



## King's Research Portal

DOI:

[10.1016/j.neuroimage.2018.12.028](https://doi.org/10.1016/j.neuroimage.2018.12.028)

*Document Version*

Peer reviewed version

[Link to publication record in King's Research Portal](#)

*Citation for published version (APA):*

Selvaggi, P., Hawkins, P. C. T., Dipasquale, O., Rizzo, G., Bertolino, A., Dukart, J., Sambataro, F., Pergola, G., Williams, S. C. R., Turkheimer, F., Zelaya, F., Veronese, M., & Mehta, M. A. (2019). Increased cerebral blood flow after single dose of antipsychotics in healthy volunteers depends on dopamine D2 receptor density profiles. *NeuroImage*, 188(0), 774-784. <https://doi.org/10.1016/j.neuroimage.2018.12.028>

### **Citing this paper**

Please note that where the full-text provided on King's Research Portal is the Author Accepted Manuscript or Post-Print version this may differ from the final Published version. If citing, it is advised that you check and use the publisher's definitive version for pagination, volume/issue, and date of publication details. And where the final published version is provided on the Research Portal, if citing you are again advised to check the publisher's website for any subsequent corrections.

### **General rights**

Copyright and moral rights for the publications made accessible in the Research Portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognize and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the Research Portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the Research Portal

### **Take down policy**

If you believe that this document breaches copyright please contact [librarypure@kcl.ac.uk](mailto:librarypure@kcl.ac.uk) providing details, and we will remove access to the work immediately and investigate your claim.

# Accepted Manuscript

Increased cerebral blood flow after single dose of antipsychotics in healthy volunteers depends on dopamine D2 receptor density profiles

Pierluigi Selvaggi, Peter C.T. Hawkins, Ottavia Dipasquale, Gaia Rizzo, Alessandro Bertolino, Juergen Dukart, Fabio Sambataro, Giulio Pergola, Steven C.R. Williams, Federico Turkheimer, Fernando Zelaya, Mattia Veronese, Mitul A. Mehta

PII: S1053-8119(18)32172-4

DOI: <https://doi.org/10.1016/j.neuroimage.2018.12.028>

Reference: YNIMG 15496

To appear in: *NeuroImage*

Received Date: 8 June 2018

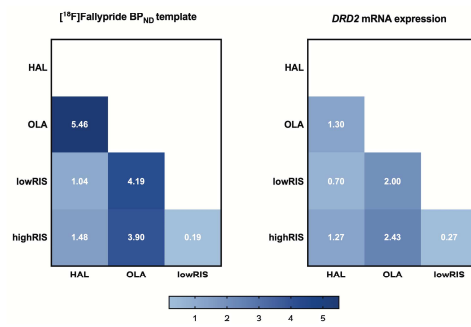
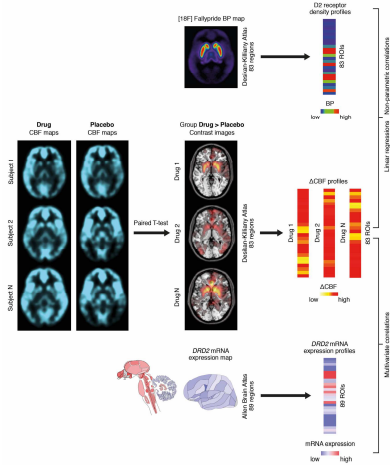
Revised Date: 5 December 2018

Accepted Date: 12 December 2018

Please cite this article as: Selvaggi, P., Hawkins, P.C.T., Dipasquale, O., Rizzo, G., Bertolino, A., Dukart, J., Sambataro, F., Pergola, G., Williams, S.C.R., Turkheimer, F., Zelaya, F., Veronese, M., Mehta, M.A., Increased cerebral blood flow after single dose of antipsychotics in healthy volunteers depends on dopamine D2 receptor density profiles, *NeuroImage* (2019), doi: <https://doi.org/10.1016/j.neuroimage.2018.12.028>.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.





**Increased Cerebral Blood Flow after single dose of antipsychotics in healthy volunteers depends on dopamine D2 receptor density profiles.**

Pierluigi Selvaggi<sup>1</sup>, Peter C.T. Hawkins<sup>1</sup>, Ottavia Dipasquale<sup>1</sup>, Gaia Rizzo<sup>2,3</sup>, Alessandro Bertolino<sup>4</sup>, Juergen Dukart<sup>5</sup>, Fabio Sambataro<sup>6</sup>, Giulio Pergola<sup>4</sup>, Steven C.R. Williams<sup>1</sup>, Federico Turkheimer<sup>1</sup>, Fernando Zelaya<sup>1</sup>, Mattia Veronese<sup>1\*</sup> and Mitul A. Mehta<sup>1\*</sup>

<sup>1</sup>Department of Neuroimaging, Institute of Psychiatry, Psychology and Neuroscience, King's College London, SE5 8AF, London, United Kingdom

<sup>2</sup>Invicro, W12 0NN, London, UK

<sup>3</sup>Division of Brain Sciences, Department of Medicine, Imperial College London, SW72AZ, London, UK

<sup>4</sup>Department of Basic Medical Science, Neuroscience and Sense Organs, University of Bari Aldo Moro, IT-70124, Bari, BA, Italy

<sup>5</sup>F. Hoffmann-La Roche, pharma Research Early Development, Roche Innovation Centre Basel, CH-4070, Basel, Switzerland

<sup>6</sup>Department of Experimental and Clinical Medical Sciences, University of Udine, IT-33100, Udine, Italy

\* Designated equal contribution as senior author.

Corresponding author:

**Pierluigi Selvaggi**

Centre for Neuroimaging Sciences  
Institute of Psychiatry, Psychology & Neuroscience  
King's College London  
De Crespigny Park; SE5 8AF; London, UK  
pierluigi.selvaggi@kcl.ac.uk

**Keywords: CBF, Antipsychotics, ASL, PET, D<sub>2</sub>R, mRNA expression, DRD2**

## Abstract

As a result of neuro-vascular coupling, the functional effects of antipsychotics in human brain have been investigated in both healthy and clinical populations using haemodynamic markers such as regional Cerebral Blood Flow (rCBF). However, the relationship between observed haemodynamic effects and the pharmacological action of these drugs has not been fully established. Here, we analysed Arterial Spin Labelling (ASL) rCBF data from a placebo-controlled study in healthy volunteers, who received a single dose of three different D<sub>2</sub> receptor (D<sub>2</sub>R) antagonists and tested the association of the main effects of the drugs on rCBF against normative population maps of D<sub>2</sub>R protein density and gene-expression data. In particular, we correlated CBF changes after antipsychotic administration with non-displaceable binding potential (BP<sub>ND</sub>) template maps of the high affinity D<sub>2</sub>-antagonist Positron Emission Tomography (PET) ligand [<sup>18</sup>F]Fallypride and with brain post-mortem microarray mRNA expression data for the *DRD2* gene from the Allen Human Brain Atlas (ABA). For all antipsychotics, rCBF changes were directly proportional to brain D<sub>2</sub>R densities and *DRD2* mRNA expression measures, although PET BP<sub>ND</sub> spatial profiles explained more variance as compared with mRNA profiles (PET R<sup>2</sup> range= 0.20-0.60, mRNA PET R<sup>2</sup> range 0.04-0.20, pairwise-comparisons all p<sub>corrected</sub><0.05). In addition, the spatial coupling between  $\Delta$ CBF and D<sub>2</sub>R profiles varied between the different antipsychotics tested, possibly reflecting differential affinities. Overall, these results indicate that the functional effects of antipsychotics as measured with rCBF are tightly correlated with the distribution of their target receptors in striatal and extra-striatal regions. Our results further demonstrate the link between neurotransmitter targets and haemodynamic changes reinforcing rCBF as a robust in-vivo marker of drug effects. This work is important in bridging the gap between pharmacokinetic and pharmacodynamics of novel and existing compounds.

## Introduction

Antipsychotics are still the preferred choice for the treatment of conditions such as schizophrenia and other mental health disorders with psychotic features (Stroup et al., 2009). The main target of most of these compounds is the dopamine D<sub>2</sub> receptor (D<sub>2</sub>R) (Burt et al., 1977; Farde et al., 1986) where they act as antagonists or partial agonists. D<sub>2</sub>R occupancy of antipsychotics has been assessed *in vivo* with emission tomography in healthy controls and clinical populations (Agid et al., 2007; Kapur et al., 1995; Nyberg et al., 1995) and it has also been linked with treatment response (Kapur et al., 2000). While antipsychotics have been well characterized in terms of their pharmacokinetics (PK) and clinical response, their impact on brain physiology and function is still not well understood. A deeper understanding of these effects is crucial to uncover biological mechanisms driving their clinical efficacy as well as their side effects.

Functional effects of antipsychotics in the brain have been investigated using different neuroimaging tools. Seminal work using Positron Emission Tomography (PET) [<sup>18</sup>F]fluorodeoxyglucose and [<sup>15</sup>O]H<sub>2</sub>O showed that drug naïve first episode psychosis patients, had increased glucose utilization after treatment with antipsychotics (DeLisi et al., 1985; Holcomb et al., 1996) and greater perfusion (Goozee et al., 2014; Miller et al., 2001; 1997) in the basal ganglia. Similar results were also obtained in healthy volunteers using a single dose of antipsychotics (Kim et al., 2013; Mehta, 2003). Studies using Arterial Spin Labelling (ASL), a Magnetic Resonance Imaging (MRI) sequence designed to quantitatively measure regional cerebral blood flow (rCBF), found results in line with the earlier PET studies. In particular, (Fernández-Seara et al., 2011) reported increased rCBF in the striatum and thalamus in healthy volunteers after a single oral dose of 10 mg of metoclopramide (a D<sub>2</sub>R antagonist) and (Handley et al., 2013) showed that both haloperidol 3 mg (a D<sub>2</sub>R

antagonist) and aripiprazole 10 mg (a D<sub>2</sub>R partial agonist with 5-HT<sub>2a</sub> antagonism properties) increased rCBF in the striatum in healthy volunteers with a larger effect size for haloperidol. Recently, we tested the effects of a single clinical effective dose of different antipsychotics (Hawkins et al., 2018). Consistent with the existing literature, 3 mg haloperidol, 2 mg risperidone and 0.5 mg risperidone increased striatal rCBF as compared with placebo.

