



Dopaminergic modulation of regional cerebral blood flow: An arterial spin labelling study of genetic and pharmacological manipulation of COMT activity

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ABSTRACT

Dopamine has direct and complex vasoactive effects on cerebral circulation. Catechol-O-methyltransferase (COMT) regulates cortical dopamine, and its activity can be influenced both genetically and pharmacologically. COMT activity influences the functional connectivity of the PFC at rest, as well as its activity during task performance, determined using blood oxygen level-dependent (BOLD) fMRI. However, its effects on cerebral perfusion have been relatively unexplored. Here, 76 healthy males, homozygous for the functional COMT Val¹⁵⁸Met polymorphism, were administered either the COMT inhibitor tolcapone or placebo in a double-blind, randomised design. We then assessed regional cerebral blood flow at rest using pulsed arterial spin labelling. Perfusion was affected by both genotype and drug. COMT genotype affected frontal regions (Val¹⁵⁸ > Met¹⁵⁸), whilst tolcapone influenced parietal and temporal regions (placebo > tolcapone). There was no genotype by drug interaction. Our data demonstrate that lower COMT activity is associated with lower cerebral blood flow, although the regions affected differ between those affected by genotype compared with those altered by acute pharmacological inhibition. The results extend the evidence for dopaminergic modulation of cerebral blood flow. Our findings also highlight the importance of considering vascular effects in functional neuroimaging studies, and of exercising caution in ascribing group differences in BOLD signal solely to altered neuronal activity if information about regional perfusion is not available.

1. Introduction

The catechol-O-methyltransferase (COMT) enzyme metabolises catecholamines and is important for determining cortical dopamine levels (Tunbridge et al., 2004; Yavich et al., 2007). As well as being linked with cognitive function, albeit inconsistently (Tunbridge et al., 2006b), it has been the focus of numerous imaging genetics studies (e.g. (Egan et al., 2001; Mier et al., 2009; Tunbridge et al., 2013)), in part because the human gene contains a coding polymorphism (Val¹⁵⁸Met; rs4680) that directly influences its enzyme activity (Val¹⁵⁸ > Met¹⁵⁸) (Tunbridge et al., 2019). In addition, COMT activity, and therefore cortical dopamine function, can also be manipulated using tolcapone, a selective, brain-penetrant COMT inhibitor used in the adjunctive treatment of Parkinson's disease (Zurcher et al., 1990). Tolcapone administration improves

cognitive function in a genotype-dependent manner (Farrell et al., 2012; Giakoumaki et al., 2008) and has also been used in COMT pharmacofMRI studies (e.g. (Apud et al., 2006; Magalona et al., 2013)). Many studies demonstrate an association between COMT activity and the cortical BOLD response (Mier et al., 2009).

Associations between COMT activity and the BOLD signal are typically ascribed to COMT's effects on cortical dopamine (Tunbridge et al., 2004; Yavich et al., 2007). BOLD fMRI measures the haemodynamic response (Ogawa and Lee, 1990), which is coupled to neural activity (Logothetis et al., 2001). However, the BOLD signal is determined by complex interactive effects of cerebral blood flow (CBF), cerebral blood volume and oxygen metabolism (Wang et al., 2011a). CBF is determined by biomechanical, neurochemical, and physiological variables acting at both systemic and local levels (Khalili-Mahani et al., 2017),

