Imaging Model of Antidepressant Effects: a Pre-clinical Investigation using Pharmacological Magnetic Resonance Imaging

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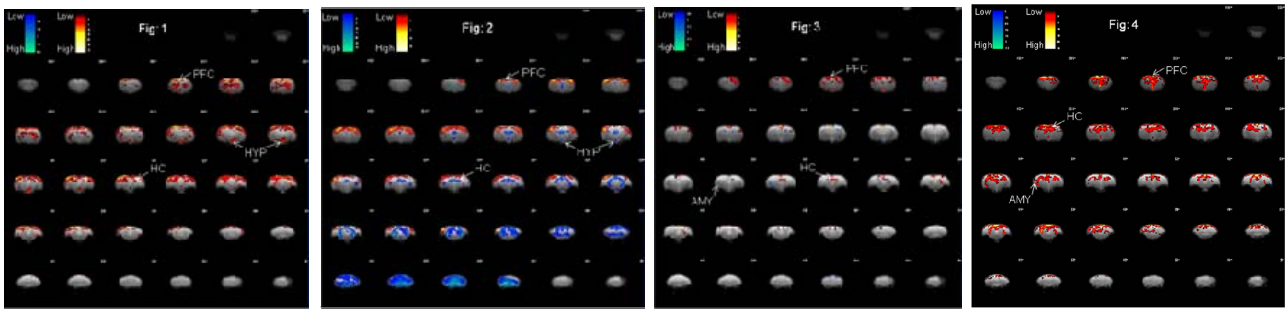
INTRODUCTION: Pharmacological magnetic resonance imaging (phMRI) allows examination of the central effects of systemically administered drugs based on the coupling between hemodynamic parameters & the neural activity, resulting in localized change in the oxygenated blood perfusion in the capillaries & the subsequent production of localized blood oxygenation level depended (BOLD) contrast in the image. Dysfunction of brain monoamine systems are implicated in various psychiatric disorders, including major depression & anxiety. Antidepressants inhibiting the reuptake of monoamines (serotonin (5-HT), noradrenalin (NE) & dopamine (DA)) in the CNS are widely accepted to be effective in treatment of such dysfunction; however the exact neuropsychological mechanisms of action of many of these compounds, resolving the depressive symptomatology still remains to be elucidated. Thus the study of adaptive changes in the system & its receptor site, aids in understanding the mechanism of action of antidepressant compunds which is important in improving the therapeutic efficacy of the compounds. Our present study aims to use phMRI in rats to investigate the regional brain response to antidepressants: citalopram (selective serotonin reuptake inhibitor -SSRI), reboxetine (selective noradrenaline reuptake inhibitor - sNRI) & bupropion (a non-5-HT antidepressant, with potent effects on NE &/or DA) in naïve, acute & chronically treated subjects, in order to characterize the neuronal mechanisms that underlie their clinical effects. Moreover to pharmacologically characterize the specific involvement of 5-HT_{1A} receptors, a highly selective 5-HT_{1A} receptor antagonist WAY 100635 has been co-administered with citalopram to examine if this combination would alter the progression of any dynamic changes observed following chronic citalopram exposure.

EXPERIMENTAL PROTOCOL & DATA ANALYSIS: The brain phMRI response to antidepressant was measured in thirteen experimental groups of male Lister Hooded rats (6 per treatment group). Chronic treatment groups received 2 weeks pre-treatment with antidepressants {citalopram (20mg/kg, i.p.), reboxetine (30mg/kg, i.p.), bupropion (30mg/kg, i.p.) or saline (i.p.), before the day of scanning; while in-magnet they had an acute i.p. injection, of the same study compound. Acute treatment groups were tested in 2 doses; while in-magnet, subjects received acute citalopram (10 or 20mg/kg, i.p), reboxetine (10 or 30mg/kg, i.p.), bupropion (15 or 30mg/kg, i.p.) or saline (i.p.) injection. Acute antagonist + antidepressant test groups received WAY 100635 (0.3 mg/kg s.c.) + citalopram (20 mg/kg, i.p., ~5 minutes following WAY 100635) or WAY 100635 (0.3 mg/kg s.c.) injection. Subjects were catheterized with i.p. &/or s.c. lines (for injection) & were scanned in a 7T MRI (Bruker) scanner (Gradient Echo (GE); TR: 807ms; TE: 17ms; FOV: 25x25mm; Matrix size: 64x64; Slice thickness; 0.5mm; 32 slices). The acquisition lasted ~ 2 hours, under isoflurane anesthesia & continuous physiological monitoring. A total of 145 functional scans were acquired (25 baseline scans, followed by drug injection & continuous scanning). Images were registered (realigned & spatially normalized) to a chosen template using SPM99. A vascular mask, derived by applying a coefficient of variance threshold of 15% was used to suppress signal changes associated with macroscopic vessels [1]. Brain masks, were subsequently applied to each subjects' time series to obtain the intra-cerebral structures; which were Gaussian smoothed to impose a normal distribution. Parametric maps of stastical significance were obtained using fixed effects general linear model, using SPM99. The condition tested was a simple comparison between pre & post test injection. Global muscle signal intensities were determined for each subject by determining a threshold as 1 S.D. below the mean of an arbitrary area of the muscle & they were used as covariates [2]. Subject movement was considered by providing the realignment parameters as nuisance variable. Group statistical maps were overlaid on anatomical structural images & corrected for multiple comparisons with a threshold of $p < 0.05$.

