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An investigation of regional cerebral blood flow and tissue structure changes after acute administration of antipsychotics in healthy male volunteers

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Abstract

Chronic administration of antipsychotic drugs has been linked to structural brain changes observed in patients with schizophrenia. Recent MRI studies have shown rapid changes in regional brain volume following just a single dose of these drugs. However, it is not clear if these changes represent real volume changes or are artefacts ('apparent' volume changes) due to drug induced physiological changes, such as increased cerebral blood flow (CBF). To address this, we examined the effects of a single, clinical dose of three commonly prescribed antipsychotics on quantitative measures of T1 and regional blood flow of the healthy human brain. Males (n=42) were randomly assigned to one of two parallel groups in a double-blind, placebo-controlled, randomised, three-period cross-over study design. One group received a single oral dose of either 0.5mg, 2mg of risperidone or placebo during each visit. The other received olanzapine (7.5mg), haloperidol (3mg) or placebo. MR measures of quantitative T1, CBF, and T1-weighted images were acquired at the estimated peak plasma concentration of the drug. All three drugs caused localised increases in striatal blood flow, although drug and region specific effects were also apparent. In contrast, all assessments of T1 and brain volume remained stable across sessions, even in those areas experiencing large changes in CBF. This illustrates that a single clinically relevant oral dose of an antipsychotic has no detectable acute effect on T1 in healthy volunteers. We further provide a methodology for applying quantitative imaging methods to assess the acute effects of other compounds on structural MRI metrics.

1 Introduction

Although debate continues around the underlying cause and dynamics of the structural brain changes that have been associated with schizophrenia (Chan, et al., 2011; Filippi, et al., 2014; Nenadic, et al., 2015; Torres, et al., 2013; Zhang, et al., 2015), there is a consistent body of evidence that indicates antipsychotics contribute to these changes in the long term. A full review of the literature of these chronic structural changes is outside the scope of this paper, but drug subtype and dose appear to be important factors, with the earlier developed ‘typicals’, such as haloperidol, associated with greater increases in basal ganglia volumes and more pronounced cortical thinning compared to the newer ‘atypical’ medications such as risperidone and olanzapine (for recent large studies and meta-analyses see Fusar-Poli, et al. (2013); Haijma, et al. (2013); Ho, et al. (2011); (2015); Vita, et al. (2015)). Identification of the unique contribution antipsychotics make to these structural brain changes is inherently problematic to study in patient populations, where placebo control and manipulation of dose and drug type are not fully possible. However, several well controlled animal studies have indicated that chronic exposure to antipsychotics at clinically relevant doses may cause structural changes over time in the absence of disease pathology, mapped both *post-mortem* (Dorph-Petersen, et al., 2005) and through longitudinal *in-vivo* MRI assessment (Vernon, et al., 2011, 2012, 2014).

These observed changes in brain structure due to antipsychotic medication may occur on a far more rapid time scale than previously thought. Recent studies have shown dopaminergic medication causing apparent changes in grey matter (GM) volume or density in healthy humans as soon as 1-2 hours after administration (Salgado-Pineda, et al., 2006; Tost, et al., 2010), typically in areas heavily innervated by midbrain dopaminergic neurons. These data resonate with other reports of acute administration of baclofen (Franklin, et al., 2013), lithium (Cousins, et al., 2013) and even cigarette smoking (Franklin, et al., 2014) causing apparent rapid localised changes in GM volume or density. Similar reversible changes following single doses of either sodium valproate or levetiracetam have

been reported in rhesus monkeys (Tang, et al., 2015). Changes on this time scale are not limited to pharmacological interventions – other factors such as dehydration (Duning, et al., 2005; Kempton, et al., 2011), learning (Kwok, et al., 2011) and environmental enrichment (Scholz, et al., 2015) have been shown to rapidly influence *in vivo* measures of brain volume when measured using longitudinal MRI. There is also evidence that this apparent MR measured plasticity is correlated with histologically assessed structural changes (Blumenfeld-Katzir, et al., 2011; Lerch, et al., 2011; Sagi, et al., 2012).