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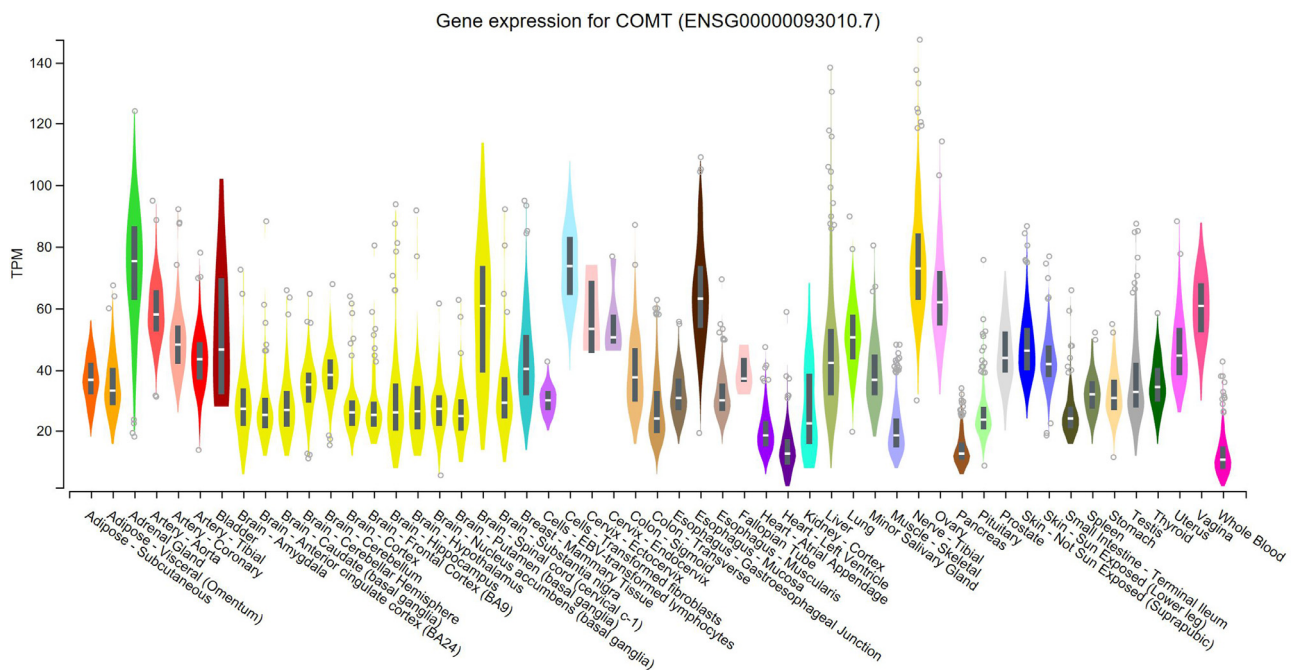


Fig. 1. COMT is expressed at similar levels across human tissues. Analysis of RNASeq data from the GTEx project reveals moderate expression of COMT (expressed as transcripts per million; TPM) across the majority of human tissues.

and many of the underlying factors can be readily altered, either directly or indirectly, by genetic factors (Detre et al., 2012) or the administration of drugs (Khalili-Mahani et al., 2017). Thus, interpreting differences in neural activity measured by BOLD fMRI can be complicated by between-group variation in total or regional CBF (Murphy and Mackay, 2011). Notably in this regard, COMT is expressed widely, including in the cerebral vasculature (Figure 1; (GTEx Consortium et al., 2017)). Consistent with this pattern of expression and the importance of catecholamines for blood pressure regulation, a number of studies report associations between COMT Val¹⁵⁸Met and blood pressure: most (but not all; (Hagen et al., 2007)) show higher blood pressure associated with the Met¹⁵⁸ allele (e.g. (Annerbrink et al., 2008; Htun et al., 2011)). There is also evidence that dopamine agonists (Schouw et al., 2013) and antagonists (Fernández-Seara et al., 2011; Handley et al., 2013) influence regional CBF, although the effects are complex and non-linear (Dukart et al., 2018). These considerations raise the possibility that some of COMT's effects in BOLD fMRI studies may be explained by differences in resting CBF. However, few studies have examined whether resting CBF differs between COMT Val¹⁵⁸Met genotype groups and, in those which have, findings are inconsistent (Eisenberg et al., 2010; Raz et al., 2017; Thomason et al., 2009). Furthermore, to our knowledge, no studies have examined whether acute COMT inhibition alters resting CBF. Therefore, here we report the separate and interactive effects of COMT genotype and tolcapone administration on regional CBF using arterial spin labelling (ASL). In addition, we examined whether there were any differences in grey matter partial volume maps between genotype groups, given some reports of structural differences between COMT Val¹⁵⁸Met genotype groups (Ohnishi et al., 2005; Rowe et al., 2010) (although see e.g. (Barnes et al., 2012; Tunbridge et al., 2013; Zinkstok et al., 2008)).

2. Methods

2.1. Participants

Ethical approval was granted by the Central University Research Ethics Committee (CUREC) of the University of Oxford (MSD-IDREC: R26521/RE006). All participants gave informed consent.