One of the major limitations of pharmacological MRI studies stands on the haemodynamic nature of the main functional measures (e.g. BOLD and rCBF). According to the neurovascular coupling model (Attwell and Iadecola, 2002; Logothetis et al., 2001) changes in haemodynamic MRI measures reflect a complex cascade of cellular, metabolic and vascular events associated with changes in neuronal activity (Heeger and Ress, 2002; Hoge et al., 1999; Singh, 2012). In line with this model, the main effects of drug may be interpreted as the result of dose-dependent enhanced or reduced pre- or post-synaptic activity due to the action of the drug on its targets (Khalili-Mahani et al., 2017). Although many antipsychotics bind to numerous receptors, the effects on rCBF have been tacitly attributed to D<sub>2</sub>R blockade. In particular, D<sub>2</sub>R antagonism would lead to enhanced neurotransmitter turnover in the dopaminergic synapses inducing metabolic activity and therefore perfusion demands (Goozee et al., 2014; Handley et al., 2013). The findings of altered dopamine synthesis capacity after acute antipsychotics administration in human volunteers and rats support this hypothesis (Hertel et al., 1996; Ito et al., 2009; Vernaleken et al., 2008). However, since MRI does not measure neuronal activity directly, making a link between neuro-receptor binding and haemodynamic effects of these compounds requires a degree of conjecture. In addition, in the case of dopaminergic drugs, MRI hemodynamic changes have also been related to non-neuronal mechanisms including the action of D<sub>1</sub>-like receptors on vessels and D<sub>2</sub>-like receptors (mainly D<sub>3</sub>R) on perivascular astrocytes and endothelial cells

(Choi et al., 2006; Krimer et al., 1998). For these reasons, despite the body of evidence, the neurochemical mechanisms underlying CBF changes after antipsychotics administration remain unclear. The use of multimodal approaches, e.g. combining MR measures with *ex-vivo* autoradiography (Dukart et al., 2018), have started to fill this gap of knowledge.

Receptor occupancy theory posits that the magnitude of the drug response is a function of receptor availability (Clark, 1970; Ploeger et al., 2009). In other words, incremental changes in functional response correspond to increments of the fraction of receptors bound. This relationship also depends on specific characteristics of each compound, such as receptor affinity (i.e.  $K_i$ ). This basic pharmacology principle has been used to characterize the spatial profile of drug effects in the brain (also called “drug fingerprinting”) assuming that brain regions with high density of the target receptor will show higher magnitude of drug effects (Khalili-Mahani et al., 2017). This approach has been proposed to describe MRI changes to dopaminergic drugs in preclinical data (Mandeville et al., 2013). Indeed, a monotonic increase in regional Cerebral Blood Volume (rCBV) has been observed following injection of an increasing dose of the  $D_2/D_3$  antagonist radiotracer [ $^{11}C$ ]raclopride in the striatum of two male rhesus macaques (Sander et al., 2013). Interestingly, [ $^{11}C$ ]raclopride non-displaceable binding potential changes ( $BP_{ND}$ ) reflecting changes in  $D_2/D_3$  receptor occupancy, correlated with the amplitude of hemodynamic changes (i.e. rCBV): the higher the dose the larger rCBV increase; and in time:  $BP_{ND}$  variation and changes in rCBV showed similar temporal profiles. The same group also found an inverse relationship between receptor occupancy and rCBV with the selective  $D_2/D_3$  agonist quinpirole in male rhesus macaques (i.e. rCBV decrease in face of dose-dependent increase of receptor occupancy) (Sander et al., 2016). Both studies provide evidence for a neurovascular coupling mechanism linking MR haemodynamic changes and  $D_2/D_3$  receptors pharmacological modulation in non-human primates although they are limited to the striatal region.

The aim of the present work is to test in humans whether rCBF changes induced by a single dose of different antipsychotics co-varies with *in vivo* measures of D<sub>2</sub>R distribution. In particular, we employed two datasets from healthy volunteers (Hawkins et al., 2018) to evaluate the spatial correlation between rCBF variation in the placebo vs antipsychotic comparison against the population-based receptor density profiles derived from human PET scans using the high affinity D<sub>2</sub>/D<sub>3</sub> antagonist [<sup>18</sup>F]Fallypride (Mukherjee et al., 1995). We also investigated the same relationship at the gene expression level using *post-mortem* mRNA expression measures of the *DRD2* gene (the gene coding for D<sub>2</sub>R) extracted from the Human Allen Brain Atlas (ABA) (Hawrylycz et al., 2012). Brain mRNA expression variation across brain regions has been shown to be associated with resting state fMRI networks suggesting that brain hemodynamic response may be linked to the architecture of the human brain transcriptome (Hawrylycz et al., 2015; Richiardi et al., 2015). Here, brain microarray mRNA expression data was chosen as a proxy to protein-level receptor density in the human brain. In fact, while post-transcriptional events may alter the relationship between gene expression and protein synthesis (Liu et al., 2016), brain mRNA expression maps have been shown to predict *in vivo* proteins level as measured with PET (Beliveau et al., 2017; Rizzo et al., 2014).

Following the receptor occupancy theory and the neurovascular coupling model proposed by (Sander et al., 2013) we hypothesized that there will be a detectable linear relationship between main effects of antipsychotics on CBF measures and D<sub>2</sub>R receptor density profiles evaluated at the protein and gene expression level. Even though brain microarray mRNA expression data from the ABA is noisier and more discrete (i.e. limited number of samples) than the PET BP<sub>ND</sub> maps, we expect CBF increases after antipsychotics to be linearly associated also with *DRD2* mRNA expression spatial profiles. However, given the fact that mRNA expression only approximates cellular protein levels due to post-

transcriptional regulatory mechanisms we predict microarray data to explain less variance in CBF changes than PET derived maps.

ACCEPTED MANUSCRIPT

## Materials and Methods

### Participants and study design

Data were collected as part of a project approved by the National Research Ethics Service Committee London – Brent (REC reference: 13/LO/1183). Details about participants, protocol and study design have been described in detail in (Hawkins et al., 2018). Briefly, forty-two healthy male subjects were enrolled in a double-blind, placebo-controlled, randomised, fully counterbalanced, three-session crossover design. Participants were randomised into two equal parallel study groups (Group 1 age mean/SD 27.6/6.9; Group 2 age mean/SD 28.3/6.3). In the first group participants received placebo, 7.5 mg of olanzapine (OLA), or 3 mg of haloperidol (HAL) on each study day. In the second group, participants received placebo, 0.5 or 2 mg risperidone (lowRIS and highRIS respectively). All but lowRIS dosage were chosen in order to achieve on average at least 60% of D2 receptor occupancy (Kapur et al., 2000; Tauscher et al., 2004). On dosing day, participants followed a standardised regime. The MRI scan was performed at the time of predicted peak level of plasma concentration of the drug after oral administration ( $T_{max}$ ): approximately 5 hours after drug administration for Group 1 and 2 hours for Group 2 (de Greef *et al.*, 2011; Midha *et al.*, 1989; Nyberg *et al.*, 1997; Tauscher *et al.*, 2002). Study days were seven days apart to allow washout between sessions. After the final visit, a follow-up phone call was made to monitor potential adverse events related to the study drugs.

### MRI acquisition and pre-processing

All scans were conducted on a GE MR750 3 Tesla scanner using a 12-channel head coil. ASL image data were acquired using a 3D pseudo-continuous ASL sequence (3DpCASL)

with a multi-shot, segmented 3D stack of axial spirals (8-arms) readout with a resultant spatial resolution (after re-gridding and Fourier Transformation) of 2x2x3mm. Four control-label pairs were used to derive a perfusion weighted difference image. The labelling RF pulse had a duration of 1.5s and a post-labelling delay of 1.5s. The sequence included background suppression for optimum reduction of the static tissue signal. A proton density image was acquired in 48s using the same acquisition parameters in order to compute the CBF map in standard physiological units (ml blood/100g tissue/min). Pre-processing of all CBF data was performed exactly as described in (Hawkins et al., 2018) (for further details please see Supplementary Materials).

### Receptor density profiles

Figure 1 shows the general framework of the analysis. D<sub>2</sub>R profiles were extracted from an independent [<sup>18</sup>F]Fallypride PET template obtained by averaging six binding potential (BP<sub>ND</sub>) whole brain maps acquired in healthy young volunteers (age range: 18-30 years) who did not participate in the drug study (Dunn et al., 2009). [<sup>18</sup>F]Fallypride is a D<sub>2</sub>/D<sub>3</sub> receptor antagonist and it is a well-established PET radiotracer in the study of D<sub>2</sub>-like receptor distribution in the brain (Mukherjee et al., 1995). Compared with other D<sub>2</sub>-like antagonist radiotracers (e.g. [<sup>11</sup>C]raclopride), [<sup>18</sup>F]Fallypride has higher affinity and higher signal-to-noise ratios *in vivo* and therefore provides reliable quantitative measures of D<sub>2</sub>R concentration including extra-striatal brain regions (Mukherjee et al., 2002; Stark et al., 2018). The [<sup>18</sup>F]Fallypride template was segmented with the Desikan-Killiany Atlas (Desikan et al., 2006) and for each of the 85 Regions of Interest (ROIs) of the template, the voxel-wise mean BP<sub>ND</sub> value was extracted with the *fslmeants* function implemented in the Functional Software Library suite (FSL, FMRIB, Oxford, UK). Conventional parametric modelling of regional BP<sub>ND</sub> was performed by using the cerebellum as reference region (Ichise et al.,

2003). Therefore, both left and right cerebellar ROIs (namely “rh\_cerebellum\_cortex” and “lh\_cerebellum\_cortex”) were excluded from correlation analyses with CBF profiles. To compare the contribution of striatal vs extra-striatal regions to this association, we also extracted  $BP_{ND}$  profiles from a [ $^{11}C$ ]raclopride template obtained from a matched group of healthy controls (Grecchi et al., 2014) (see Supplementary Material).

### **CBF profiles**

CBF profiles were obtained from maps of CBF changes at the group level. In particular, for each antipsychotic, a group-wide paired T-test was performed in SPM12 for each drug vs placebo. Whole brain total blood flow was added as a covariate of no interest in the model to account for peripheral (global) drug effects and between-subjects variability in global brain perfusion (Handley et al., 2013; Viviani et al., 2013; 2009). For each antipsychotic the main effect of the drug was tested using group voxel-wise paired t-tests with cluster-level FWE correction ( $\alpha = 0.05$ , cluster defining threshold = 0.001) as implemented in SPM12. In agreement with previous studies (Handley et al., 2013; Hawkins et al., 2018), we identified statistically significant increases in CBF after drug administration (against placebo) and have thus focussed on this contrast in the remainder of this work. Therefore, DRUG>PLACEBO contrast maps for each antipsychotic were segmented by using the Desikan-Killiany Atlas (Desikan et al., 2006) using the same approach used for the extraction of receptor profiles. This resulted in 85  $\Delta$ CBF profiles measuring the antipsychotic induced increase of CBF in each ROI. For consistency with the receptor profile data, we carried out correlation analyses excluding the two cerebellum ROIs.