Figures represent the group stastical parametric map (SPM) t -distribution of BOLD changes (threshold set at p<0.05, corrected for multiple comparisons) overlaid on anatomical image



Analysis Model: Multi-group analysis (chronic saline treated control group vs. antidepressant treated group) looked for post injection increase and decrease in BOLD in chronic antidepressant [citalopram (**Fig 1**); reboxetine (**Fig 3**), bupropion (**Fig 4**)] treated group. **Fig 2:** WAY 100635 controls vs. (WAY 100635 + citalopram combination) looked for post-injection BOLD changes in (WAY 100635 + citalopram) treated group.

RESULTS: The BOLD activation following acute citalopram dosage's produced a dose dependent wide spread activation throughout the brain. Chronic treatment with citalopram for a period of 14 days produced highly significant positive changes in phMRI BOLD contrast, localized to specific brain regions: hypothalamus (HYP), hippocampus (HC) & cortex (PFC) [Fig 1] integral in 5-HT neurotransmission. Citalopram in combination with the 5-HT_{1A} antagonist, WAY 100635 produced increased BOLD activation in the cortical regions & a significant decreased BOLD contrast in the HC, AMY & more prominently in hindbrain structures [Fig 2]. The acute reboxetine (10 & 30mg/kg i.p.) challenge in the lower dose produced positive BOLD activation specifically in the HYP, whereas the larger dose produced activations in the HYP, anterior HC & PFC. Chronic reboxetine (30mg/kg i.p.) treatment increased BOLD activation in posterior HC & PFC, while no activation was observed in the HYP & a significant decrease was apparent in the amygdala (AMY) [Fig 3]. In contrast, acute bupropion (15 & 30mg/kg i.p.) challenge in both doses produced no significant activation in the regions of interests. However, chronic bupropion treatment (30mg/kg i.p.) produced robust increases in BOLD activations in the regions of HC, AMY & PFC [Fig 4]. Observed regional chronic effects were also apparent when modeled vs. corresponding acute group (instead of control group).

DISCUSSION & CONCLUSION: The main finding to emerge from this study is the identification of the regions implicated in the mechanism of action of antidepressants: citalopram, reboxetine & bupropion, and the non-invasive utility of phMRI technique in investigation of monoamine (5-HT, NE & DA) mechanisms in depression & its treatment. Regional adaptations observed following chronic administration of the antidepressants potentially suggests the gradual desensitization of neurotransmitter receptors (SSRI's - 5-HT1A receptors [3]; sNRI's - α2-adrenoceptors - sNRI's [4]). Our observations are consistent with the central effects of SSRI's: citalopram [5, 6], fluoxetine or paroxetine [7] in hippocampus, cortical & thalamic areas; sNRI: reboxetine [8] and bupropion [9] in the regions of cortex & amygdala of the brain observed in human fMRI studies. Similar results have also been reported in in-vitro animal studies [10] identifying dorsal hippocampus & frontal cortex integral in NE neurotransmission. Earlier imaging studies in depression have also reported altered blood flow &/or metabolism [11] and in response to antidepressant treatment [12 - 14] in the regions of PFC & AMY in clinical settings. Responses observed in the cortical regions following chronic citalopram treatment could also be emulated by acutely combining citalopram with the 5-HT1A antagonist, WAY 100635. These observations are of potential importance in understanding clinical drug efficacy & will contribute significantly in the development of more efficacious clinically active antidepressants with the potential to combine with other pharmacological agents (specific receptor antagonists) to reduce the delay in the onset of antidepressant response in clinic.

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