# Reproducibility and sensitivity of pain-related FMRI-BOLD activation responses due to noxious Nd:YAP laser stimulation: a possible tool for analgesic drug discovery

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**INTRODUCTION:** Chronic pain is one of the largest medical health problems in the world but management and treatment is limited due to the low efficacy of pain medications [1]. Neural investigation of pain drug mechanisms using pharmacological FMRI (phMRI) can potentially focus the development of new drugs by providing a more objective marker of pain perception [2]. Most phMRI experiments take place over the course of several hours/days requiring repeated measurements on and off the drug. However the sensitivity and intra−subject reproducibility of FMRI to acute experimental pain stimulation is still unknown and is crucial to the future development and application of phMRI.

**METHODS:** 5 healthy volunteers (3 female, 2 male, mean age ± SD= 27.2 ± 6.1 years) underwent painful laser stimulation during FMRI-BOLD data acquisition on a 3T Varian INOVA MRI system at three time points. Sessions 1 and 2 took place on the same day followed by a session 3 one week later. Nd:YAP laser pulses (5ms duration, 2.75mm diameter, 1.34μm wavelength) were delivered to the dorsum of the left hand. Prior to each session, the laser stimulus energy required to achieve the individual's perception energy of low, medium and high pain was determined. Two runs of laser stimulation were delivered in each experimental session, with each run containing 12 repetitions of the three stimulus intensities presented in a pseudo-randomised order. Volunteers were asked to rate the intensity of each stimulus 9s after presentation using an eleven point (0-10) numerical rating scale (NRS) anchored with '0=no sensation', '1 = pain threshold' and '10=most intense sensation'. For each session, the volunteers' psychophysical responses relating to the low, medium and high subjective pain intensities from both laser runs were averaged. Repeated measures ANOVAs were performed on the subjective ratings and laser stimulus energy to investigate for any effects of session and subjective stimulus intensity.

A gradient-echo EPI sequence providing whole brain and cerebellum coverage was used for functional scans (TE = 34 ms, TR = 3s, 41 contiguous coronal oblique slices, field of view 224 x 224mm, image matrix 64 x 64, resulting in voxel size of 3x3x3.5mm). Analysis of FMRI datasets was carried out using FSL [3] to identify regions exhibiting BOLD signal changes that correlated with the stimulus timings. In order to investigate parametric modulation of pain-related brain activity, 6 contrasts were set-up for each laser run corresponding to the three subjective pain intensities and their differences (i.e. high-low etc.). A second-level fixed-effects (FE) analysis was then carried out to investigate the mean activation for each session as well as paired-t tests to identify differences between sessions, resulting in 36 contrasts for each subject. A further FE analysis was then performed to calculate the Z-statistical maps across subjects for each of the contrasts. Cluster thresholding was performed with a Z threshold of 2.3 and a corrected *p*-value of <0.05.

**RESULTS:** The average numerical ratings as a function of session and subjective pain intensity obtained from the volunteers are shown in Figure 1. After correction for multiple comparisons using a repeated measures ANOVA, subjective pain intensity was found to be a significant effect ($F=27.2$, $p<0.001$) and show a significant linear correlation ($F=41.3$, $p<0.005$). Corrected post-hoc revealed that all pair-wise intensity comparisons were significant at $p<0.05$ level. Session was found not to be a significant effect ($F=2.239$ and $p=0.169$) when examining the subjective ratings. The laser stimulus energy required to achieve the desired subjective pain level (presented for all sessions in Table 1) was tested with a repeated measures ANOVA. Similarly, session was not found to be a significant effect ($F=1.18$, $p=0.348$) whereas, after Greenhouse-Geisser correction as Mauchly's test of Sphericity revealed a significant effect ($p=0.01$), subjective pain intensity level was found have a significant effect on the laser stimulus energy ($F=18.0$, $p<0.01$) and show a significant linear correlation ($F=18.5$, $p<0.01$). Significant linear regressions of laser energy against average numerical rating were also found for all sessions with $R^2= 0.993$, $p=0.05$ for session 1, $R^2= 1$, $p=0.004$ for session 2 and $R^2= 0.996$, $p=0.04$ for session 3.