### **Statistical analysis for the $\Delta$ CBF/receptor density profiles correlations**

To test the associations between  $\Delta$ CBF profiles (derived for each antipsychotic) and  $D_2/D_3$  receptor density profiles, linear regression models as implemented in SPSS were used (IBM, SPSS Statistics, Version 23). Normal distribution of the residuals of the regression models were tested by Shapiro-Wilk test.  $BP_{ND}$  data were transformed using a natural logarithmic function ( $\ln$ ) so that the residuals conformed to a normal distribution. For all linear regression models Mahalanobis distance and Cook's distance were computed in order to explore the presence of multivariate outliers and estimate the presence of highly influential data points. To identify multivariate outliers, Mahalanobis distance values were compared to a chi-square distribution with degrees of freedom equal to the number of variables (two in this case) with  $p=0.001$  (Finch, 2012; Tabachnick and Fidell, 2013). Any data point with Cook's distance higher than 1 was considered as highly influential outlier and excluded from the analysis (Cook and Weisberg, 1982). To further control for the effect of extreme observation we also used the bias-corrected accelerated bootstrap technique as implemented in SPSS (Efron and Tibshirani, 1986) with 10,000 resamples. Non-parametric Spearman's correlations between  $\Delta$ CBF profiles and receptor  $BP_{ND}$  profiles were also performed as a countercheck. In addition, Fisher's r-to-z transformation was performed to test pairwise significance of the difference between correlation coefficients of  $\Delta$ CBF profiles between different antipsychotics. Asymptotic covariance method was adopted to account for the fact that correlations had one variable in common (Lee and Preacher, 2013)

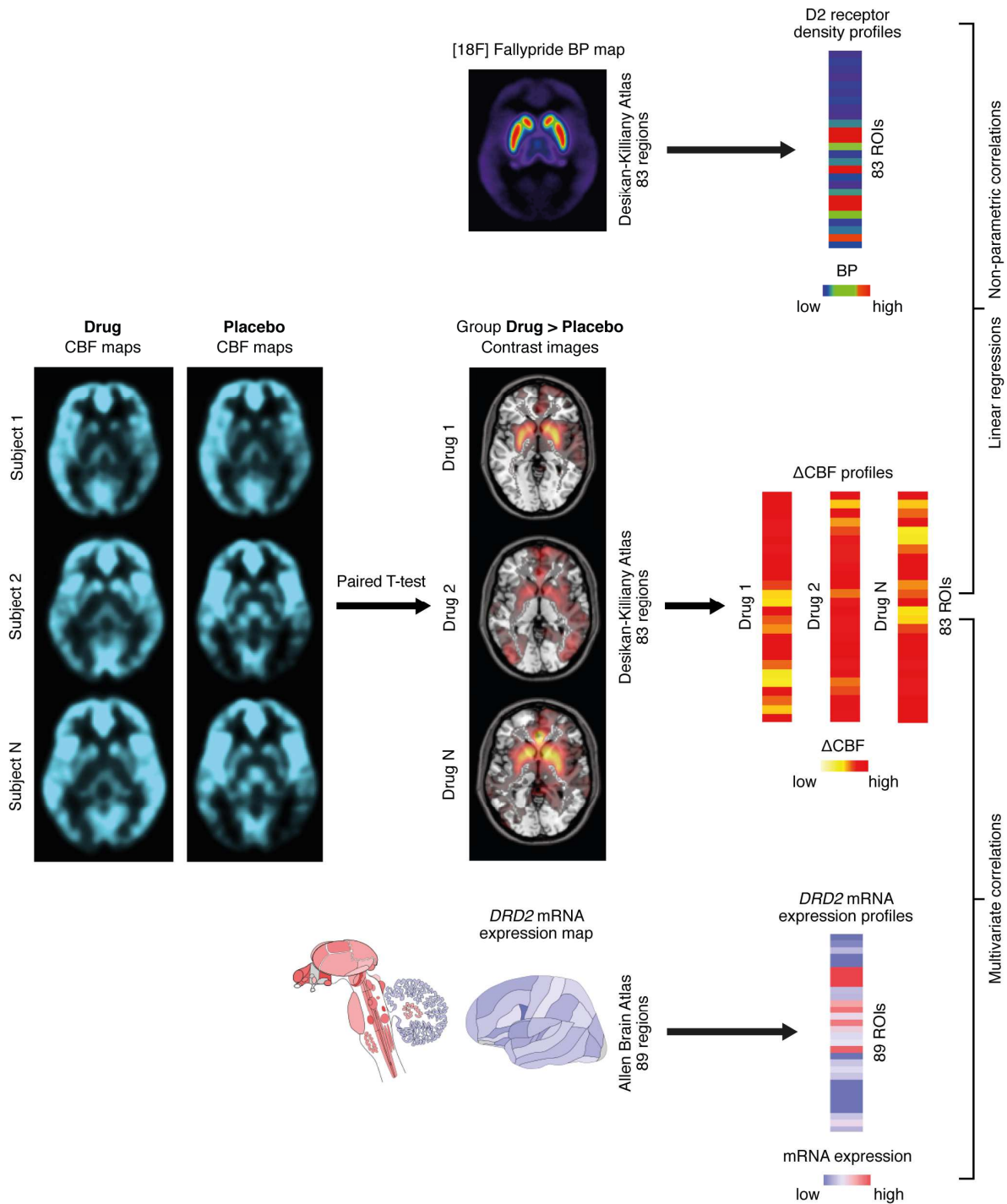
### **mRNA profiles and genetic correlations**

*DRD2* gene brain microarray mRNA expression values were extracted from ABA data (<http://human.brain-map.org>) by using the Multimodal Environment for Neuroimaging and Genomic Analysis (MENGA) toolbox (<http://www.nitrc.org/projects/menga/>) (Rizzo et al., 2016). The same toolbox was used to carry out correlations with  $\Delta$ CBF profiles of each

antipsychotic. First, antipsychotics' contrast images (DRUG>PLACEBO) were resampled in the ABA space. Then, each CBF image sample was spatially matched with the corresponding genomic ABA sample within a search window of a sphere of 5mm radius centred on the MNI coordinates of the ABA sample. Both CBF contrast image and ABA data were then segmented using the list of structures (N= 169) provided by the ABA (Hawrylycz et al., 2012). A subset of 89 structures (ROIs), each containing at least one genomic sample for all the six ABA brains (donors), was selected to perform correlations between  $\Delta$ CBF profiles and gene expression. For each donor, samples data were converted from their original log<sub>2</sub> intensity in z-scores using mean and standard deviation as normalization factor for a given subject. This was done to minimize bias related to inter-donor variability in the ABA dataset. For the *DRD2* gene a unique profile was obtained by selecting the probe which expression values were highly consistent across donors. In particular, distributions of expression values for all the probes were evaluated across donors, retaining the probe with the most symmetric and least skewed distribution. In the case of multiple samples within the same ROI, the average between samples within the ROI was calculated (Rizzo et al., 2016).

After completing the matching and the extraction of  $\Delta$ CBF and gene expression profiles, two different correlations were performed: 1) between-donors correlation or gene auto-correlation returning the biological variability of the spatial profile of mRNA expression between donors (the higher the gene autocorrelation the lower the heterogeneity in mRNA expression spatial profile between donors); 2) correlation between each gene expression and the  $\Delta$ CBF by ROIs also called cross-correlation. A Principal Component Analysis (PCA) on mRNA expression measures of the 6 ABA donors was performed beforehand in order to extract the component that accounted at least for the 95% of the total variance in the mRNA expression data. In particular, the PCA was performed on an 89x6 matrix (89 ROIs by 6 donors) and represented a consistent spatial mRNA expression profile across all donors. This component was then

used in the regression model against CBF profiles. Significance was assessed with a bootstrapping approach resulting in a chance likelihood of the correlation coefficient expressed as a %. More specifically, ROIs were permuted within donors repeating the correlation between PCA component and CBF profiles 1,000 times, in order to obtain a measure of the likelihood that the correlation found was different from chance level. For further details on ABA mRNA processing and analysis see Supplementary Material and (Rizzo et al., 2016). A multivariate spatial correlation using MENGA was also performed between *DRD2* gene expression profiles and [<sup>18</sup>F]Fallypride BP<sub>ND</sub> maps (see Supplementary Material). As for protein density profile analysis, Fisher's r-to-z transformation was performed to test pairwise significance of the difference between correlation coefficients of  $\Delta$ CBF profiles between different antipsychotics and also between PET and mRNA profiles.



**Figure 1.** General framework of the analysis. Group-level map showing main effect of drug was computed for each antipsychotic. The resulting maps and the [18F]Fallypride PET template were segmented into 83 ROIs by using the Desikan-Killiany Atlas (Desikan et al., 2006). For each ROI  $\Delta$ CBF profiles and [18F]Fallypride  $BP_{ND}$  template values were extracted and then correlated. *DRD2* gene brain microarray mRNA expression values were extracted from ABA data (<http://human.brain-map.org>) (Hawrylycz et al., 2012). MENGA software (<http://www.nitrc.org/projects/menga/>) (Rizzo et al., 2016) was used to extract *DRD2* mRNA expression profiles which then entered multivariate correlation analysis against  $\Delta$ CBF profiles (please refer to Material and methods and Supplementary Material for a more detailed description).

