices PFC (52%), insula (50%), ACC (52%), SSCII (49%) and PAG (52%), which are related to affect and cognition (Figure 7). Thalamus is considered to be the higher centre involved in reception and processing of nociceptive (ventral posterolateral nuclei) and motivational affective (ventral posteromedial nuclei) information in neuropathic pain. Previous PET studies have reported increased thalamic and post central gyrus activity in response to

painful conditions [2, 34]. By reducing the glucose metabolism and in turn thalamic activity it may be hypothesised that SCS may have an effect on reduced thalamic nociceptive/affect input in this receptive area of the pain matrix. Furthermore, a similar proportion of SSCI areas (59.2%) demonstrated reduced metabolism in line with thalamus, perhaps attributed to reduced conscious perception of sensory-discriminative pain signals received from thalamus.

An equivocal uptake pattern in the areas responsible for affect/cognition (PFC, ACC, Insula and SSCII) could be due to variability in the exchange of excitatory or inhibitory neurotransmitters following SCS pain relief in line with multimodal aspects of pain perception. In some fMRI studies, rheumatic pain reduction has been associated with increased activation of ACC and PFC; the activation has been attributed to the inhibition of signal to midbrain PAG and RVM [35]. A recent FDG PET study has also demonstrated increased activation of medial affect/cognition pathways with burst SCS therapy [3].
We demonstrated that at 40 Hz, a higher proportion (61.9%) of selected higher pain matrix centres showed reduction in metabolic activity from baseline. Proportionately higher number of patients showed reduction in the key pain matrix regions at 40Hz i.e. thalamus, prefrontal cortex, insula, ACC, SSC1, SSCII and PAG (9, 9, 9, 9, 9, 8, 9 respectively) compared to other frequencies (53.1% at 4000 Hz and 45.6% at 10,000 Hz) [36]. This could perhaps be related to paraesthesia and in turn expectation/anticipation related placebo responses [29].
Further in a fMRI model, it has been demonstrated, that paraesthesia based stimulation may cause higher deactivation of the cortical areas (frontal brain regions: limbic, sensory, motor and diencephalon) and in turn default mode network (DMN) and its connectivity to thalamus, which has been found responsible for the dysfunctional DMN chronic back pain [37].

We demonstrated the least charge burden at 40 Hz (least neural dose) and this is in line with previous published work [15]. Furthermore, although there was a negative correlation of neural dose with absolute PET uptake at 40 Hz, we were unable to demonstrate any correlation at any frequency when the uptake was compared to baseline. Correlation between neural dose and absolute SUVmax is exploratory, which needs to be validated in a larger cohort and establish the biological rationale/correlate. As highlighted in the results, 40Hz was the only frequency which showed a significant correlation and that too for all the 18 brain regions, which demonstrate a consistent, significant correlation trend which provides some degree of reassurance, whereas for other frequencies (4000 Hz and 10000Hz) there was no significant correlation. The increasing neural dose related decrease brain metabolism may well be due to enhanced paraesthesia responses at 40Hz as discussed above. No correlation at higher frequencies may suggest that, although the high charge burden reduces pain (given we demonstrated consistent and statistical significant pain relief across all of frequencies), the resultant decrease in brain metabolism does not follow a pattern with neural dose. The higher neural doses required at the higher frequencies appear rather inefficient in terms of objective outcome measurement. However, neurons, and their voltage controlled ionic gates, are unable to respond as quickly as those high frequency pulses, requiring therefore many pulses to cause an effect. Hence, rather than having any simplistic view of only frequency or charge burden related effects on brain metabolism, it needs to be considered that other factors may also be involved in differential uptakes between patients and across the various pain matrices.

All frequencies had a statistical improvement in the clinical outcomes, pain scores and quality of life parameters as compared to baseline. This study was not designed to investigate the clinical efficacy following SCS and it was not unreasonable to see clinical benefits of a therapy that already has a strong evidence base [8,9].

Although we demonstrated significant positive correlation for %change in PET uptake (SUVmax) and NRS leg at 10,000Hz (cut off at median NRS 2), this correlation was not seen in any other frequency in either back or leg pain. Hence, though we report some signals, the ability of PET as a biomarker for SCS therapy is yet to be substantiated. It may need further and perhaps larger numbers to establish any correlation between pain relief and changes in metabolic activity in the brain following SCS. Further studies with a larger sample size and power calculation based on this pilot results, may benefit from employing appropriate

correction to the statistical p-values. Using a median value as a cut-off, provides for an unbiased approach where equal proportion of patients are above and below the cut-off. Being significant for the median cut-off, in future studies and with larger patient population, one could then perhaps employ other more optimised techniques to derive cut-offs.
Similarly, we were unable to demonstrate any correlation between the quality of life index and glucose metabolism, perhaps indicating that one-month follow up could be inadequate for such measure.

