

Paradoxical increase in amygdala responsiveness to unpleasant stimuli through peripheral beta-blockade: a pharmacological fMRI study

Rebecca Susan Dewey^{1,2}, Olga Pollatos³, Akram A. Hosseini¹, Susan T. Francis², and Dorothee P. Auer¹

¹Radiological and Imaging Sciences, University of Nottingham, Nottingham, Nottinghamshire, United Kingdom, ²Sir Peter Mansfield Magnetic Resonance Centre, University of Nottingham, Nottingham, Nottinghamshire, United Kingdom, ³Department of Psychology, Potsdam University, Potsdam, Germany

Introduction: Autonomic nervous system responses to emotional stimuli (e.g. heart rate changes) can be predicted by the level of activity in the limbic system including the amygdala, brainstem and salience network (anterior insula and anterior cingulate), as demonstrated by neuroimaging (1). Pharmacological blockade of autonomous nervous responsiveness is an elegant way to test the James-Lange theory (2). Several studies have demonstrated reduced amygdala responsiveness, and emotional memory encoding and retrieval under the influence of beta-blockade. Amygdala responsiveness to verbal emotional stimuli is shown to significantly decrease with administration of the centrally acting beta blocker, propranolol (3). These studies are however ambiguous as propranolol does not act selectively in the periphery, and therefore the modulatory effects seen may be direct central effects. To disentangle these effects, we studied the effect of the peripherally acting beta blocker, nadolol, on the fMRI response to neutral, pleasant and unpleasant stimuli. Simultaneous acquisition of BOLD and Pseudo-Continuous Arterial Spin Labelling (PCASL) data provided a novel approach to study functional changes to beta blockers. Preliminary results on valence and drug-dependent fMRI responses will be presented.

Aim and Main Hypothesis: We aim to assess how neural responses to pleasant and unpleasant emotional visual stimuli are modulated by peripheral beta-blockade. We hypothesise that Nadolol reduces bodily arousal to emotional stimuli, and will suppress neural activity in the amygdala and salience network thought to subservise emotional perception through perception of bodily feelings.

Methods: This study was approved by the University of Nottingham Medical School Ethics Committee. 20 healthy, non-smoking, volunteers (age 22 ± 2 years, $n=10$ male) participated, and were asked to abstain from any psychoactive substances (including alcohol, caffeine, pain killers) on the day of each scan. Scans took place between 4 and 8pm. Volunteers were screened for contra-indications in their medical history, cardiac examination and ECG. Volunteers each attended 2 sessions, separated by between 7 and 21 days (mean \pm stdev: 13 ± 5). Sessions consisted of an initial resting blood pressure (BP) measurement, followed by the administration of a single tablet containing 80mg Nadolol or placebo (double-blinded, randomised crossover). BP was measured again 2 hours following tablet administration. Subjects underwent training for the fMRI task. After 2.5 hours, when peak plasma concentration of the drug is expected, the subject was scanned. fMRI data was collected on a Philips Achieva 3.0T MRI scanner with an 8-element SENSE head coil using a double echo PCASL-BOLD EPI scheme (TE=13/36.5ms, flip angle 90° , resolution $3 \times 3 \times 5$ mm, 13 axial slices; PCASL label duration 1500ms, post-label delay 1350ms, label gap 85mm, TR 3500ms). Data were collected for a 5 min resting scan to assess baseline perfusion, and a 14 minute fMRI emotional task. Physiological data were recorded (respiratory rate, vector cardiogram) throughout. The emotional stimulus consisted of neutral, pleasant and unpleasant images from the International Affective Picture System (IAPS) test battery (4). Each image was displayed for 3.5s, with images grouped into blocks of 4 images of the same valence, resulting in an "on" period of 14s, followed by an "off" period (fixation cross) of 14s, during which the volunteer was prompted to rate arousal on a scale of 1-9 for each block.

Analysis: Analysis was performed using SPM8 and software toolboxes coded in Matlab (5). BOLD and PCASL data were corrected for physiological artefacts using RETROICOR (6), motion corrected, co-registered to a standard EPI template, and smoothed using a Gaussian kernel of FWHM 8mm. Statistical analysis was performed using a GLM including motor responses and motion parameters as covariates of no interest. Second level analysis was performed for neutral, pleasant and unpleasant stimuli, and a paired t-test was used to assess differences in responses between drug conditions.