## Results

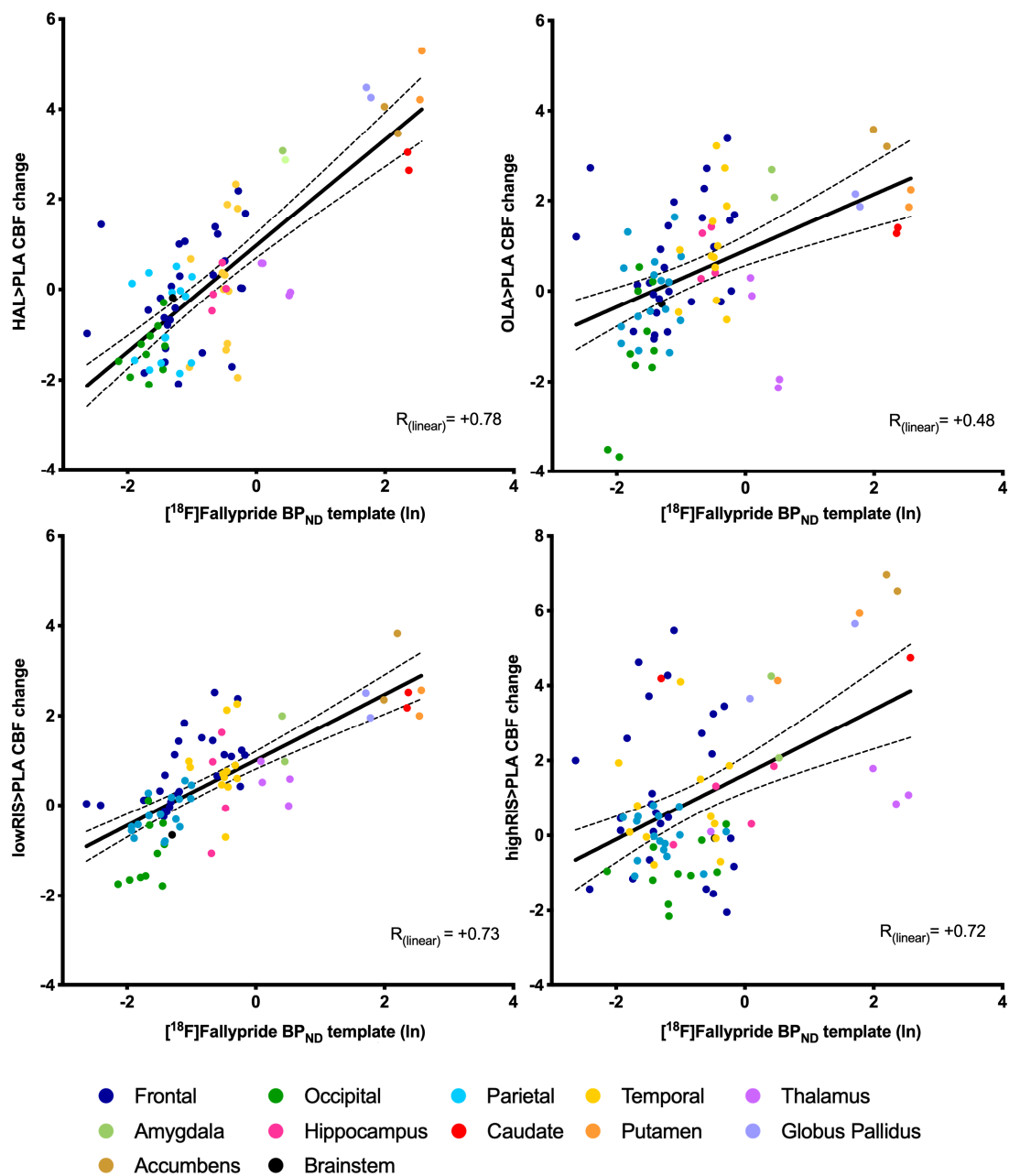
### Main effect of antipsychotic on CBF

Group paired T-tests revealed that all antipsychotics but olanzapine, produced a statistically significant increase of CBF in basal ganglia, in particular in the striatum with haloperidol causing the largest increase as compared with lowRIS and highRIS. In particular HAL>PLA t-contrast revealed a significant cluster in the left Putamen; OLA>PLA t-contrast revealed a significant cluster in the right parietal cortex and in the right parahippocampus; lowRIS>PLA t-contrast revealed a significant cluster in the left caudate; highRIS>PLA t-contrast revealed a significant cluster in the left caudate. None of the DRUG<PLA t-contrast revealed statistically significant clusters. Full statistics and voxel-wise t-maps are in Supplementary Material.

### CBF changes with D<sub>2</sub> receptor profiles correlations

All antipsychotic  $\Delta$ CBF profiles significantly correlated with [<sup>18</sup>F]Fallypride BP<sub>ND</sub> template values (Table 1 and Figure 2). HAL  $\Delta$ CBF had the strongest correlation with [<sup>18</sup>F]Fallypride BP<sub>ND</sub> template ( $R_{\text{linear}}=0.78$ ) followed by RIS ( $R_{\text{linear}}=0.73$  and  $R_{\text{linear}}=0.72$  for lowRIS and highRIS respectively) and OLA ( $R_{\text{linear}}=0.48$ ). Results from linear models and non-parametric Spearman correlations were consistent. In all linear regressions, none of the data points were identified as a highly influential outlier (all Cook's distances > 1) or multivariate outliers (all Mahalanobis distances  $p>0.01$ ). Results were retained after 10,000 resamples (all bootstrapped  $p<0.01$ ). The rank of order and  $R_{\text{linear}}$  values matched the variation in affinity with D<sub>2</sub> receptor (McCormick et al., 2010), with the stronger the association between  $\Delta$ CBF profiles and D<sub>2</sub> receptor densities the lower the  $K_i$  (Table 1 and Figure 4). In the pairwise correlation comparisons we found significant difference for HAL vs OLA ( $z=5.46$ ), lowRIS

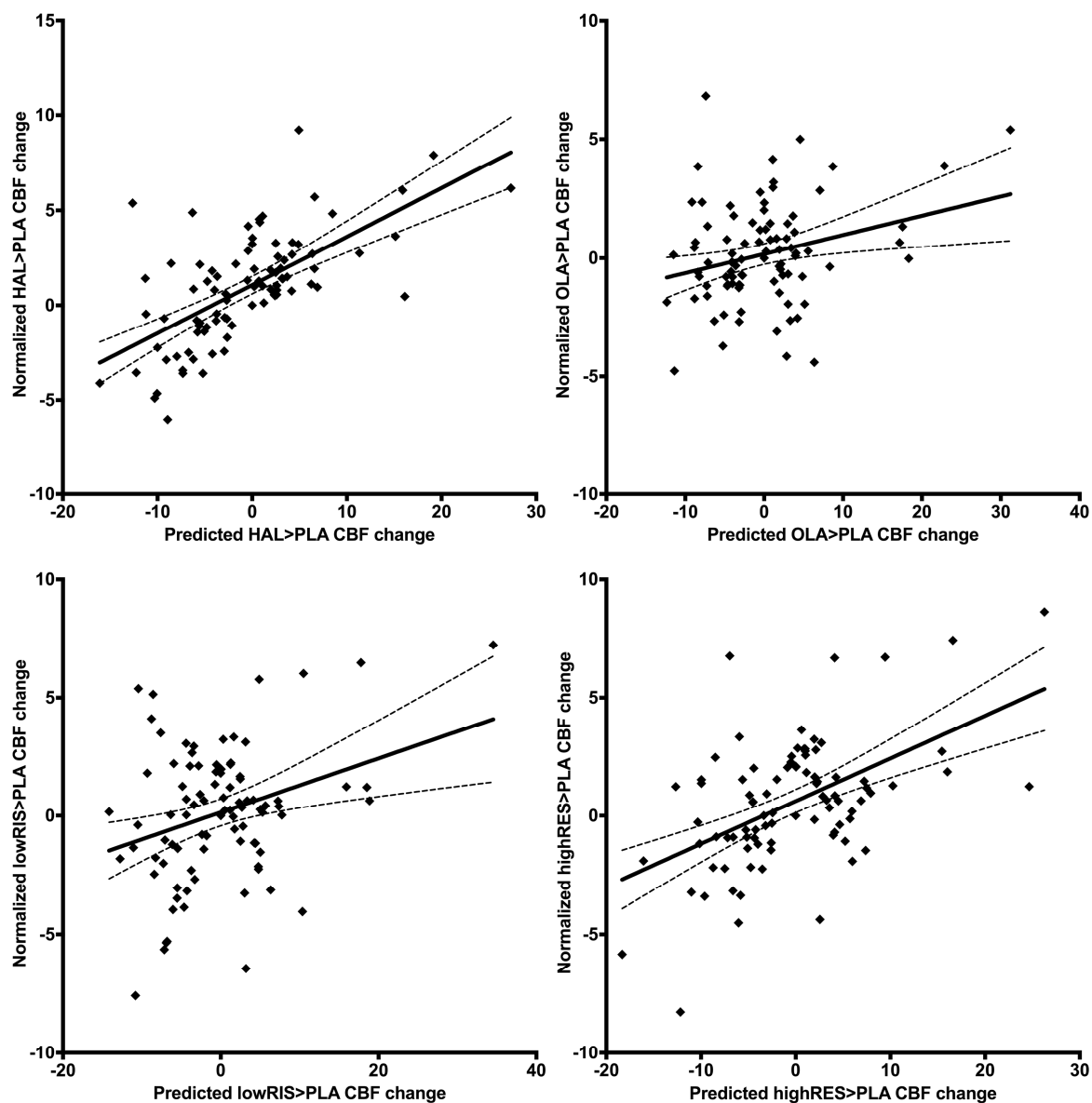
vs OLA ( $z= 4.19$ ) and vs highRIS vs OLA ( $z= 3.90$ ) (all  $p_{\text{two-tailed}} < 0.01$ , Bonferroni corrected, Figure 4). All the other comparisons were not significant. To compare striatal vs extra-striatal contributions to this association we also performed correlations between  $\Delta\text{CBF}$  and receptor density profiles by extracting  $\text{BP}_{\text{ND}}$  values from a [ $^{11}\text{C}$ ]raclopride PET template. We found weaker correlation with [ $^{11}\text{C}$ ]raclopride  $\text{BP}_{\text{ND}}$  template as compared with [ $^{18}\text{F}$ ]Fallypride  $\text{BP}_{\text{ND}}$  template (see Supplementary material).



**Figure 2.** Scatterplots of  $\Delta$ CBF/receptor density profiles correlations. Top row: scatterplot of the correlation between HAL  $\Delta$ CBF profiles and [ $^{18}$ F]Fallypride  $BP_{ND}$  template (left) and of the correlation between OLA  $\Delta$ CBF profiles and [ $^{18}$ F]Fallypride  $BP_{ND}$  template (right). Bottom row: scatterplot of the correlation between lowRIS  $\Delta$ CBF profiles and [ $^{18}$ F]Fallypride  $BP_{ND}$  template (left) and of the correlation between highRIS  $\Delta$ CBF profiles and [ $^{18}$ F]Fallypride  $BP_{ND}$  template (right). Dashed lines indicate 95% confidence bands.