In terms of antipsychotic induced changes, two critical questions are i) whether these changes occur *in vivo* after exposure to *clinically relevant* doses of antipsychotics in the human and ii) if so do they alter the MR signal sufficiently to then influence macro-level measurement MRI analysis techniques (Thomas and Baker, 2013). It is important to note here that MRI does not measure brain structure directly, but relies on the magnetic resonance properties of the surrounding tissue environment. These properties may be influenced by several non-structural factors which are yet to be fully explored (Weinberger and Radulescu, 2016). One such factor is potential pharmacologically induced changes in blood flow, which could exert an influence on the MR signal used to construct standard structural images, leading to an apparent acute remodelling at the macro-level. Indeed, Franklin, et al. (2013) posit that changes in blood flow induced by pharmacological agents may be ‘masquerading as volumetric changes.’ Their study reported changes in CBF, measured using arterial spin labelling (ASL), overlapping with same-direction changes in GM volume from standard T1-weighted images following acute administration of the GABA agonist, baclofen. This notion is particularly relevant in the context of volumetric changes induced by acute antipsychotic administration, as rapid alterations in blood flow produced by single doses of these drugs have consistently been reported (Fernández-Seara, et al., 2011; Handley, et al., 2013; Mehta, et al., 2003; Viviani, et al., 2013). Resolving this issue requires the assessment of the acute effect of antipsychotics on both regional blood flow and structural MR metrics in a placebo controlled setting (Hoflich, et al., 2017).

How might blood flow influence the outcome of structural MR techniques? Typical methods used to gauge structural changes *in vivo* in both human and animal subjects using MRI are morphometry based techniques such as voxel based morphometry (VBM). These analysis techniques make use of gradient recalled, high resolution anatomical images in which the signal intensity in each voxel is primarily governed by the longitudinal relaxation or spin lattice relaxation time, T1 (and to a lesser extent the inhomogeneous transverse relaxation time T2*). T1 is highly sensitive to the physical properties of the tissue surrounding the ¹H spins that generate the MR signal. In brief, the ¹H longitudinal relaxation is faster in densely packed matter (such as white matter and bone), slower in grey matter tissue, and slower still in less-restricted fluid environments (such as the ventricles). VBM relies on classification of images into tissue types based on the distribution of these T1-weighted image intensity values, as well as the *a priori* information from probabilistic tissue prior maps. Theoretically, pharmacological related blood flow changes may alter the relaxation times of ¹H spins in certain regions due to the relative change in the movement of blood in that region. Although the related increase in cerebral blood volume (CBV) itself is unlikely to be sufficient to register a change in GM at this resolution, a biophysical influence of the change in CBF could alter the MR signal to result in such apparent changes. For instance, Franklin et al. (2013) point out the similarity of the T1 relaxation time of blood and grey matter (Stanisz, et al., 2005), which may contribute to the apparent probability of a given voxel belonging to a particular tissue class, leading to an artefactual change in volumetric outcomes.

Allowing the accurate assessment of the potential influence of blood flow on the measurement of brain structure requires careful consideration of how exactly this structural information is gathered using MRI. A T1-weighted image, the standard for structural MR acquisition, is a qualitative measure heavily dependent on the TR and TE parameters of the acquisition protocol. However, recent developments in relaxometry imaging have allowed the relatively rapid acquisition of *quantitative* T1 maps, which provide a precise metric of the T1 relaxation time within each voxel. These absolute measures are more readily comparable across time points and could give a more informative

measure of the underlying structure and possible drug-dependant tissue changes than T1-weighted values can provide (Draganski and Kherif, 2013; Draganski, et al., 2014; Lorio, et al., 2016; Tardif, et al., 2016; Weiskopf, et al., 2015). Several studies have used quantitative MR techniques to attempt to assess brain microstructure changes on a short-term time scale in both humans and animals (Blumenfeld-Katzir, et al., 2011; Ding, et al., 2013; Hofstetter, et al., 2013; Sagi, et al., 2012), although the majority of these examine use- or experience-dependant neuroplasticity. By using quantitative MR methods to assess T1 values following a single dose of an antipsychotic, it would allow the clear assessment of the pharmacological affect an acute dose has on the MRI signal. To our knowledge, only Fujimoto, et al. (1987) have assessed the effect of an acutely administered antipsychotic on quantitative T1, reporting increased T1 in the striate body of dogs following a single large 20mg IV dose of haloperidol, albeit at a very low spatial resolution by current standards. Furthermore, by concurrently assessing cerebral blood flow using ASL (also a quantitative method), the impact of potential changes in blood flow on T1 can also be explored.

In this study we examine the effect of a single clinically relevant dose of three commonly prescribed antipsychotics on CBF and qualitative T1 measures of the brain using a multi-modal placebo controlled design in healthy participants. Using a parallel group design, we were able to assess the dose response effect of a single drug (risperidone) in one group, and the subtype effect between two different drugs (haloperidol and olanzapine) in the other. Following drug administration, we determined the likely contribution of regional CBF changes to the local spin lattice relaxation time, T1, by quantitatively measuring the T1 of each voxel using 'Driven equilibrium single pulse observation of T1' (DESPOT). We also determined regional CBF using 3D pseudo-continuous Arterial Spin Labelling, which has been shown to be sensitive to the effects of a single dose of pharmacological agents (Zelaya, et al., 2015). For completeness, we further carried out a range of automated morphometric analyses on standard T1-weighted images to allow a more direct comparison with the majority of published literature on acute structural changes following antipsychotic exposure.