Non-smoking, healthy men, aged 18 to 40 years old were recruited by advertisement. Women were excluded from the study given marked sexually dimorphic effects of COMT (Laatikainen et al., 2013; Tunbridge and Harrison, 2011) and the need to ensure sufficient statistical power given the available resources. Participants were mailed study packs and provided buccal swabs by post. They were genotyped for the COMT Val¹⁵⁸Met polymorphism (rs4680) using the appropriate Taqman SNP Genotyping Assay. All potential participants (homozygotes) underwent telephone screening to establish inclusion and exclusion criteria. Mental health was assessed using the Structured Clinical Interview for DSM-IV. Exclusion criteria included: current or past history of psychiatric or neurological disorder; use of psychotropic medication or medication that might affect the stress response (e.g. corticosteroids, beta-blockers, since this study formed part of a larger study examining stress responses); a history of heart disease or hypertension; current or past liver disease, or other significant current medical illness, alcohol intake greater than 30 units/week; smoker, currently or within past three months; illicit drug use in the last three months; MRI safety concerns or an inadequate command of spoken English. The participants' general practitioners were asked to check that participants did not meet any of the exclusion criteria and that there were no medical reasons why it would be unsafe for them to take part in the study.

351 males were genotyped; genotypes were in Hardy-Weinberg equilibrium ($n=84$ Val¹⁵⁸/Val¹⁵⁸; $n=167$ Val¹⁵⁸/Met¹⁵⁸ and $n=100$ Met¹⁵⁸/Met¹⁵⁸; $\chi^2=0.76$; $p=0.38$). After screening, 76 homozygotes were eligible and randomised (by PJH) to receive either placebo or tolcapone. The participants and the main study researcher (MM) were blind to both genotype and drug allocation.

2.2. Study design

Participants attended the laboratory at noon and were administered tolcapone (200 mg by mouth) or visually-matched placebo. Tolcapone has an elimination half-life of 2.0 ± 0.8 h; the dose given produces 70–80% peripheral blood COMT inhibition between 1 and 4 h (Ceravolo et al., 2002; Dingemans et al., 1995), and influences cognitive function within this period (Farrell et al., 2012). Therefore, scan-

ning commenced 90 min after administration. Participants were asked to refrain from caffeine in the 3.5 h before scanning.

2.3. Neuroimaging protocol

Scanning was performed at the Oxford Centre for Human Brain Activity (OHBA), University of Oxford, using a 3-Tesla Siemens Prisma scanner with a 32-channel head-coil. The neuroimaging protocol comprised functional and structural sequences as follows.

Whole-brain perfusion imaging was performed using Siemens' pulsed arterial spin labelling (ASL) product PICOE-Q2TIPS pulsed arterial spin labelling (ASL) product sequence, with a 2D gradient spin echo read-out (QUIPSS II (quantitative imaging of perfusion using a single subtraction, version 2), thin slice T11, periodic saturation (Q2TIPS) using a proximal inversion with a control for off-resonance effects (PICOE) labelling scheme)(Luh et al., 1999). ASL data were collected as tag-control pairs with a TI of 1.8 s and a bolus duration of 0.7 s (TR = 2500 ms, TE = 12 ms, FA 90°, field of view = 256 × 256 mm², 13 slices, voxel dimensions = 4.0 × 4.0 × 8.0 mm, 120 repeats, acquisition time = 10 min 7 s). A calibration image was acquired with identical parameters but without labelling. In addition, structural scans were acquired via T1-weighted MR images (TR = 1900 ms, TE = 3.97 ms, flip angle = 8°, field of view = 192 mm, voxel dimension = 1 mm isotropic, acquisition time = 5 min 31 s). Participants were allowed to close their eyes but instructed not to fall asleep. Images were distortion corrected by an acquired fieldmap (echos at 4.92 and 7.38 ms, TR=482 ms, flip angle = 46°).

During the scan protocol an eye-tracker monitored whether the participant's eyes were open (blinking normally). Physiological measures including pulse, respiration, and skin conductance were also recorded throughout using a photoplethysmograph (TSD200-MRI, Biopac, Goleta, CA, US), respiration bellows (TSD221-MRI, Biopac, Goleta, CA, US), and electrodes strapped by Velcro (EDA100C-MRI amplifier; MR-conditional Leads: 2 x LEAD108C 30 cm; 2 x radio-translucent Electrodes: EL509 disposable dry; electrodermal gel: GEL101, all Biopac, Goleta, CA, US) to the participant's fingertips.

2.4. Data analysis

Pulse rate (beats per minute) and mean skin conductance level (μ S) for the ASL scan duration were extracted using a custom Matlab script and analysed using between-subjects analysis of variance (ANOVA) using SPSS version 25 (IBM, Portsmouth, UK). Skin conductance measures were non normally-distributed and so were log-transformed prior to analysis. MRI data were analysed using FSL (FMRIB Software Library v6.0) tools (<https://fsl.fmrib.ox.ac.uk/fsl>). Analyses were conducted blind to information about how the four subject groups mapped to genotype and drug allocation.