### mRNA expression correlations

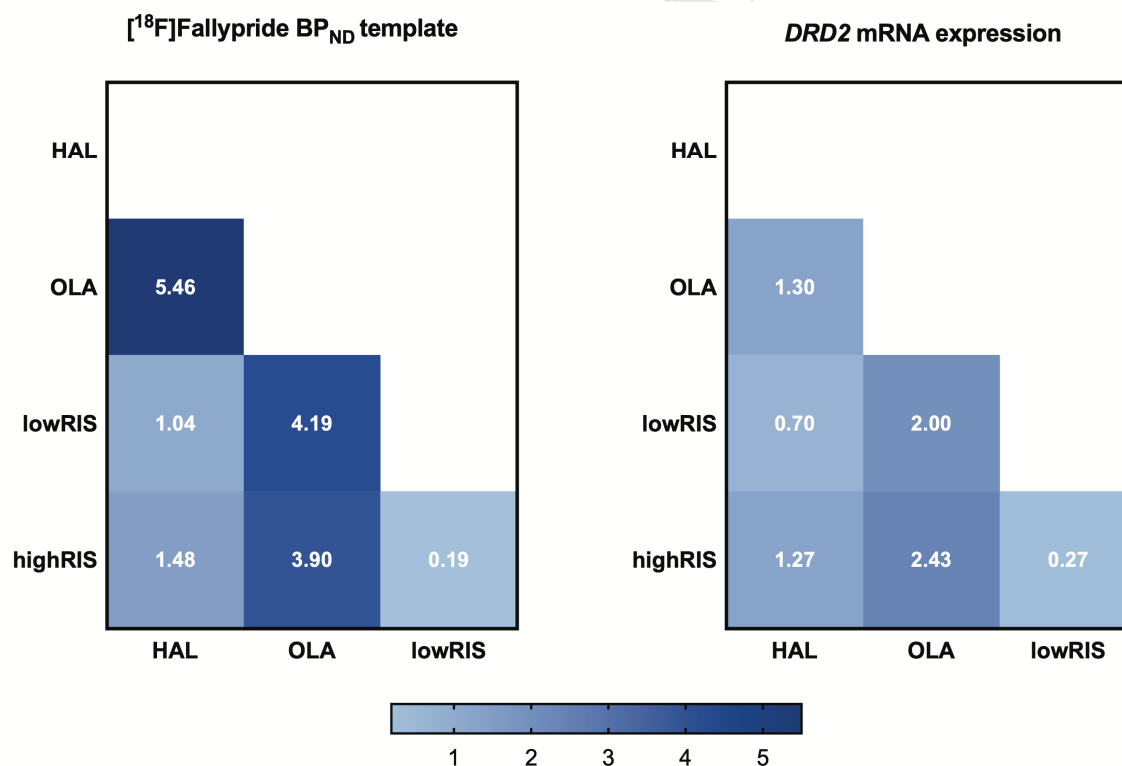
The average correlation coefficient ( $R^2$ ) of the genomic autocorrelation analysis for the *DRD2* gene was 0.575 (standard deviation= 0.058) for the six donors. This result indicated good stability between donors of *DRD2* mRNA expression spatial profile (Rizzo et al., 2016). For the different antipsychotic drugs, the correlation coefficients were all positive and statistically significant (Figure 3 and Table 1) and also significantly lower than those obtained for the PET template (PET  $R^2$  range= 0.20-0.60; mRNA PET  $R^2$  range 0.04-0.20; pairwise-comparisons all  $p < 0.05$ ). As for the correlation with [ $^{18}$ F]Fallypride values, genomic mRNA expression correlations qualitatively mirrored  $K_i$  differences (McCormick et al., 2010) between antipsychotics at  $D_2R$  (Figure 4). However, none of the pairwise comparisons between correlation coefficients between antipsychotics were statistically significant after correction for multiple comparisons.



**Figure 3.** Scatterplots of the genomic correlation. For all scatterplots on the y axis normalized DRUG>PLACEBO CBF changes and on the x axis DRUG>PLACEBO CBF changes predicted by the first Principal Component of the mRNA expression measures of the 6 ABA donors. Top right: HAL; Top left: OLA; Bottom right: lowRIS; Bottom left: highRES. Dashed lines indicate 95% confidence bands.

**Table 1.** Summary of the correlations of  $\Delta$ CBF profiles  $D_2$  receptor profiles ( $[^{18}\text{F}]$ Fallypride  $\text{BP}_{\text{ND}}$  template) and of genomic multivariate cross-correlations of antipsychotics'  $\Delta$ CBF profiles with  $\text{DRD2}$  mRNA expression profiles.

DRUG>PLA contrast	$[^{18}\text{F}]$ Fallypride $\text{BP}_{\text{ND}}$ template				$\text{DRD2}$ mRNA expression	
	$R_{\text{linear}}$	$p_{\text{linear}}$	Spearman rho	$p_{\text{Spearman}}$	R	Chance likelihood
HAL	+ 0.78	$p < 0.001$	0.61	$p < 0.001$	+ 0.34	0 %
OLA	+ 0.48	$p < 0.001$	0.51	$p < 0.001$	+ 0.21	0 %
lowRIS	+ 0.73	$p < 0.001$	0.76	$p < 0.001$	+ 0.43	0 %
highRIS	+ 0.72	$p < 0.001$	0.61	$p < 0.001$	+ 0.45	2 %



**Figure 4.** Differential strength of association between  $\Delta$ CBF and  $D_2$ R profiles. Heat maps showing z-values for the pairwise comparison of correlations. Colour bar indicates z-scores. On the left correlations with  $[^{18}\text{F}]$ Fallypride  $\text{BP}_{\text{ND}}$  template values, on the right correlations with mRNA microarray data from the ABA. HAL= haloperidol, OLA= olanzapine, lowRIS = low dose of risperidone, highRIS = high dose of risperidone. The following tests were performed HAL vs OLA, HAL vs lowRIS, HAL vs highRIS, lowRIS vs OLA, highRIS vs OLA, highRIS vs lowRIS.

## Discussion

The aim of the present study was to investigate the relationship between the effects of single clinical effective doses of antipsychotics on rCBF and receptor distribution profiles in the brain as indexed by [<sup>18</sup>F]Fallypride BP<sub>ND</sub> values extracted from a template map and brain *DRD2* mRNA expression profiles. Consistently with our hypothesis, we found that for all compounds there was a spatial coupling between drug-induced CBF changes and D<sub>2</sub> receptor density profiles (at both protein and gene expression level). In addition, we found that mRNA data explained less variance in CBF changes than PET derived map.

### Receptor density profiles

The association between CBF changes induced by all antipsychotics and receptor brain spatial distribution of D<sub>2</sub>R matches earlier evidence in non-human primates showing large CBF increases after injection of the D<sub>2</sub> antagonist PET tracer [<sup>11</sup>C]raclopride in brain regions with high D<sub>2</sub>R density (Sander et al., 2013). This suggests that the relationship between the physiological response to D<sub>2</sub>R antagonist and D<sub>2</sub>R availability described by (Sander et al., 2013) in preclinical models also exists *in vivo* in humans. Furthermore, we have shown that the relationship between ASL-CBF increases after antipsychotic administration also matched [<sup>18</sup>F]Fallypride BP<sub>ND</sub> template values in extra-striatal ROIs significantly populated by D<sub>2</sub>R such as the thalamus and the amygdala, even though they show lower BP<sub>ND</sub> template values as compared with striatal ROIs. These results extend earlier evidence (Sander et al., 2016)

and suggest that the linear coupling between CBF response to dopaminergic drugs and D<sub>2</sub>R concentration might also be a valid model outside the striatum. This interpretation is also supported by the weaker correlation of  $\Delta$ CBF with [<sup>11</sup>C]raclopride BP<sub>ND</sub> template as compared with [<sup>18</sup>F]Fallypride BP<sub>ND</sub> template (Supplementary Material). Of note, in Sander et al., (2016; 2013) the functional measure was rCBV instead of rCBF. However, given the tight association between rCBV changes and rCBF changes (Ito et al., 2005), we believe this difference will not affect the interpretation of our findings.

### Microarray mRNA expression data

We found that for all antipsychotics, the  $\Delta$ CBF profiles also correlated with microarray mRNA expression data extracted from the ABA. All genomic correlations were positive and therefore in the same direction of [<sup>18</sup>F]Fallypride BP<sub>ND</sub> template linear models. Notably, microarray mRNA expression measures explained less variance in  $\Delta$ CBF than [<sup>18</sup>F]Fallypride BP<sub>ND</sub> template values. This is consistent with our hypothesis motivated by the existence of a large variability between mRNA expression and protein synthesis due to post-transcriptional regulation mechanisms (Liu et al., 2016). Our findings are consistent with previous works that have linked the spatial architecture of the brain transcriptome to brain structure (Grecchi et al., 2017; Veronese et al., 2015), function (Hawrylycz et al., 2015; Richiardi et al., 2015) and *in vivo* measures of brain proteins (Gryglewski et al., 2018; Rizzo et al., 2016; 2014; Veronese et al., 2016). Here we could show that *post mortem* brain mRNA expression data may potentially be used to map also variations in MRI functional response to drug stimulation. However, such mapping has still many limitations to be addressed in order to adopt it extensively in the context of pharmacological-MRI studies. For instance, it might be difficult to use mRNA expression mapping in neurotransmitter systems where protein synthesis is highly dependent on post-transcriptional regulation mechanisms (e.g. serotonin

system) (Beliveau et al., 2017; Rizzo et al., 2014). While further studies are needed to fully validate this mRNA expression-MRI approach, it might be especially valuable for profiling the functional effects of drugs with poorly characterized or unknown targets. Interestingly, as for the correlation with receptor density profiles, the rank order of correlations between  $\Delta$ CBF and *DRD2* mRNA expression measures mirrored affinity profiles of the compounds, although these differences were only numerical. It is worth noting that the variance explained by genomic correlation was lower than the PET correlations (Table 1), which might have reduced the chance of detecting any significant difference on the pairwise comparison between the correlation coefficients.

These findings represent a further evidence supporting the hypothesized PK/PD model of antipsychotic effect of CBF measures (Mandeville et al., 2013). Indeed, we were able to show that quantitative measures of brain functional effect of antipsychotics (i.e. CBF changes) are directly associated with receptor density measures. However, intrinsic limitations of the ABA dataset should be considered when evaluating mRNA/ $\Delta$ CBF relationships. First the number of available ABA donors is limited (N= 6) and therefore only approximates population-level brain mRNA expression templates. However, despite this limitation, ABA data has been showed to be able to predict brain protein densities (Beliveau et al., 2017; Gryglewski et al., 2018; Rizzo et al., 2016; 2014; Veronese et al., 2016) and other neuroimaging measures (Hawrylycz et al., 2015; Richiardi et al., 2015; Ritchie et al., 2018). Another limitation is that different approaches have been proposed to analyse this dataset (French and Paus, 2015; Gryglewski et al., 2018). The fact that different strategies in ABA dataset processing could affect reproducibility of the findings is under debate (Arnatkeviciute et al., 2018). We have also analysed the mRNA/ $\Delta$ CBF correlations using a different methods that employs whole-brain voxel-wise mRNA expression maps obtained from variograms (Gryglewski et al., 2018). The results of this analysis were comparable with

the analysis performed in MENGA (Supplementary Materials). Therefore we believe that it is unlikely that this methodological issue could have biased our results.