In addition to unbiased whole-brain analyses, we also analysed three *a priori*, anatomically defined bilateral striatal ROIs: the caudate, putamen and ventral striatum. These ROIs were chosen based on (i) the high density of D2 receptors in the striatum, (ii) well replicated reports of acute and chronic structural changes due to antipsychotic administration in patients, healthy volunteers and preclinical subjects in this region and (iii) previously observed changes in striatal blood flow in these regions following antipsychotic administration. Blood flow changes were hypothesised to increase in the striatum in response to antipsychotic administration. If quantitative T1 measures change and correlate with the quantitative measures of blood flow, it would suggest that the primary MRI signal used to assess brain volume is influenced by transient drug induced CBF changes. Alternatively, if T1 remains stable in the face of the expected blood flow changes, this would suggest CBF changes are not sufficient to influence structural metrics alone. This would provide some validation of the structural methods employed in studies examining the chronic effects of brain structure, as it would suggest these measures are not unduly influenced by acute blood flow changes at the point of measurement, over and above more pervasive long term effects.

2 Methods

2.1 Participants

Forty-two healthy right-handed males were pseudo-randomly assigned to one of two parallel groups in a double blind, placebo-controlled, fully counterbalanced three-period crossover study design. All subjects were scanned three times, with seven days separating each scan. Scanning was conducted at the same time of day per visit. During each visit, each volunteer received a single capsule given orally with water. In one group the capsule contained either a single dose of risperidone 0.5 mg or risperidone 2 mg or placebo (herein referred to as group RIS-H/L); while in the other group participants received either a single oral dose of olanzapine (7.5 mg) or haloperidol (3 mg) or placebo (herein referred to as OLAN/HAL). Within-group treatment order was randomised using a Williams square design. Two subjects were discounted from the HAL/OLAN group due to DESPOT

protocol unavailability, while a further subject was removed due to structural image artefacts, leaving 18 subjects in this group (age range 19 – 42 years, mean 28.8, SD +/- 6.3 years). All 21 subjects from RIS-H/L were available for full analysis (age range 19 – 41 years, mean 27.6, SD +/- 6.9 years). The study was approved by the London (Brent) Human Research Ethics committee (REC reference: 13/LO/1183).

Inclusion criteria required normal ECG, standard laboratory blood screens and urinalysis, and alcohol consumption within the recommended guidelines at the time of the study (less than 21 units per week). Exclusion criteria included, a history of neurological or psychiatric illness, physical illness, and positive drugs of abuse or alcohol breath test on the screening or study days. Three volunteers from each group reported smoking one cigarette per day. However, smoking was not permitted on the study days so it is unlikely the potential acute effects reported by Franklin, et al., (2014) would be a factor here.

MRI data were acquired at the approximate point after dosing when the agents would be at their predicted peak plasma concentrations - 5 hrs post dose for olanzapine (de Greef, et al., 2011; Nyberg, et al., 1993; Tauscher, et al., 2002) and haloperidol (de Greef, et al., 2011; Midha, et al., 1989), and 2 hrs post dose for risperidone (de Greef, et al., 2011; Kodaka, et al., 2011; Nyberg, et al., 1993). Blood plasma samples were taken 90, 230 and 510 minutes post-dose for RIS-H/L, and 90, 270 and 600 minutes post-dose for HAL/OLAN to allow modelling of total drug exposure at the time of MRI acquisition. The half-life for oral risperidone, olanzapine and haloperidol is 22 hours, 33 hours and 37 hours respectively (Kudo and Ishizaki, 1999; Mauri, et al., 2014), allowing for full washout of treatment between scans.

2.2 MRI Acquisition

All scans were conducted on a GE MR750 3Tesla scanner using a 12-channel head coil. A T2-weighted image (FOV = 240mm, TR/TE = 4380/46.992ms, 320x256x156 matrix, slice thickness = 2mm), required for the preprocessing of the ASL images, was acquired during the first visit. A T1-

weighed MPRAGE scan, for use in DARTEL normalisation, (FOV = 270mm, TR/TE/TI = 7.312/3.016/400ms, 256x256x156 matrix, slice thickness = 1.2mm) was acquired on the second visit.

ASL image data was acquired using a pseudo-continuous Arterial Spin Labelling sequence (pCASL) with a multi-shot, segmented 3D stack of axial spirals (8-arms) readout with a resultant spatial resolution of 2x2x3mm. Three control-label pairs were used to derive a perfusion weighted difference image (Dai, et al., 2008). The labelling RF pulse had duration of 1.5s and a post-labelling delay of 1.5s was also used. The sequence included background suppression for optimum reduction of the static tissue signal. A proton density image was acquired in 48sec using the same acquisition parameters to compute the CBF map in standard physiological units (ml blood/100gm tissue/min). Two runs were acquired per visit.