We used ^{18}FDG as a radiotracer as it directly measures neuronal activity, hence making it less susceptible to indirect effects of blood volume changes, used in other functional neuroimaging techniques, whilst PET is unique in its ability to evaluate central neuronal processing by directly measuring their excitation and exchange of neuro-transmitters [18].
Standardized uptake value (SUV) is used to measure FDG uptake in order to compensate for factors contributing to variation [31]. We chose maximum standardized uptake value (SUV max) as the marker to measure metabolic activity as it is the most common variable used in clinical practice and has significantly better reproducibility compared to mean standardized uptake value (SUV mean) since maximum value within a region of interest (ROI) is invariant with respect to small spatial shift of the ROI. There is a limited bias and variance associated with SUV max due to noise correlations introduced during the image reconstruction process, making it a robust metric for assessing treatment response. We used the PMOD software package (PMOD Technologies, Zurich, Switzerland) for semi-quantitative analysis of the 18F FDG PET/CT. PMOD is a validated research software and the PET/CT methodology used in this study has been previously reported in SCS model with validation of metabolic mapping via semi-quantitative assessment method using PMOD [39].

It is worthwhile to remember that PET-CT is mainly used for oncology clinical model, that has multi-fold changes in the SUVmax in patients with tumours with subsequent effect on glucose metabolism. This is in contrast to chronic pain (non-oncology) models that will have only subtle changes in the given short duration of therapy [36]. Hence future studies will need to explore and include PET quantification such as metabolic tissue volume measures and texture based radiomic features which could reflect tissue heterogeneity and pick up subtle features not perceivable to the naked eye, which otherwise may be lost from maximum and average SUV uptake measures avoiding any false positives. Further, due to very limited evidence on SCS and its relation to FDG PET metabolism, inconsistent response across the pain matrix regions and ethical considerations of multiple PET scans, it becomes challenging to set a sample size. Therefore, this study was not designed to compare individual frequencies or powered to investigate any brain metabolism differences between these frequencies.
Another limitation of this study was that each frequency was exposed for four weeks only. Although the randomised model would ordinarily allow adequate wash out time i.e. 30 days for each frequency before PET-CT, it remains to be seen if any further increase in wash in time would show remarkably substantial differences in PET uptake and in turn correlation to NRS and neural doses at differential frequencies. Perhaps, changes in SUV max would have been more evident, had each therapy been instituted for relatively longer duration by providing sufficiently enough time to brain to respond to central sensitisation and neuroplasticity which generally takes long time to develop.

We performed pairwise comparisons, given this was an initial proof of concept, exploratory study and we simply wanted to see if the pairwise comparison yielded any interesting, significant difference and this is a limitation of the study. However, having observed some significant difference, we would suggest the next logical step will be to look at a longitudinal analysis. This being a pilot exploratory study, no statistical correction was employed, which should be undertaken in a future, properly powered study as part of the validation.

It remains a challenge to construct a study with multiple locations each specific to frequency, however in this study design, all patients had dual leads with 16 contacts placed over the dorsal column extending from T8-T10 to ensure coverage of T8 and T9 disc space. The Precision Plus ^{R} IPG device allowed us to program with differential frequencies and obtain

coverage as per the patient's clinical symptoms. In a clinical setting, the SCS device, is able to provide compensation for the relative rostro-caudal orientation between the same lead group. By varying the longitudinal position of the lead and the position of the cathode and the stimulation-induced cathodal field, the population of fibres recruited in the dorsal columns and dorsal roots and the corresponding paraesthesia coverage can be varied. The number of contacts on the lead allowed for different combinations of programming options. The increased contact systems lead to an exponential increase in possible electrode combinations to shape the field.

In conclusion, we were able to see statistically significant differences in brain metabolic activity at 40 Hz and 4000Hz from baseline, with effect on both nociceptive and affect-cognitive pathways, highlighting a possible mechanism of SCS in chronic pain. A trend of reduction in 18FDG and in turn brain metabolic activity across all the frequencies from baseline is most likely due to the effect of therapy. This is considering cross over design and patients being their own control for the changing frequency hence reduced subject variations. The strength of this study lies in its high numbers of PET-CT brain scans, high number of areas analysed, and the rigorously consistent methodology. Our findings indicate that the effect of SCS on brain activity is linked with a pattern of reduced metabolism in pain matrices with the pooled analysis most significant reduction at 4000Hz, whilst proportionately more areas showed reduction at 40 Hz, highest being thalamus and SSC1.

This study alludes to the objective mechanism behind SCS, a therapy with an established clinical evidence base. We would suggest that further studies are designed to establish imaging and metabolic activity as a meaningful biomarker to predict the responders to SCS. With an adequately powered study, it would be useful to establish significant quantitative differences with a pattern in specific pain matrices and to set up a biological marker of therapy response.

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