### **Differential strength of association between $\Delta$ CBF and receptor density profiles.**

The correlation strength between  $\Delta$ CBF and receptor density measured with PET varied between the different antipsychotics tested (Figure 4). One possible interpretation of this difference might be related to the differential affinities for these compounds to D<sub>2</sub>R (Dukart et al., 2018). Our data seems to be in line with this hypothesis. In fact, the strengths of the association were higher for antipsychotics with higher affinity for D<sub>2</sub>R (Table 1 and Figure S1). In particular, HAL was the drug with the highest correlation coefficient and the lowest K<sub>i</sub>, whereas OLA showed the lowest correlation coefficient and the highest K<sub>i</sub>. Both lowRIS and highRIS were in the middle between HAL and OLA. Another possible interpretation might be that related with the different secondary affinities between the drugs. For instance, for compounds with an high affinity with 5HT<sub>2a</sub> receptors like risperidone and olanzapine, part of the effect on CBF might also be linked with a mechanism different from D<sub>2</sub>R blockade (Goozee et al., 2014). In fact, both OLA and RIS as well as other second-generation antipsychotics (e.g. aripiprazole) showed decreases in CBF especially in cortical areas with a mechanism that is possibly mediated by 5HT<sub>2a</sub> receptors (Handley et al., 2013; Lahti et al., 2005). We did not find differences in correlations between the two different doses of risperidone, despite the fact that they do show different effect size in CBF increase (as shown in Figure 2 bottom row). This might indicate that the coupling between the measurable physiological effects and target receptor distribution can be detected regardless the dose of the compound if that dose is able to produce detectable functional effects. Nonetheless, differences in brain disposition of antipsychotics have been reported in previous studies (Kornhuber et al., 2006; Rodda et al., 2006). In particular, animal data suggested that

antipsychotics (including the ones considered in the present work) show different blood-brain barrier penetration and brain clearance leading to dissimilar spatial distribution in the brain (Loryan et al., 2016). Even though these dissimilarities have been reported to be only moderate (Loryan et al., 2016), brain disposition is a factor to be considered in addition to receptor affinity when linking the pharmacodynamics of each antipsychotic with receptor occupancy. Therefore, without maps of brain deposition variation, conclusions regarding the D<sub>2</sub>R affinities and strength of associations between receptors and CBF are necessarily incomplete.

### **Limitations**

A number of limitations need to be considered for the present study. First, we used population-based profiles of D<sub>2</sub>R density as the spatial architecture of D<sub>2</sub>R is typically consistent across individuals (Rizzo et al., 2014; Veronese et al., 2016). For example, the striatum always has higher D<sub>2</sub>R density than the thalamus which in turn has higher D<sub>2</sub>R density than the cortex. However, variance in density between individuals within the same brain region (Farde et al., 1995) may drive inter-individual differences in the drug functional response. Individualized receptor profiles mapping is therefore necessary to bring more precision to the method and to further validate the present findings. Nevertheless, normative atlases for protein and mRNA expressions have proven useful in many different applications, suggesting that the core *spatial* architecture of the brain receptor systems is consistent across individuals (Beliveau et al., 2017; Rizzo et al., 2016; 2014; Veronese et al., 2016).

In addition, we considered only local effects by matching CBF changes and D<sub>2</sub>R receptor density measures with the same ROIs. However, studies in patients and healthy volunteers showed that antipsychotics also produce changes in functional connectivity in rs-fMRI

suggesting the existence of downstream effects (Cole et al., 2013; Sarpal et al., 2015) that were not included in our analyses.

As discussed above, increases in CBF after acute antipsychotic challenge have been usually interpreted as the result of a neuronal metabolic changes due to D<sub>2</sub>R blockade. D<sub>2</sub>-like receptors are also present in perivascular astrocytes and endothelial cells modulating brain hemodynamic changes. However these receptors are mainly D<sub>3</sub>R and not D<sub>2</sub>R (Choi et al., 2006). Together with the knowledge that the affinity of these compounds for D<sub>2</sub>R is higher than for D<sub>3</sub>R we expect the change in CBF is mainly neuronal in origin. In addition, both risperidone and olanzapine, but not haloperidol, act as antagonists at the serotonin-2a receptors (5HT<sub>2a</sub>) (Meltzer, 1999). Blockade of 5HT<sub>2a</sub> receptors on smooth-muscle cells of brain arteries has been shown to induce relative vasodilation in animal models (i.e. blocking vasoconstriction caused by the endogenous ligand serotonin) (Kovács et al., 2012). Therefore, the degree to which these non-neuronal effects contribute to the associations described here is not known. Finally, we believe that the limited sample size offered by the ABA (N = 6) could have undermined power in detecting a linear relationship between mRNA data and rCBF changes. This factor could also be an alternative explanation of the difference we found between mRNA and PET correlations with rCBF.

## Conclusion

Understanding the link between neurochemical changes and brain function is crucial to uncover mechanisms underlining the effects of psychopharmacological treatment and between-subjects variability in response. In this work we investigated the case of antipsychotics whose functional effect, evaluated as changes in CBF measures, mirror the

well-known spatial distributions of their main target (i.e. D<sub>2</sub>R). The characterisation of the haemodynamic response using this multimodal approach might be applicable also for other classes of drugs and it becomes particularly valuable for profiling compounds known to bind to multiple targets or with unknown or poorly characterised targets. Finally, our work demonstrates that the use of MRI 3D pCASL as a measure of regional CBF offers an efficient, non-invasive tool to investigate CNS penetration in the process of psychotropic drug development that is also linked with brain chemistry.

## **Funding**

Contract grant sponsor: Hoffmann – LaRoche Pharmaceuticals.

This paper represents independent research part funded by the National Institute for Health Research (NIHR) Biomedical Research Centre at South London and Maudsley NHS Foundation Trust and King's College London that support PS, OD, SCRW, FZ, MV and MAM. The views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health and Social Care. PS is supported by a PhD studentship jointly funded by the NIHR-BRC at SLaM and the Department of Neuroimaging, King's College London.

## **Competing interest statement**

AB is a stockholder of Hoffmann-La Roche Ltd. He has also received consulting fees from Biogen and lecture fees from Otsuka, Janssen, Lundbeck. FS is a former employee of F. Hoffmann-La Roche Ltd. GP has been the academic supervisor of a Roche collaboration grant (years 2015-16) that funds his salary. JD is current employees of F. Hoffmann-La Roche Ltd.

and received support in form of salaries. SCRW has received grant funding from the Medical Research Council (UK), Wellcome Trust (UK), National Institute for Health Research (UK) and support for investigator led studies from Takeda, Pfizer, Lundbeck, PIVital, Roche and Eli Lilly. In the past 3 years MAM has acted as an advisory board member for Lundbeck and Forum Pharmaceuticals. He also holds research funding from Lundbeck, Takeda and Johnson & Johnson. No other conflict of interested are disclosed.

### **Acknowledgments**

MRI data were collected at the NIHR/Wellcome Trust King's Clinical Research Facility (CRF). Authors are grateful to the CRF team and to Dr Ndabezinhle Mazibuko and Stephanie Stephenson (Department of Neuroimaging, Institute of Psychiatry, Psychology and Neuroscience, King's College London, London, United Kingdom) for their help on data acquisition. We also gratefully acknowledge the work of Dr Joel Dunn (Kings College London and Guy's and St Thomas' PET Centre, School of Biomedical Engineering and Imaging Sciences, King's College London, London, UK) who contributed to the creation of PET templates. We also thank Gill Brown (London College of Communication, UAL, London, UK) for her help on illustrations. Finally, we thank all the volunteers who took part in the study.

## References

- Agid, O., Mamo, D., Ginovart, N., Vitcu, I., Wilson, A.A., Zipursky, R.B., Kapur, S., 2007. Striatal Vs Extrastriatal Dopamine D2 Receptors in Antipsychotic Response|[mdash]|A Double-Blind PET Study in Schizophrenia. *Neuropsychopharmacology* 32, 1209–1215. doi:10.1038/sj.npp.1301242
- Arnatkeviciute, A., Ben D Fulcher, Fornito, A., 2018. A practical guide to linking brain-wide gene expression and neuroimaging data. *bioRxiv* 380089. doi:10.1101/380089
- Attwell, D., Iadecola, C., 2002. The neural basis of functional brain imaging signals. *Trends in Neurosciences* 25, 621–625. doi:10.1016/S0166-2236(02)02264-6
- Beliveau, V., Ganz, M., Feng, L., Ozenne, B., Højgaard, L., Fisher, P.M., Svarer, C., Greve, D.N., Knudsen, G.M., 2017. A High-Resolution In Vivo Atlas of the Human Brain's Serotonin System. *Journal of Neuroscience* 37, 120–128. doi:10.1523/JNEUROSCI.2830-16.2017
- Burt, D.R., Creese, I., Snyder, S.H., 1977. Antischizophrenic drugs: chronic treatment elevates dopamine receptor binding in brain. *Science*.
- Choi, J.-K., Chen, Y.I., Hamel, E., Jenkins, B.G., 2006. Brain hemodynamic changes mediated by dopamine receptors: Role of the cerebral microvasculature in dopamine-mediated neurovascular coupling. *NeuroImage* 30, 700–712. doi:10.1016/j.neuroimage.2005.10.029
- Clark, A.J., 1970. *Methods of General Pharmacology, General Pharmacology*. Springer Berlin Heidelberg, Berlin, Heidelberg. doi:10.1007/978-3-662-28641-8\_2
- Cole, D.M., Oei, N.Y.L., Soeter, R.P., Both, S., van Gerven, J.M.A., Rombouts, S.A.R.B., Beckmann, C.F., 2013. Dopamine-dependent architecture of cortico-subcortical network connectivity. *Cerebral Cortex* 23, 1509–1516. doi:10.1093/cercor/bhs136
- Cook, R.D., Weisberg, S., 1982. Residuals and influence in regression.
- DeLisi, L.E., Holcomb, H.H., Cohen, R.M., Pickar, D., Carpenter, W., Morihisa, J.M., King, A.C., Kessler, R., Buchsbaum, M.S., 1985. Positron emission tomography in schizophrenic patients with and without neuroleptic medication. *J Cereb Blood Flow Metab* 5, 201–206. doi:10.1038/jcbfm.1985.26
- Desikan, R.S., Ségonne, F., Fischl, B., Quinn, B.T., Dickerson, B.C., Blacker, D., Buckner, R.L., Dale, A.M., Maguire, R.P., Hyman, B.T., Albert, M.S., Killiany, R.J., 2006. An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. *NeuroImage* 31, 968–980. doi:10.1016/j.neuroimage.2006.01.021
- Dukart, J., Holiga, Š., Chatham, C., Hawkins, P., Forsyth, A., McMillan, R., Myers, J., Lingford-Hughes, A.R., Nutt, D.J., Merlo-Pich, E., Risterucci, C., Boak, L., Umbricht, D., Schobel, S., Liu, T., Mehta, M.A., Zelaya, F.O., Williams, S.C., Brown, G., Paulus, M., Honey, G.D., Muthukumaraswamy, S., Hipp, J., Bertolino, A., Sambataro, F., 2018. Cerebral blood flow predicts differential neurotransmitter activity. *Sci Rep* 8, 4074. doi:10.1038/s41598-018-22444-0
- Dunn, J.T., Stone, J., Cleij, M., Marsden, P.K., O'Doherty, M., Reed, L.J., 2009. Differential occupancy of striatal versus extrastriatal dopamine D2/D3 receptors by the typical antipsychotic Haloperidol in man measured using [18F]-Fallypride PET. *NeuroImage* 47, S176. doi:10.1016/S1053-8119(09)71925-1
- Efron, B., Tibshirani, R., 1986. *Bootstrap Methods for Standard Errors, Confidence Intervals, and Other Measures of Statistical Accuracy*. *Statistical Science* 1, 54–75. doi:10.1214/ss/1177013815