For the creation of the quantitative T1 maps, a spoiled gradient recalled T1-weighted image (SPGR; FOV = 220mm, TR/TE = 8.1/3.7ms, 220x220x172 matrix, slice thickness = 1mm) at two flip angles (4° & 18°) and an inversion recovery (IR) SPGR image (FOV = 220mm, TR/TE/TI = 8.1/3.7/450ms, 220x110x86 matrix, slice thickness = 2mm) were acquired at each visit. The 4° flip angle SPGR image from this scanning protocol was also utilised for the automated morphometric analysis (see Supplementary Materials).

2.3 Preprocessing and ROI definition

All preprocessing and analysis of imaging data was conducted in SPM8 (Functional imaging Laboratory, UCL, London, UK), running on Matlab v7.12.0.635 (MathWorks, Natick, MA) unless stated otherwise.

Probabilistic bilateral putamen and caudate ROIs were defined from the FSL Harvard-Oxford subcortical atlas, thresholded at 0.20 and binarised using the *fslmaths* tool as implemented in *fslutils* (Jenkinson, et al., 2012). A bilateral ventral striatum ROI was also defined as described in Montgomery, et al. (2006), based on previous work by Mawlawi, et al. (2001). As CSF produces

extremely high values on T1 maps, ROIs were also combined with the SPM probabilistic grey matter mask (thresholded at 0.20) to ensure any areas extending into non-grey matter areas such as CSF were removed.

2.4 Arterial Spin Labelling analysis

The *New Segment* tool in SPM8 (an extension of unified segmentation (Ashburner and Friston, 2005)) was used to create grey, white and CSF class images from each MP-RAGE T1-weighted image. These were used in the Diffeomorphic Anatomical Registration Through Exponentiated Lie Algebra toolbox (DARTEL; Ashburner (2007)) to create a study specific template and set of flow fields to later transform each subject's ASL data into standard space.

The T2-weighted images were also co-registered to this MP-RAGE image. Each raw Proton Density image (acquired at the end of the pCASL sequence) was then co-registered to the T2 image, as this provided superior contrast for the mutual information cost function. The parameters for this transformation were then applied to the CBF maps (as they were already in alignment with the Proton Density image) prior to their normalisation using the earlier generated DARTEL flow fields. Finally, the normalised CBF maps were smoothed using a 6mm full width at half maximum (FWHM) kernel. As increasing the number of pCASL scans per session has been previously shown to increase sensitivity to drug effects (Marquand, et al., 2012), image calculator in SPM was then used to create an average image of the two CBF maps produced per visit for each volunteer.

Global CBF values were extracted using the MarsBar toolbox (Brett, et al., 2002) and the default whole brain mask provided in SPM8, and analysed using a one-way repeated measures ANOVA for each group in SPSS. Mean CBF values for each of the *a priori* ROIs were also extracted from unsmoothed images and analysed by the same means. Absolute CBF values were used as the main metric of interest in this analysis as they provide the most meaningful comparison with T1 values from the same region. However, CBF calculated relative to whole brain CBF has been shown to be more sensitive in detecting regional differences (Aslan and Lu, 2010; Stewart, et al., 2015).

Therefore, for completeness whole brain relative ROI CBF was also examined, calculated simply as whole brain CBF minus ROI CBF, and analysed in the same manner as the absolute values in a one-way repeated measures ANOVA.

Non-parametric whole brain analysis was conducted using Threshold Free Cluster Enhancement (TFCE; (Smith and Nichols, 2009) within FSL's RANDOMISE (Winkler, et al., 2014), based on recent recommendations (Eklund, et al., 2016). 10,000 permutations were conducted for each treatment-placebo comparison in order to create a non-parametric null distribution and calculate a 5% significance threshold, familywise error corrected. Exchangeability blocks were specified to ensure permutations would only occur within subject, to take account of the repeated measures nature of the data. Data were modelled using a General Linear Model (GLM) with global CBF values added as a covariate, in order to account for inter-individual differences in global perfusion (Handley, et al., 2013). Paired t-tests were conducted between each drug and placebo condition to assess treatment effects on perfusion.

2.5 Quantitative T1 map analysis

Driven Equilibrium Single-Pulse Observation of T1 (DESPOT1; Deoni (2007)) was used to create voxel-wise quantitative T1 maps for each subject, in all scanning sessions. Briefly, this involved resetting the origin of the IR image and the SPGR image at both flip angles to the anterior commissure and reorienting the images to the AC-PC line, and then registering and resampling all images within subject to the SPGR acquired on the second visit. The DESPOT1HIFI protocol (described in detail in Deoni (2007)) was then used to create a T1 map for each visit. An example T1 map compared with a T1-weighted image is illustrated in Figure 1.