Distortion and motion corrected resting perfusion maps in units of mL/100g/min were calculated using Oxford_AS_L (part of the Bayesian Inference for Arterial Spin Labelling (BASIL) tool, <https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/BASIL>; (Chappell et al., 2009) for each participant, which performs label-control subtraction, inference of voxelwise perfusion, and voxelwise calibration to obtain absolute perfusion maps. FSL's Anatomical Processing Script (FSL_Anat, https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/fsl_anat) was used to pre-process each participant's high resolution T1 structural image (includes bias-field correction, brain extraction and registration to standard space via FMRIB's Linear Image Registration Tool (FLIRT) and FMRIB's Non-linear Image Registration Tool (FNIRT). After visual examination to confirm that perfusion values were within the expected range (all scans were), the processed perfusion images were non-linearly aligned with MNI-152 standard space via an initial linear transformation T1 structural space (using FLIRT), followed by application of the non-linear warp from *fsl_anat*. A Gaussian smoothing kernel of 2.35 mm was applied to all the normalised images (to match functional data).

Data were interrogated using voxel-wise generalized linear model (GLM) permutation nonparametric testing (5000 permutations) with randomise (FSL's tool for nonparametric permutation inference on neuroimaging data), correcting for multiple comparisons across space (cluster-based thresholding using TFCE and a family-wise error (FWE)-corrected cluster significance threshold of $p < 0.05$ applied to the suprathreshold clusters). This results in spatial maps characterising the between-subject/group differences. The GLM was set up with four groups (COMT-Val-placebo, COMT-Val-tolcapone, COMT-Met-placebo, COMT-Met-tolcapone) to allow comparisons of the main effects of drug (placebo vs. tolcapone) and genotype (COMT-Val vs. COMT-Met), as well as their interaction. No other covariates were included in the analysis. Each of the contrasts of interest was tested for group averages and difference between groups. To further visualise the results, individual perfusion values were extracted from their custom maps, using significant clusters as binary masks. These represent differences in blood flow between groups. All co-ordinates are given in MNI space.

Global grey matter (GM) perfusion was defined as mean GM perfusion and calculated by thresholding GM partial volume maps in ASL native space at 0.75 (taking the average perfusion in voxels with more than 75% grey matter). These were compared by univariate ANOVA using SPSS.

Structural data were analysed with FSL's tool for Voxel Based Morphometry (FSL-VBM; (Douaud et al., 2007), an optimised VBM protocol (Good et al., 2001) carried out with FSL tools (Smith et al., 2004) using default settings as described at <http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSLVBM>). In brief, structural images were brain-extracted and GM segmented before being registered to the MNI 152 standard space using non-linear registration (Smith et al., 2004). The resulting images were averaged and flipped along the x-axis to create a left-right symmetric, study-specific GM template. Second, all native GM images were non-linearly registered to this study-specific template and "modulated" to correct for local expansion (or contraction) due to the non-linear component of the spatial transformation (Good et al., 2001). The modulated GM images were then smoothed with an isotropic Gaussian kernel with a sigma of 3 mm. Finally, voxel wise GLM, using the same groups of interest and comparisons described above for ASL analysis, was applied using permutation-based non-parametric testing (5000 permutations), correcting for multiple comparisons across space (cluster-based thresholding using TFCE and a family-wise error (FWE)-corrected cluster significance threshold of $p < 0.05$ applied to the suprathreshold clusters).

3. Results

The demographic details of the final participants are given in Table 1. There were no main or interactive effects of genotype or drug on heart rate or skin conductance (F 's < 1; p 's > 0.4).