- Farde, L., Hall, H., Ehrin, E., Sedvall, G., 1986. Quantitative analysis of D2 dopamine receptor binding in the living human brain by PET. *Science* 231, 258–261.
- Farde, L., Hall, H., Pauli, S., Halldin, C., 1995. Variability in D<sub>2</sub> dopamine receptor density and affinity: A PET study with [<sup>11</sup>C]raclopride in man. *Synapse* 20, 200–208. doi:10.1002/syn.890200303
- Fernández-Seara, M.A., Aznárez Sanado, M., Mengual, E., Irigoyen, J., Heukamp, F., Pastor, M.A., 2011. Effects on resting cerebral blood flow and functional connectivity induced by metoclopramide: a perfusion MRI study in healthy volunteers. *Br. J. Pharmacol.* 163, 1639–1652. doi:10.1111/j.1476-5381.2010.01161.x
- Finch, W.H., 2012. Distribution of variables by method of outlier detection. *Front. Psychol.* 3, 211. doi:10.3389/fpsyg.2012.00211
- French, L., Paus, T., 2015. A FreeSurfer view of the cortical transcriptome generated from the Allen Human Brain Atlas. *Front Neurosci* 9, 323. doi:10.3389/fnins.2015.00323
- Goozee, R., Handley, R., Kempton, M.J., Dazzan, P., 2014. A systematic review and meta-analysis of the effects of antipsychotic medications on regional cerebral blood flow (rCBF) in schizophrenia: Association with response to treatment. *Neuroscience & Biobehavioral Reviews* 43, 118–136.
- Grecchi, E., Doyle, O.M., Bertoldo, A., Pavese, N., Turkheimer, F.E., 2014. Brain shaving: adaptive detection for brain PET data. *Phys. Med. Biol.* 59, 2517–2534. doi:10.1088/0031-9155/59/10/2517
- Grecchi, E., Veronese, M., Bodini, B., García-Lorenzo, D., Battaglini, M., Stankoff, B., Turkheimer, F.E., 2017. Multimodal partial volume correction: Application to [<sup>11</sup>C]PIB PET/MRI myelin imaging in multiple sclerosis. *J Cereb Blood Flow Metab* 37, 3803–3817. doi:10.1177/0271678X17712183
- Gryglewski, G., Seiger, R., James, G.M., Godbersen, G.M., Komorowski, A., Unterholzner, J., Michenthaler, P., Hahn, A., Wadsak, W., Mitterhauser, M., Kasper, S., Lanzenberger, R., 2018. Spatial analysis and high resolution mapping of the human whole-brain transcriptome for integrative analysis in neuroimaging. *NeuroImage* 176, 259–267. doi:10.1016/j.neuroimage.2018.04.068
- Handley, R., Zelaya, F.O., Reinders, A.A.T.S., Marques, T.R., Mehta, M.A., O’Gorman, R., Alsop, D.C., Taylor, H., Johnston, A., Williams, S., McGuire, P., Pariante, C.M., Kapur, S., Dazzan, P., 2013. Acute effects of single-dose aripiprazole and haloperidol on resting cerebral blood flow (rCBF) in the human brain. *Hum. Brain Mapp.* 34, 272–282. doi:10.1002/hbm.21436
- Hawkins, P.C.T., Wood, T.C., Vernon, A.C., Bertolino, A., Sambataro, F., Dukart, J., Merlo-Pich, E., Risterucci, C., Silber-Baumann, H., Walsh, E., Mazibuko, N., Zelaya, F.O., Mehta, M.A., 2018. An investigation of regional cerebral blood flow and tissue structure changes after acute administration of antipsychotics in healthy male volunteers. *Hum. Brain Mapp.* 39, 319–331. doi:10.1002/hbm.23844
- Hawrylycz, M., Miller, J.A., Menon, V., Feng, D., Dolbeare, T., Guillozet-Bongaarts, A.L., Jegga, A.G., Aronow, B.J., Lee, C.-K., Bernard, A., Glasser, M.F., Dierker, D.L., Menche, J., Szafer, A., Collman, F., Grange, P., Berman, K.A., Mihalas, S., Yao, Z., Stewart, L., Barabási, A.-L., Schulkin, J., Phillips, J., Ng, L., Dang, C., Haynor, D.R., Jones, A., Van Essen, D.C., Koch, C., Lein, E., 2015. Canonical genetic signatures of the adult human brain. *Nature Neuroscience* 18, 1832–1844. doi:10.1038/nn.4171
- Hawrylycz, M.J., Lein, E.S., Guillozet-Bongaarts, A.L., Shen, E.H., Ng, L., Miller, J.A., van de Lagemaat, L.N., Smith, K.A., Ebbert, A., Riley, Z.L., Abajian, C., Beckmann, C.F., Bernard, A., Bertagnolli, D., Boe, A.F., Cartagena, P.M., Chakravarty, M.M., Chapin, M., Chong, J., Dalley, R.A., Daly, B.D., Dang, C., Datta, S., Dee, N., Dolbeare, T.A., Faber, V., Feng, D., Fowler, D.R., Goldy, J., Gregor, B.W., Haradon, Z., Haynor, D.R.,

- Hohmann, J.G., Horvath, S., Howard, R.E., Jeromin, A., Jochim, J.M., Kinnunen, M., Lau, C., Lazarz, E.T., Lee, C., Lemon, T.A., Li, L., Li, Y., Morris, J.A., Overly, C.C., Parker, P.D., Parry, S.E., Reding, M., Royall, J.J., Schulkin, J., Sequeira, P.A., Slaughterbeck, C.R., Smith, S.C., Sodt, A.J., Sunkin, S.M., Swanson, B.E., Vawter, M.P., Williams, D., Wohnoutka, P., Zielke, H.R., Geschwind, D.H., Hof, P.R., Smith, S.M., Koch, C., Grant, S.G.N., Jones, A.R., 2012. An anatomically comprehensive atlas of the adult human brain transcriptome. *Nature* 489, 391–399. doi:10.1038/nature11405
- Heeger, D.J., Ress, D., 2002. What does fMRI tell us about neuronal activity? *Nat Rev Neurosci* 3, 142–151. doi:10.1038/nrn730
- Hertel, P., Nomikos, G.G., Iurlo, M., Svensson, T.H., 1996. Risperidone: Regional effects in vivo on release and metabolism of dopamine and serotonin in the rat brain. *Psychopharmacology* 124, 74–86. doi:10.1007/BF02245607
- Hoge, R.D., Atkinson, J., Gill, B., Crelier, G.R., Marrett, S., Pike, G.B., 1999. Linear coupling between cerebral blood flow and oxygen consumption in activated human cortex. *Proc. Natl. Acad. Sci. U.S.A.* 96, 9403–9408. doi:10.1073/pnas.96.16.9403
- Holcomb, H.H., Cascella, N.G., Thaker, G.K., Medoff, D.R., Dannals, R.F., Tamminga, C.A., 1996. Functional sites of neuroleptic drug action in the human brain: PET/FDG studies with and without haloperidol. *Am J Psychiatry* 153, 41–49. doi:10.1176/ajp.153.1.41
- Ichise, M., Liow, J.-S., Lu, J.-Q., Takano, A., Model, K., Toyama, H., Suhara, T., Suzuki, K., Innis, R.B., Carson, R.E., 2003. Linearized reference tissue parametric imaging methods: application to [<sup>11</sup>C]DASB positron emission tomography studies of the serotonin transporter in human brain. *J Cereb Blood Flow Metab* 23, 1096–1112. doi:10.1097/01.WCB.0000085441.37552.CA
- Ito, H., Kanno, I., Fukuda, H., 2005. Human cerebral circulation: Positron emission tomography studies. *Annals of nuclear medicine* 19, 65–74. doi:10.1007/BF03027383
- Ito, H., Takano, H., Takahashi, H., Arakawa, R., Miyoshi, M., Kodaka, F., Okumura, M., Otsuka, T., Suhara, T., 2009. Effects of the Antipsychotic Risperidone on Dopamine Synthesis in Human Brain Measured by Positron Emission Tomography with L-[<sup>11</sup>C]DOPA: A Stabilizing Effect for Dopaminergic Neurotransmission? *J. Neurosci.* 29, 13730–13734. doi:10.1523/JNEUROSCI.4172-09.2009
- Kapur, S., Remington, G., Zipursky, R.B., Wilson, A.A., Houle, S., 1995. The D2 dopamine receptor occupancy of risperidone and its relationship to extrapyramidal symptoms: A pet study. *Life Sciences* 57, PL103–PL107.
- Kapur, S., Zipursky, R., Jones, C., Remington, G., Houle, S., 2000. Relationship Between Dopamine D2 Occupancy, Clinical Response, and Side Effects: A Double-Blind PET Study of First-Episode Schizophrenia. *American Journal of Psychiatry* 157, 514–520.
- Khalili-Mahani, N., Rombouts, S.A.R.B., van Osch, M.J.P., Duff, E.P., Carbonell, F., Nickerson, L.D., Becerra, L., Dahan, A., Evans, A.C., Soucy, J.-P., Wise, R., Zijdenbos, A.P., van Gerven, J.M., 2017. Biomarkers, designs, and interpretations of resting-state fMRI in translational pharmacological research: A review of state-of-the-Art, challenges, and opportunities for studying brain chemistry. *Hum. Brain Mapp.* 38, 2276–2325. doi:10.1002/hbm.23516
- Kim, E., Howes, O.D., Turkheimer, F.E., Kim, B.H., Jeong, J.M., Kim, J.W., Lee, J.S., Jang, I.J., Shin, S.G., Kapur, S., Kwon, J.S., 2013. The relationship between antipsychotic D2 occupancy and change in frontal metabolism and working memory : A dual [(11)C]raclopride and [(18)F]FDG imaging study with aripiprazole. *Psychopharmacology* 227, 221–229.