A ROI approach was primarily conducted to assess T1 times in the a priori regions in the striatum, in addition to an exploratory voxel-wise whole brain approach. For whole brain analyses a DARTEL template was created for each group using the 4-degree flip angle SPRG images from each participant's second visit. The subsequent flow fields were applied to the native space T1 maps to

warp them into standard space before smoothing with a 6mm FWHM kernel for the voxel-wise analysis, allowing for assessment of T1 values throughout the whole brain. For the ROI analysis, the same flow fields were utilised to inverse-warp standard space ROIs into each individual's native space using nearest neighbour interpolation, producing a set of ROIs for each individual T1 map. These native space ROIs were eroded with a 3x3x1 kernel in FSL to ensure the warping procedure did not extend any of the ROIs into CSF. This allowed more precise assessment of *a priori* regions while controlling for any errors related to the normalisation or smoothing steps of the T1 maps (Aribisala, et al., 2011). After extraction, mean T1 from each ROI was entered into a two way repeated measures ANOVA in SPSS (within subject factors: drug and ROI) for each group. For completeness the same analysis was conducted on ROI values extracted from the normalised T1 maps using the standard space ROIs described above to rule out any potential issues related to the ROI warping procedure.

2.6 T1-weighted analysis

Although drug influence on quantitative T1 is the primary outcome for this study, the T1-weighted images were also assessed using VBM, the most common automated approach for analysing structural change in MRI, to allow direct comparison with much of the existing literature. However, there are several other approaches used for assessing structural changes in this modality. Given the scarcity of studies concerning volumetric changes on this time scale, three additional exploratory methods were employed to fully elucidate any *apparent* structural changes due to drug exposure and account for any differences in analysis methodology: 1) Longitudinal Registration within SPM12 (this feature being unavailable in SPM8); 2) Structural Image Evaluation, using Normalization, of Atrophy (SIENA) within FSL; 3) Longitudinal stream analysis for cortical thickness and subcortical volume within Freesurfer. The processing steps for each of these methods, and their relative strengths and weaknesses for the addressing the hypotheses presented here, are discussed in full within supplementary materials.

3 Results

All data are mean +/- standard deviation, unless otherwise stated.

3.1 Plasma levels

Total drug exposure calculated using the trapezoid method as AUC from administration to 4.5 hours (30 minutes before start of the scan) for haloperidol and olanzapine, and from administration to 3.8 hours (end of the scan) for risperidone were as follows: Haloperidol 1530 +/-720 (pg*hr)/ml; Olanzapine 16.8 +/-6.83 (hr*ng)/ml; Risperidone 0.5mg 13.8 +/- 4.53 (ng*hr)/ml; Risperidone 2mg 54.0 +/-17.6 (ng*hr)/ml.

3.2 Cerebral Blood Flow

3.2.1 Global blood flow

Group average global mean CBF values (ml/100g/min) for the RIS-H/L group were: Risperidone 2mg 47.98 +/- 10.37, Risperidone 0.5mg 49.52 +/- 8.71, placebo 49.21 +/- 8.90; and for the HAL/OLAN group: Olanzapine 7.5mg 43.10 +/- 7.76, Haloperidol 45.3 +/- 7.93, placebo 46.29 +/- 7.3.

A one-way repeated measure ANOVA revealed there were no global differences in CBF in the RIS-H/L group ($F(2,40) = 1.380$ $p < .263$), but a borderline significant difference was observed in the OLAN/HAL group ($F(2,34) = 3.395$ $p < .045$). Pairwise comparisons with Bonferroni adjustment revealed the largest reduction in CBF was after olanzapine compared to placebo, a non-significant reduction of 3.1 (95% CI, 0.36 to -6.43) ml/100g/min, $p = .056$.

3.2.2 ROI analysis

Absolute CBF values (figures 2 & 3, left) sampled from the *a priori* ROIs were entered into a two-way repeated measures ANOVA (within subject factors: drug and ROI) which revealed a significant main effect of drug on CBF in both the RIS-H/L group ($F(2,40) = 6.476$, $p = 0.004$) and in the OLAN/HAL group ($F(2,34) = 6.838$, $p = 0.003$). Pairwise comparisons revealed a significant increase in CBF compared to placebo after 2mg risperidone (4.545 ml/100g/min (95% CI 1.117 to 7.973) $p = 0.07$),

0.5mg risperidone (3.243 ml/100g/min (95% CI 0.553 to 5.933) $p = 0.015$), and haloperidol (4.125 ml/100g/min (95% CI 0.926 to 7.324) $p = 0.01$). There was no significant pairwise difference between olanzapine and placebo. However, when ROI values for olanzapine were analysed relative to whole brain values, a significant increase did emerge compared to placebo (2.755ml/100g/min (95% CI 0.788 to 4.722) $p = 0.005$). Whole brain relative ROI blood flow after risperidone and haloperidol also produced similar significant increases in the ROIs to that of the absolute values.