When examining the FMRI data, clear activation of the brain areas associated with pain perception including the cingulate, insula and somatosensory cortices, thalamus and cerebellum was observed or all three subjective pain intensity conditions and was found to reproducible across sessions. When the high-low pain contrast that details the areas demonstrating a general linear increase across subjective pain intensity levels (i.e. a [-1 0 1] contrast) was examined, robust activation was observed in the cingulate and insula cortices, thalamus, caudate, putamen and cerebellum. Differences in the activation maps between sessions were explored for the high pain condition. It was found that the observed differences in the mean psychophysical ratings between sessions correlated with the degree of activation of the brain areas associated with pain perception, particularly in the insula cortex. For example the largest difference between the mean psychophysical ratings for the high pain condition was found to be 1.02 points on the 0-10 NRS between sessions 2 and 3. The corresponding Z-statistical map (presented in figure 2) showed the highest degree of activation in the insula when compared with the results of the other paired-t tests.

**DISCUSSION:** Volunteers were able to reliably discriminate the three different stimulus intensities and examination of the FMRI data reveals robust activation of brain regions classically associated with pain perception [4]. As expected the subjective ratings showed a significant linear correlation with both subjective pain intensity and laser stimulus energy [5]. Whilst the effect of session on the psychophysical ratings was not found to be significant, the FMRI data did reveal significant differences that appeared to be correlated with small differences in online pain ratings. Preliminary FMRI data closely match the observed psychophysics particularly in the insula cortex, a well known region for encoding pain intensity. A region of interest (ROI) analysis is underway to investigate the areas demonstrating parametric modulation with stimulus intensity and is currently focussed on the insula, somatosensory and cingulate cortices.

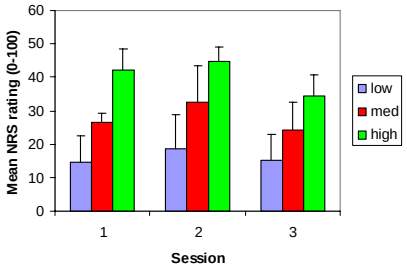


***Figure 1**: Average subjective ratings obtained from n=5 volunteers for the laser stimuli delivered at low, medium and high subjective pain for all sessions.*

| Session | Mean laser stimulus energy (J) | | |
|---|---|---|---|
| | Low | Medium | High |
| 1 | 3.20 ± 0.35 | 3.65 ± 0.31 | 4.10 ± 0.38 |
| 2 | 3.23 ± 0.30 | 3.65 ± 0.30 | 4.03 ± 0.35 |
| 3 | 3.58 ± 0.31 | 3.90 ± 0.28 | 4.20 ± 0.33 |

***Table 1:** Session variation of the mean laser energy ± standard deviation required to achieve the desired subjective pain intensity levels for n = 5 volunteers. Laser stimulus energy was not found to be significantly different across sessions.*

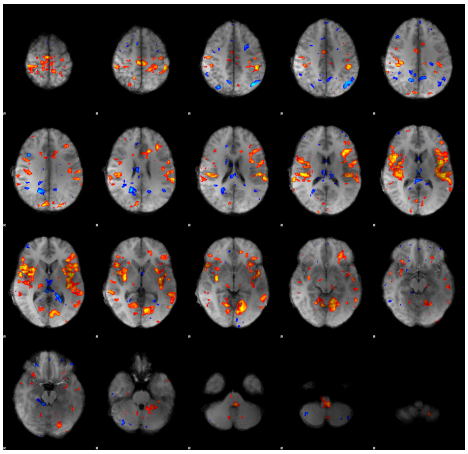

***Figure 2:** Paired-t test between session 2 and 3 for high pain condition. Activation in red details where activity in session 2 > activity in session 3 and blue where activity in session 3 > activity in session 2. Z statistic range from 1.5-2.5 with p= 0.05.*

**REFERENCES:**

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