- Kornhuber, J., Wiltfang, J., Riederer, P., Bleich, S., 2006. Neuroleptic drugs in the human brain: clinical impact of persistence and region-specific distribution. *Eur Arch Psychiatry Clin Neurosci* 256, 274–280. doi:10.1007/s00406-006-0661-7
- Kovács, A., Hársing, L.G., Szénási, G., 2012. Vasoconstrictor 5-HT receptors in the smooth muscle of the rat middle cerebral artery. *Eur. J. Pharmacol.* 689, 160–164. doi:10.1016/j.ejphar.2012.05.031
- Krimer, L.S., Muly, E.C., Williams, G.V., Goldman-Rakic, P.S., 1998. Dopaminergic regulation of cerebral cortical microcirculation. *Nature Neuroscience* 1, 286–289. doi:10.1038/1099
- Lahti, A.C., Weiler, M.A., Medoff, D.R., Tamminga, C.A., Holcomb, H.H., 2005. Functional effects of single dose first- and second-generation antipsychotic administration in subjects with schizophrenia. *Psychiatry Research: Neuroimaging* 139, 19–30. doi:10.1016/j.psychresns.2005.02.006
- Lee, I.A., Preacher, K.J., 2013. Calculation for the test of the difference between two dependent correlations with one variable in common [Computer software]. Retrieved July 18 2016.
- Liu, Y., Beyer, A., Aebersold, R., 2016. On the Dependency of Cellular Protein Levels on mRNA Abundance. *Cell* 165, 535–550. doi:10.1016/j.cell.2016.03.014
- Logothetis, N.K., Pauls, J., Augath, M., Trinath, T., Oeltermann, A., 2001. Neurophysiological investigation of the basis of the fMRI signal. *Nature* 412, 150–157. doi:10.1038/35084005
- Loryan, I., Melander, E., Svensson, M., Payan, M., König, F., Jansson, B., Hammarlund-Udenaes, M., 2016. In-depth neuropharmacokinetic analysis of antipsychotics based on a novel approach to estimate unbound target-site concentration in CNS regions: link to spatial receptor occupancy. *Nature Publishing Group* 21, 1527–1536. doi:10.1038/mp.2015.229
- Mandeville, J.B., Sander, C.Y.M., Jenkins, B.G., Hooker, J.M., Catana, C., Vanduffel, W., Alpert, N.M., Rosen, B.R., Normandin, M.D., 2013. A receptor-based model for dopamine-induced fMRI signal. *NeuroImage* 75, 46–57. doi:10.1016/j.neuroimage.2013.02.036
- McCormick, P.N., Kapur, S., Graff-Guerrero, A., Raymond, R., Nobrega, J.N., Wilson, A.A., 2010. The antipsychotics olanzapine, risperidone, clozapine, and haloperidol are D2-selective ex vivo but not in vitro. *Neuropsychopharmacology* 35, 1826–1835. doi:10.1038/npp.2010.50
- Mehta, M., 2003. Systemic sulpiride modulates striatal blood flow: relationships to spatial working memory and planning. *NeuroImage* 20, 1982–1994. doi:10.1016/j.neuroimage.2003.08.007
- Meltzer, H.Y., 1999. The role of serotonin in antipsychotic drug action. *Neuropsychopharmacology* 21, 106S–115S. doi:10.1016/S0893-133X(99)00046-9
- Miller, D.D., Andreasen, N.C., O'Leary, D.S., Watkins, G.L., Boles Ponto, L.L., Hichwa, R.D., 2001. Comparison of the effects of risperidone and haloperidol on regional cerebral blood flow in schizophrenia. *BPS* 49, 704–715. doi:10.1016/S0006-3223(00)01001-5
- Miller, D.D., Andreasen, N.C., O'Leary, D.S., Rezai, K., Watkins, L., Boles Ponto, L.L., Hichwa, R.D., 1997. Effect of antipsychotics on regional cerebral blood flow measured with positron emission tomography. *Neuropsychopharmacology* 17, 230–240.
- Mukherjee, J., Christian, B.T., Dunigan, K.A., Shi, B., Narayanan, T.K., Satter, M., Mantil, J., 2002. Brain imaging of 18F-fallypride in normal volunteers: blood analysis, distribution, test-retest studies, and preliminary assessment of sensitivity to aging effects on dopamine D-2/D-3 receptors. *Synapse* 46, 170–188. doi:10.1002/syn.10128

- Mukherjee, J., Yang, Z.-Y., Das, M.K., Brown, T., 1995. Fluorinated benzamide neuroleptics—III. Development of (S)-N-[(1-allyl-2-pyrrolidinyl)methyl]-5-(3-[18F]fluoropropyl)-2,3-dimethoxybenzamide as an improved dopamine D-2 receptor tracer. *Nuclear Medicine and Biology* 22, 283–296.
- Nyberg, S., Farde, L., Halldin, C., Dahl, M.L., 1995. D2 dopamine receptor occupancy during low-dose treatment with haloperidol decanoate. *American Journal of ...*
- Ploeger, B.A., van der GRAAF, P.H., and, M.D.D.M., 2009, 2009. Incorporating receptor theory in mechanism-based pharmacokinetic-pharmacodynamic (PK-PD) modeling. Elsevier 24, 3–15. doi:10.2133/dmpk.24.3
- Richiardi, J., Altmann, A., Milazzo, A.C., Chang, C., 2015. Correlated gene expression supports synchronous activity in brain networks. *Science* 348, 1241–1244. doi:10.1126/science.1255905
- Ritchie, J., Pantazatos, S.P., French, L., 2018. Transcriptomic characterization of MRI contrast with focus on the T1-w/T2-w ratio in the cerebral cortex. *NeuroImage* 174, 504–517. doi:10.1016/j.neuroimage.2018.03.027
- Rizzo, G., Veronese, M., Expert, P., Turkheimer, F.E., Bertoldo, A., 2016. MENGA: A New Comprehensive Tool for the Integration of Neuroimaging Data and the Allen Human Brain Transcriptome Atlas. *PLoS ONE* 11, e0148744–20. doi:10.1371/journal.pone.0148744
- Rizzo, G., Veronese, M., Heckemann, R.A., Selvaraj, S., Howes, O.D., Hammers, A., Turkheimer, F.E., Bertoldo, A., 2014. The predictive power of brain mRNA mappings for in vivo protein density: a positron emission tomography correlation study. *Journal of Cerebral Blood Flow & Metabolism* 34, 827–835. doi:10.1038/jcbfm.2014.21
- Rodda, K.E., Dean, B., McIntyre, I.M., Drummer, O.H., 2006. Brain distribution of selected antipsychotics in schizophrenia. *Forensic Sci. Int.* 157, 121–130. doi:10.1016/j.forsciint.2005.03.017
- Sander, C.Y., Hooker, J.M., Catana, C., Normandin, M.D., Alpert, N.M., Knudsen, G.M., Vanduffel, W., Rosen, B.R., Mandeville, J.B., 2013. Neurovascular coupling to D2/D3 dopamine receptor occupancy using simultaneous PET/functional MRI. *Proc. Natl. Acad. Sci. U.S.A.* 110, 11169–11174. doi:10.1073/pnas.1220512110
- Sander, C.Y., Hooker, J.M., Catana, C., Rosen, B.R., Mandeville, J.B., 2016. Imaging Agonist-Induced D2/D3 Receptor Desensitization and Internalization In Vivo with PET/fMRI. *Neuropsychopharmacology* 41, 1427–1436. doi:10.1038/npp.2015.296
- Sarpal, D.K., Robinson, D.G., Lencz, T., Argyelan, M., Ikuta, T., Karlsgodt, K., Gallego, J.A., Kane, J.M., Szeszko, P.R., Malhotra, A.K., 2015. Antipsychotic treatment and functional connectivity of the striatum in first-episode schizophrenia. *JAMA Psychiatry* 72, 5–13. doi:10.1001/jamapsychiatry.2014.1734
- Singh, K.D., 2012. Which “neural activity” do you mean? fMRI, MEG, oscillations and neurotransmitters. *NeuroImage* 62, 1121–1130. doi:10.1016/j.neuroimage.2012.01.028
- Stark, A.J., Smith, C.T., Petersen, K.J., Trujillo, P., van Wouwe, N.C., Donahue, M.J., Kessler, R.M., Deutch, A.Y., Zald, D.H., Claassen, D.O., 2018. [18F]fallypride characterization of striatal and extrastriatal D2/3 receptors in Parkinson's disease. *YNICL* 18, 1–42. doi:10.1016/j.nicl.2018.02.010
- Stroup, T.S., Lieberman, J.A., McEvoy, J.P., Davis, S.M., Swartz, M.S., Keefe, R.S.E., Miller, A.L., Rosenheck, R.A., Hsiao, J.K., 2009. Results of phase 3 of the CATIE schizophrenia trial. *Schizophrenia Research* 107, 1–12. doi:10.1016/j.schres.2008.10.011
- Tabachnick, B.G., Fidell, L.S., 2013. *Using Multivariate Statistics*.
- Tauscher, J., Hussain, T., Agid, O., Verhoeff, N.P.L.G., Wilson, A.A., Houle, S., Remington, G., Zipursky, R.B., Kapur, S., 2004. Equivalent Occupancy of Dopamine D1 and D2

- Receptors With Clozapine: Differentiation From Other Atypical Antipsychotics. *American Journal of Psychiatry* 161, 1620–1625. doi:10.1176/appi.ajp.161.9.1620
- Vernaleken, I., Kumakura, Y., Buchholz, H.-G., Siessmeier, T., Hilgers, R.-D., Bartenstein, P., Cumming, P., Gründer, G., 2008. Baseline [<sup>18</sup>F]-FDOPA kinetics are predictive of haloperidol-induced changes in dopamine turnover and cognitive performance: A positron emission tomography study in healthy subjects. *NeuroImage* 40, 1222–1231. doi:10.1016/j.neuroimage.2007.12.045
- Veronese, M., Bodini, B., García-Lorenzo, D., Battaglini, M., Bongarzone, S., Comtat, C., Bottlaender, M., Stankoff, B., Turkheimer, F.E., 2015. Quantification of [(11)C]PIB PET for imaging myelin in the human brain: a test-retest reproducibility study in high-resolution research tomography. *Journal of Cerebral Blood Flow & Metabolism* 35, 1771–1782. doi:10.1038/jcbfm.2015.120
- Veronese, M., Zanotti-Fregonara, P., Rizzo, G., Bertoldo, A., Innis, R.B., Turkheimer, F.E., 2016. Measuring specific receptor binding of a PET radioligand in human brain without pharmacological blockade: The genomic plot. *NeuroImage* 130, 1–12. doi:10.1016/j.neuroimage.2016.01.058
- Viviani, R., Graf, H., Wieggers, M., Abler, B., 2013. Effects of amisulpride on human resting cerebral perfusion. *Psychopharmacology* 229, 95–103. doi:10.1007/s00213-013-3091-z
- Viviani, R., Sim, E.-J., Lo, H., Richter, S., Haffer, S., Osterfeld, N., Thöne, J., Beschoner, P., 2009. Components of variance in brain perfusion and the design of studies of individual differences: The baseline study. *NeuroImage* 46, 12–22. doi:10.1016/j.neuroimage.2009.01.041