3.2.3 Whole brain analysis

Both haloperidol and risperidone produced significant increases in striatal blood flow (figure 4, top and middle panel). Haloperidol produced greater perfusion than placebo in two large bilateral clusters encompassing the right and left putamen, while 2mg risperidone produced a very large continuous cluster with a peak centred around the left caudate but extending into bilateral caudate, putamen and anterior cingulate. 0.5mg risperidone produced a similar but less pronounced pattern to 2mg, and was limited to left and right caudate and putamen. 2mg risperidone also produced large reductions in blood flow within the cerebellum (figure 4, bottom panel) – these were not found after the 0.5mg dose.

Non-parametric whole brain analysis did not reveal any significant changes in blood flow with Olanzapine compared to placebo.

3.3 Quantitative T1 analysis

There was no significant effect of drug on T1 in either group, for ROIs extracted both from native space T1 maps (RIS-H/L: $F(2,40) = 0.383$, $p=.684$; OLAN-HAL: $F(2,34) = 0.253$, $p=.778$) and normalised maps (see figures 2 & 3, right; RIS-H/L: $F(2,40) = 0.407$, $p=.668$; OLAN-HAL: $F(2,34) = 0.253$, $p=.779$).

Voxel-wise analysis of normalised T1 maps also failed to reveal any significant changes, which remained the case with an exploratory height threshold of 0.001 uncorrected (cluster threshold 50).

3.4 T1-weighted image analysis

None of the automated morphometric techniques applied to the T1-weighted images detected an effect of drug on brain structure. Full results of these analyses can be found in Supplementary Materials.

4 Discussion

4.1 Brief

In agreement with our hypothesis and the results of previous studies, acute antipsychotic administration induced pronounced dose and drug dependent changes in cerebral blood flow across the three antipsychotics tested herein. However, following a thorough examination of structural metrics, we report that in healthy individuals, no significant changes were observed in quantitative T1 relaxometry in the face of these significant blood flow changes relative to placebo. Additionally, extensive exploration of T1-weighted images using several volumetric analysis techniques also showed no volumetric changes in response to the doses administered.

4.2 CBF

The rapid increases in blood flow observed following acute antipsychotic administration replicate earlier findings in healthy humans using ASL (Handley, et al., 2013). Using PET with ^{15}O to measure CBF, Lahti, et al. (2005) also reported similar striatal increases in schizophrenic patients following a 10mg dose of haloperidol. However, a 15mg dose of olanzapine also produced increases in the ventral striatum and decreases in the thalamus, which was not replicated in the whole brain results here. This may be reflective of the differences in the dose and cohort used between Lahti, et al. (2005) and the current study. Furthermore, PET is typically corrected by the global signal, and the ROI results here when accounting for whole brain blood flow did in fact reveal an olanzapine increase in the striatal ROIs.

Increased postsynaptic metabolism in striatal areas due to the large density of D2 receptors is a possible interpretation of the CBF changes observed here (Goozée, et al., 2014), with blockade of D2 receptors in the striatum potentially resulting in disinhibition of D2 receptor containing medium spiny neurons (Fernández-Seara, et al., 2011). However, CBF may not be solely influenced by neuronal activity. Astrocyte signalling is heavily implicated (Attwell, et al., 2010) and pharmacological manipulation of these cells may also influence blood flow. For instance, D3 receptors – of the same family of receptors as D2 – are present on astroglial cells and are positioned to mediate regional blood flow, with D3 agonists having been previously shown to cause vasoconstriction (Choi, et al., 2006). The antipsychotics used in this study also exhibit affinity for D3 receptors (Girgis, et al., 2015; Stahl, 2008), so it follows that antagonism of these receptors may contribute to the observed increase in CBF through vasodilation.

In all, it appears likely that both neuronal and glial receptor expression and the differing receptor profiles of the antipsychotics in question can give rise to the CBF increases observed. The comparatively less pronounced CBF change in striatal areas produced by olanzapine could be due to its receptor profile, as it displays less affinity for D2 receptors than haloperidol and risperidone, while exhibiting a higher affinity for histaminergic, cholinergic and 5-HT_{2A} receptors. Nevertheless, the distinctive blood flow profile elicited by these three different drugs highlights the fact that broad categorisation of these drugs into either typical/first generation or atypical/second generation classes does not take into account the precise differences in receptor profiles within these groups. These profiles should prove to be more informative in understanding their physiological and therapeutic impact than the typical/atypical nomenclature.