Results: Three subjects were excluded from analysis due to excessive head motion. Nadolol induced a significant reduction in mean arterial blood pressure (MABP) two hours after tablet administration from 85.1 to 76.7 mmHg ($p < 5 \times 10^{-7}$), compared to placebo (from 84.0 to 82.1 mmHg), with a significant between effect ($p < 0.001$). Nadolol also reduced heart rate more than placebo, with a mean reduction of 7 beats per minute, ($p < 0.01$). In the fMRI task, all three stimuli elicited responses in the visual cortex. Compared to the neutral stimuli, pleasant and unpleasant stimuli elicited additional significant activation in the middle/inferior occipital and temporal gyri, anterior insula and fusiform gyrus and amygdala, as well as less significant activations in the superior medial frontal cortex and anterior cingulum. Paired t-tests elicited a reduction of anterior insula response to emotional visual stimuli for the nadolol condition compared to placebo. Regions exhibited greater activity to the emotional stimuli after nadolol compared to placebo, at MNI co-ordinates [26 -2 -14] (amygdala) and [32 -28 -4] (thalamus, hippocampus). Figure 1 shows the group paired t-test ($n = 17$) showing increased activation under nadolol compared to placebo in response to (unpleasant > neutral) stimuli. Region of interest analysis was performed using anatomically defined ROIs for the amygdala from the WFU-Pick Atlas toolbox. Group measures of BOLD signal intensity response to unpleasant stimuli in both the left and right amygdala increased significantly with the administration of nadolol compared to placebo, Figure 2. There was no significant difference in resting ASL signal in the amygdala (Figure 3).

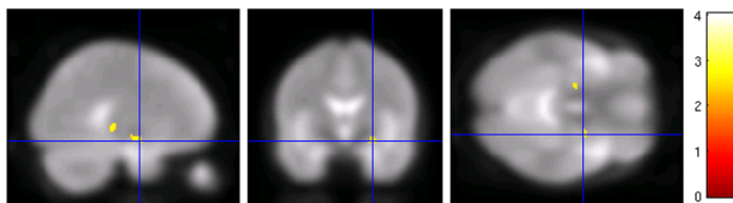


Figure 1: Region of greater activation under nadolol compared to placebo for (unpleasant > neutral). $T_{max}=2.91$, $z_{max}=2.89$, $p < 0.01$, cluster threshold = 20 voxels. Colour bar shows values of T statistic.

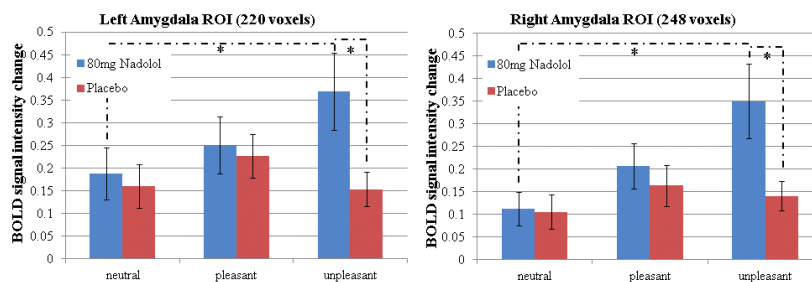


Figure 2: Group mean and standard error BOLD intensity changes in anatomical ROIs in the amygdala in response to stimuli of each valence, under each drug condition. Asterisks represent significance $p < 0.04$ using paired t test.

Discussion and Conclusions: Nadolol, as expected due to its peripheral beta-receptor antagonistic property, was found to reduce sympathetic activity (heart rate and BP). In line with its hypothesised reduced bodily arousal, we found reduced anterior insula responsiveness to emotional versus neutral visual stimuli. Interestingly, we observed an increased responsiveness selectively to unpleasant stimuli within the right and left amygdala. This finding contradicts previous studies using centrally acting beta-blockers that reported reduced amygdala response to verbal emotional stimuli (3). We suggest that impaired amygdala response in those studies was mediated by a central beta-adrenergic mechanism rather than a consequence of reduced bodily arousal. On the other hand, the increased responsiveness of the amygdala after peripheral beta-blockade is difficult to explain. One may speculate that the opposite modulation by nadolol compared to propranolol is due to an imbalance of central and peripheral beta-adrenergic arousal resulting in some form of 'sensitisation' of central beta-adrenergic amygdala arousal. The combined use of PCASL and BOLD furthermore allowed us to exclude a baseline perfusion effect that may have confounded the BOLD response. We have yet to analyse the effect of nadolol on subjective arousal ratings and objective physiological (heart rate) measures in response to the emotional stimuli.

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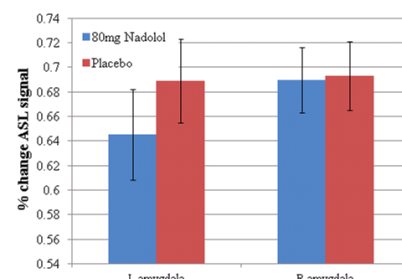


Figure 3: ROI analysis of resting perfusion: mean \pm st. err. % change ASL signal in anatomically defined amygdala ROIs.