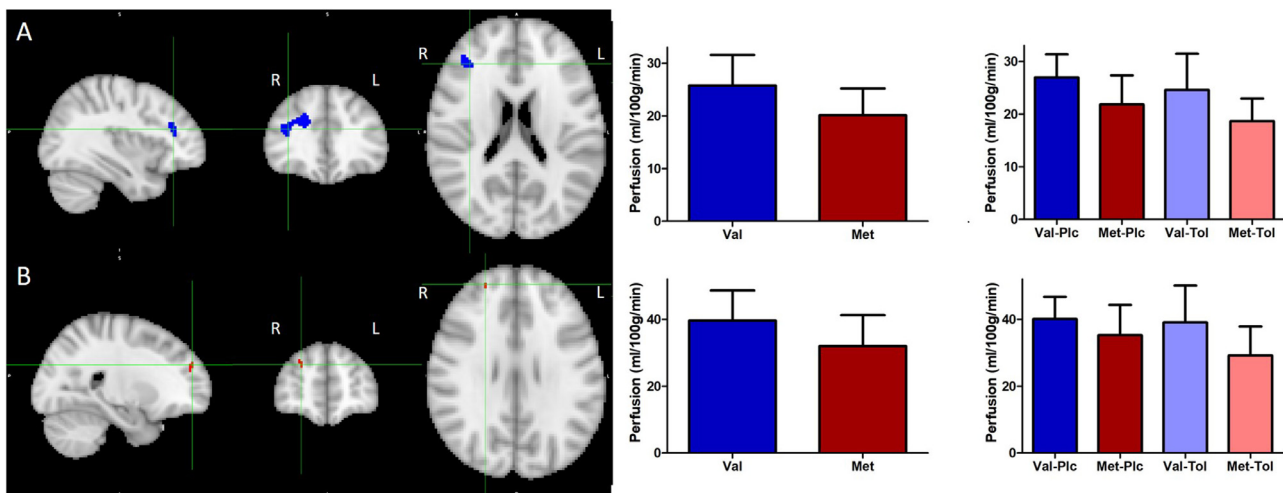
Both COMT genotype and tolcapone treatment influenced resting brain perfusion, but there was no interaction between them (see Table 2; Figs. 2 and 3). COMT genotype influenced perfusion in two frontal regions (Fig. 2). In both cases, perfusion was greater in Val¹⁵⁸ homozygotes, compared to Met¹⁵⁸ homozygotes. The largest cluster ($t=3.88$, maximum $p=0.0406$, MNI peak voxel co-ordinates: $x=36$, $y=23$, $z=20$, cluster size=274 voxels) comprises the right superior frontal and medial gyrus. The second cluster includes the superior frontal gyrus and the dorsolateral prefrontal cortex ($t=3.41$, $p=0.0497$, MNI peak voxel location: $x=24$, $y=52$, $z=28$, cluster size=9 voxels). Tolcapone administration reduced cerebral perfusion in parietal and temporal regions, compared to placebo (Fig. 3). The largest cluster was found in the right parietal lobe, surrounding the post-central gyrus ($t=4.99$, maximum $p=0.003$, MNI peak voxel location: $x=20$, $y=-34$, $z=72$, cluster size=1089 voxels). The other two clusters were found in the right superior parietal lobule ($t=3.73$, maximum $p=0.044$, MNI peak voxel location: $x=34$, $y=-48$, $z=36$, cluster size=144 voxels) and right superior temporal gyrus

Table 1
Demographic and physiological measures of participants.

Values are mean \pm SD Number	COMT-Met Placebo 17	COMT-Val placebo 19	COMT-Met tolcapone 20	COMT-Val tolcapone 20
Age (years)	22.47 \pm 3.9	23.53 \pm 4.6	23.60 \pm 6.6	25.00 \pm 6.4
NART ^a predicted Full scale-IQ (non-native speakers excluded)	113.2 \pm 3.8	112.2 \pm 5.0	111.5 \pm 5.6	113.3 \pm 4.2
Global grey matter perfusion	45.90 \pm 6.5	47.84 \pm 4.8	43.33 \pm 4.9	45.65 \pm 7.3
Pulse (beats per minute)	71 \pm 13	73 \pm 16	78 \pm 18	73 \pm 17
Skin Conductance level	3.89 \pm 2.8	3.75 \pm 2.7	4.5 \pm 3.1	4.73 \pm 5.7

^a NART: National Adult Reading Test**Table 2**
Clusters showing effects of COMT genotype or tolcapone on resting cerebral blood flow.