The decreases in CBF observed in the cerebellum following risperidone are in line with our previous observation (Shcherbinin, et al., 2015). These effects of risperidone differ from the effects of aripiprazole, a partial D2 agonist that increased CBF in the cerebellum (Handley, et al., 2013). It is noteworthy that the basal ganglia and cerebellum are more heavily integrated than previously

thought, both anatomically (Bostan, et al., 2010; Hoshi, et al., 2005) and functionally (Neychev, et al., 2008) with Dasgupta, et al. (2014) proposing their interactions are modulated by striatal dopamine release. However, such an account does not easily accommodate that cerebellar changes were only observed after risperidone, and even consideration of the involvement of other systems such as serotonin (Schweighofer, et al., 2004) does not predict that cerebellar effects would be limited to this drug. Understanding the precise mechanism behind this change and its associated impact on brain function, therapeutic or otherwise, would require concurrent measures of function, or confirmation in a patient group.

4.3 T1

In addition to the pronounced relative CBF changes observed in the unbiased whole brain analysis, risperidone and haloperidol both produced significant absolute increases in CBF in all *a priori* striatal regions, the largest being a 9.5% increase in absolute CBF for the ventral striatum following 2mg of risperidone. Olanzapine also produced significant CBF changes in these ROIs, relative to whole brain CBF. Within these same regions we acquired quantitative measures of T1, an absolute metric of the MR signal that standard volumetric analyses are based upon, and were unable to find a significant change following drug exposure. This suggests that the blood flow changes produced by clinical doses of antipsychotics do not measurably alter T1 at this resolution.

Furthermore, none of the automated volumetric T1-weighted analyses employed returned any significant changes; either using (when possible) whole brain or ROI approaches, in either standard or native space (see Supplementary Material). Given a variety of automated techniques were employed, each with their own application of registration, segmentation, modulation and statistical analysis methods, it is also unlikely the absence of any detectable changes is due to an idiosyncrasy of a methodological approach.

What are the likely causes of our apparent discrepancy with the results of other investigations? The two studies that previously reported acute changes in T1 values or GM volume in response to

antipsychotic exposure (Fujimoto, et al. (1987) and Tost, et al. (2010) respectively) both used large doses of haloperidol administered intravenously, as compared to the clinically relevant oral doses used in the current study. An intravenously administered dose produces large and almost immediate increases in drug plasma levels compared to that achieved by oral dosing, which takes several hours to reach peak concentration in the blood and is dependent on factors such as absorption rate and first pass metabolism. Consequently, oral exposure is considerably more gradual, making direct comparison between the two methodologies problematic – for instance, the physiological impact of sudden and extreme exposure to an antipsychotic compound could include factors such as highly pronounced off-target effects. High occupancy levels of serotonergic, histaminergic or adrenergic systems could potentially produce changes, transient or otherwise, to the biophysical environment which could be sufficient to influence the MR parameters underlying structural measurement, with or without a ‘real’ structural change. Indeed, Fujimoto, et al. (1987) concluded that the increase in the T1 values in the striate body of dogs 30 minutes after IV administration of haloperidol was due to the functional effects of haloperidol rather than any morphological change. However, without a clearer understanding of the physiological processes occurring after a dose of this extremity, the underlying cause of the related T1 change remains unspecified - although the clinical relevance of the impact of such doses in respect of understanding the contribution of antipsychotic doses to longer term structural changes is unclear.

It has been argued (Franklin, et al., 2013) that blood flow changes could influence accurate MR assessment of brain structure. The similarity of the T1 relaxation times of blood and grey matter (T1 of grey matter at 1820 +/-114, and blood at 1932 +/- 85 at 3T; Stanisiz, et al. (2005)) suggests the potential to influence structural metrics. However, it should be noted that to a simple approximation, the apparent T1 of each *tissue* voxel may be viewed as a weighted sum of the individual contributions of the T1 of tissue and the T1 of the capillary blood compartments. Thus, the longitudinal magnetization recovery $M_z(t)$ of the signal in each voxel, in an SPGR scan such as the one used herein, will be given by

$$M_z(t) = M_0 \sum_{i,j} (1 - 2A_{i,j} e^{-t/T_{i,j}})$$

where M_0 is the equilibrium magnetisation, $A_{i,j}$ are the relative contributions of each domain (tissue and capillary respectively) to the spin density; and $T_{i,j}$ represents the individual T1 relaxation times of ^1H spins in each compartment. Therefore, the most likely explanation for the absence of significant changes in T1 (in our study), is that the blood capillary domain contributes to the whole T1 weighted signal, with a maximum of its overall ^1H density. Since this is known to be $\sim 1\%$ in human grey matter (Alsop, et al., 2014) any changes in the T1 of blood have a low likelihood of making a measurable change in the overall signal.