	Brain area / Brodmann Area (BA)	Hemisphere	Cluster size (voxels)	Voxel max (x,y,z)	MNI max (x,y,z)	Subgroup perfusion values (ml/100 g/min; confidence intervals)	
Main effect of genotype							
Cluster 1	Middle frontal and superior frontal gyrus, BA6, BA9 and BA32	R	274	27, 80, 46	24, 52, 28	Val¹⁵⁸ 23.87–27.64	Met¹⁵⁸ 18.42–21.81
Cluster 2	Superior frontal gyrus including BA9	R	9	33, 89, 50	36, 34, 20	36.65–42.50	28.88–35.06
Main effect of drug							
Cluster 1	Post-central gyrus, including BA3, BA4, BA5, BA6 and BA7	R	1089	35, 46, 72	20, -34, 72	Placebo 14.93–19.44	Tolcapone 9.23–11.67
Cluster 2	Superior parietal lobule, including BA7 and BA40	R	144	28, 39, 54	34, -48, 36	29.94–34.29	22.55–26.94
Cluster 3	Superior temporal gyrus BA22	R	77	23, 48, 36	44, -30, 0	26.21–31.05	20.25–23.38

**Fig. 2.** COMT genotype is associated with resting CBF in frontal regions. Resting CBF was greater in Val vs. Met homozygotes in two clusters: (A) the right superior frontal and medial gyrus, and (B) a cluster including the superior frontal gyrus and the dorsolateral prefrontal cortex. In each case, thresholded images are shown in the left panel, with perfusion in the two genotype groups (pooled across drug groups) in the middle panel, and in all four groups in the right panel.

($t=4.73$, $p=0.0404$, MNI peak voxel location: $x=44$, $y=-30$, $z=0$, cluster size=77 voxels) respectively.

There were no main or interactive effects of genotype or drug on global GM perfusion (F 's < 3.0 ; p 's > 0.09 ; Table 1), nor were there any voxel-wise differences in local GM volume between genotype groups (p 's > 0.18).

4. Discussion

Our findings show that low COMT activity in men, resulting either from the presence of the Met¹⁵⁸ allele or from tolcapone administration, is associated with regional reductions in CBF, compared to high COMT activity groups. However, drug and genotype affected distinct regions, with COMT genotype influencing frontal regions, whilst tolcapone affected parietal and temporal regions. There were no interactive effects between them. There were no statistically significant effects of drug or

genotype on global GM perfusion, nor were there genotype differences in brain structure, as assessed with FSL-VBM.

The mechanism(s) by which COMT influences resting CBF remain obscure. Given its widespread expression (Fig. 1), it is plausible that its effects on CBF might be a consequence either of its effects on neuronal activity, or potential vascular effects. Although COMT Val¹⁵⁸Met genotype shows associations with blood pressure (Annerbrink et al., 2008; Hagen et al., 2007; Htun et al., 2011) it seems unlikely that this peripheral effect is the sole explanation for the COMT-related differences in regional CBF observed here, both because CBF is tightly autoregulated in normotensive individuals (Cipolla, 2009) and because blood pressure effects on CBF would be expected to be global in nature. Moreover, tolcapone does not affect systemic blood pressure (Dingemanse et al., 1995). These facts therefore strongly imply that COMT's effects are, at least in part, mediated by its central actions, although we cannot rule out some contribution from global changes in perfusion. Our findings

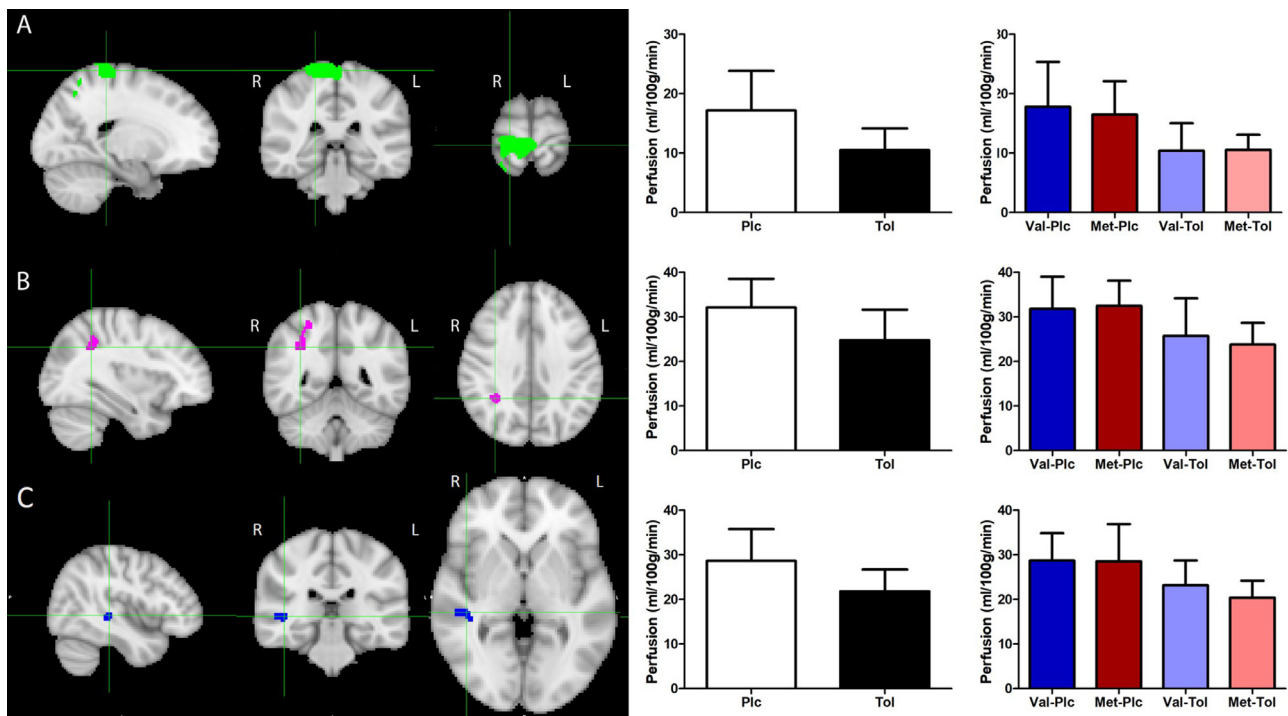


Fig. 3. Tolcapone administration was associated with lower resting CBF in in parietal and temporal regions, compared to placebo. Drug group differences were observed in three clusters: (A) the right parietal lobe, surrounding the post-central gyrus, (B) the right superior parietal lobule, and (C) the right superior temporal gyrus. In each case, thresholded images are shown in the left panel, with perfusion in the two drug groups (pooled across genotype groups) in the middle panel, and in all four groups in the right panel.

do not speak to the neurochemical basis of this effect, but it is notable that COMT has been robustly shown to alter dopamine in the absence of effects on noradrenaline (Karoum et al., 1994; Tunbridge et al., 2004; Yavich et al., 2007) making it likely that any central effects are dopaminergic in nature. Whilst it is possible that the observed changes in CBF are indirect, secondary to COMT-related differences in neuronal activity, it is also plausible that they result from direct effects on the central vasculature. Thus, at least in rats, COMT is expressed in perivascular astrocytes and capillary endothelia (Karhunen et al., 1995), as well as in the basilar artery tunica adventitia (Karhunen et al., 1994), and so is well-placed to contribute to the dopaminergic regulation of the cerebral microvasculature (Krimer et al., 1998). Our findings therefore underscore the complex dynamics linking neuronal and vascular function, and the possibility that agents that influence dopamine function may have non-linear effects involving both processes.

Dopamine projections are found throughout the primate neocortex (Lewis et al., 1987); similarly, COMT shows widespread expression (GTEx Consortium et al., 2017). We found that the regions affected by COMT genotype and tolcapone differ from one another, arguing that the mechanisms underlying drug and genotype effects on CBF are distinct. The effects of acute COMT inhibition by tolcapone presumably reflect the localisation of neuronal and/or perivascular COMT across the brain, perhaps coupled with heterogeneity in dopaminergic receptor distribution and, possibly, with regional variation in tolcapone penetrance into the brain parenchyma. In contrast, since the Val¹⁵⁸Met polymorphism likely encodes a ‘trait’ difference in COMT activity and therefore dopamine function, its effects on resting CBF may result from genotype-related compensatory changes within brain networks. For example, since cortical dopamine lesions lead to enhanced striatal dopamine function, and vice versa (Pycocok et al., 1980), the differences in cortical dopamine tone (Egan et al., 2001; Mier et al., 2009; Tunbridge et al., 2013) associated with the COMT Val¹⁵⁸Met polymorphisms might indirectly result in differences in subcortical dopamine function via these compensatory mechanisms (Tunbridge et al., 2012).

Since little is known about the timescales over which these compensatory changes in dopamine function occur, it would be of interest to examine the effects of both acute and chronic tolcapone administration in one of the mouse models of the Val¹⁵⁸Met polymorphism (Barkus et al., 2016; Gogos et al., 1998; Risbrough et al., 2014) to better understand spatial and temporal dynamics of COMT’s effects on brain function.