This of course does not rule out that structural remodelling can occur on an acute timescale, but rather that blood flow is unlikely to be driving its putative detection using MRI within the current context. Indeed, one recent study reported macro level MR assessed structural changes in response to balance training in the absence of ASL measured blood flow changes (Taubert, et al., 2016).

Several other transient processes could be responsible for the apparent acute structural change following antipsychotics observed in previous studies aside from blood flow, such as the influence of drug on cell microstructure, cell hydration, concentration of iron content or microglial activation (Cousins, et al., 2013; Salgado-Pineda, et al., 2006; Tost, et al., 2010), while numerous other biophysical factors that are known to influence T1 could be involved, such as myelination and axonal growth (Deoni, 2011). Further, while T1 is the primary contributor to contrast in T1-weighted images, the other parameters that determine MR contrast (T2, PD) were not quantitatively assessed in this study. Lorio, et al. (2016) recently reported that altering the relative contribution of $R_1(1/T_1)$, $R_2^*(1/T_2^*)$ and proton density (PD) in the creation of synthetic T1-weighted images resulted in changes in GM volume and thickness as assessed by VBM and Freesurfer, suggesting changes in these other parameters (in addition to T1) could significantly impact the structural information derived from T1-weighted images using automated methods.

Nevertheless, in the current study neither current T1 mapping techniques or T1-weighted morphometric analyses detected a change in response to clinical doses of antipsychotics. This does provide some validation for studies examining the chronic impact of clinical dosing regimens of these drugs on brain structure, as it appears acute effects of clinical doses are not capable of confounding the long-term effects assessed by current structural analysis techniques (Lovden, et al., 2013). A full understanding of the phenomena outlined above will be best achieved by future, careful studies of the effects of clinically relevant oral doses; whilst relevant interpretations from studies using large, intravenous doses are likely to remain limited.

4.4 Limitations of our study

The counterbalanced approach to treatment levels meant not all treatments followed placebo in a longitudinal fashion, and it could be argued that if there were persistent structural changes following a single dose these would remain during later scanning/treatment sessions. However, the purpose of this study was to primarily explore acute effects (which had previously shown to be reversible). As a precaution, subjects were split into groups depending on which visit they undertook their placebo scan, and separate one-way ANOVAs for each placebo ROI was conducted – the findings were not altered based on this analysis.

The ASL protocol employed in this study deviates slightly from that recommended in Alsop et al. (2014) as data collection was already in progress at the time of publication, although the parameters used remain appropriate for a healthy sample as employed herein. It should also be noted that the failure to detect a change in structure using the various methods explored above does not rule out that acute structural changes are occurring to a more finite extent than that detectable by the resolution clinical MRI currently offers. However, also worth noting is that the current sample size is superior to other studies that have reported such acute changes in structure, and includes a placebo control. Further research, potentially in larger preclinical samples with the option for histological

confirmation, will allow a more sensitive analysis of the microstructural changes that may occur after clinically-relevant doses of antipsychotic drugs.

5 Conclusions

By means of a careful and direct determination of voxel-wise values of T1 and CBF, in a within-subject, placebo controlled experiment design with healthy volunteers, we have been able to demonstrate that changes in regional blood flow as a result of acute antipsychotic administration are not likely to be the cause of the volumetric changes observed in some previous investigations. Other physiological and bio-chemical factors must be evaluated in order to gain a deeper understanding of the factors that underpin the influence of this family of compounds on brain structure.

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7 References

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8 Figure Legends

Fig 1: T1 map (left) and a T1-weighted image of a single subject in native space.

Fig 2: Absolute CBF and T1 values with SE bars in each ROI after placebo and 2mg Risperidone
(* $p < 0.05$ corrected for multiple comparisons)

Fig 3: Absolute CBF and T1 values with SE bars in each ROI after placebo and 3mg Haloperidol
(* $p < 0.05$ corrected for multiple comparisons)

Fig 4: Whole brain blood flow, 10,000 permutations, $p < 0.05$ (FWE corrected). Top panel: 2mg Risperidone>Placebo, peak -11.3, 5.6, 9; 9486 voxels. Middle panel: 3mg Haloperidol>Placebo, peaks at 30.1, -3.76, 3; 2607 voxels & -18.8, -3.76, -3; 1589 voxels. Bottom panel: Placebo>2mg Risperidone, peak at 5.64, -73.3, -21; 7800 voxels.

Table 1: TFCE whole brain analysis of CBF, 1000 permutations, $p < 0.05$ familywise error corrected.