Very few studies have investigated the impact of COMT on resting CBF and, even amongst this small number, findings are mixed. To our knowledge, only one other study has used ASL to determine the effect of COMT genotype on regional CBF. It found bidirectional effects of COMT genotype in children (Thomason et al., 2009): the Met allele was associated with greater CBF in a majority of regions, including frontal and temporal cortices, but a few, including the vermis of the cerebellum, showed the opposite pattern. However, it is difficult to extrapolate the findings of this study to adults, given marked changes in COMT’s activity and the dopamine system more generally across postnatal development including adolescence (Lewis, 1997; Tunbridge et al., 2006a). Eisenberg and colleagues (Eisenberg et al., 2010) assessed CBF using positron emission tomography and found differential effects of genotype on CBF between regions: the Met allele predicted greater CBF in the left orbital cortex and medial temporal gyrus, but lower CBF in a cingulate gyrus region. Finally, one study demonstrated reductions in CBF rate associated with the Met allele, but regional CBF was not investigated (Raz et al., 2017). Thus, further studies are required to confirm the genotype-associated changes in regional CBF that we observe here, as well as to establish whether these findings extrapolate to women. It will also be of interest to examine whether there is an allele dose effect by examining heterozygotes.

Our findings highlight the importance of considering brain perfusion in studies examining brain activation determined using BOLD fMRI. Note that, whilst perfusion differences could explain observed differences in the BOLD response, they might also act to mask or reduce genuine drug or genotype effects on neuronal activation determined using this approach. It is difficult to predict which of these options might be

expected in the case of differences in COMT activity (whether resulting from drug or genotype). For example, in the case of COMT genotype, regional CBF differences were observed in the frontal cortex, a region in which Val¹⁵⁸Met-related differences in task-related activation are frequently observed (Egan et al., 2001; Mier et al., 2009), with the Val¹⁵⁸ allele typically associated with greater task-evoked BOLD response for cognitive tasks, and the Met¹⁵⁸ allele has been linked with greater task-evoked activity for emotional processing tasks (Egan et al., 2001; Mier et al., 2009). Although the non-linear relationship between the CBF and BOLD signals (Miller et al., 2001) means that caution should be exercised when interpreting BOLD response in light of CBF findings, several studies suggest an *inverse* correlation between resting cortical perfusion and the magnitude of the BOLD response (Brown et al., 2003; Stefanovic et al., 2006). This simplistic model would predict that the higher resting perfusion seen in Val¹⁵⁸ homozygotes might result in a relatively *attenuated* task-evoked BOLD response, compared to Met¹⁵⁸ homozygotes. Notably, this is the opposite of what is typically observed for cognitive tasks (which have been better studied than emotional processing tasks) (Mier et al., 2009). Given inconsistencies in the COMT neuroimaging literature (Nickl-Jockschat et al., 2015), it will therefore be of interest to see if more reliable COMT genotype-related differences are observed in BOLD fMRI studies in which individual differences in resting CBF are determined.

From a practical point of view, we would recommend that researchers wishing to control for perfusion in their BOLD studies (be these task-related or resting state scans) do so by entering perfusion maps as voxel-dependent explanatory variables in their analyses. Wherever possible we would recommend performing the ASL scan immediately prior to BOLD measurements to minimise the impact of any state-dependent factors on perfusion measures. However, ASL measures show good between-session reproducibility between scanning sessions (Wang et al., 2011b), indicating that perfusion measures are relatively stable over time. Nevertheless, decisions about whether to use ASL data collected at a different timepoint as regressors in analyses of the BOLD response will depend on the details of the experiment in question. For example, in the case of the data presented here, it would make little sense to use ASL data collected in individuals on tolcapone as regressors for fMRI data collected in the absence of the drug, given the drug's effects on perfusion. In contrast, since the effects of COMT genotype presumably persist over time, where perfusion measures cannot be collected at the same time as functional measures, it may be valuable to include perfusion measures collected in a different scanning session to provide the most accurate estimate of the BOLD response.

When interpreting the results, it has to be taken into account that the standard Siemens ASL method used for this study has a large voxel size and runs PICORE-Q2TIPS pulsed ASL. This ASL technique was the only one available at the time of starting the study. Therefore, it was not possible to scan with the gold standard pseudo-continuous ASL (pcASL) (see (Alsop et al., 2015)). The current study did not correct for partial volume effects (PVE) in the ASL perfusion analysis, which is a potential limitation of the work. However, VBM analysis found no voxelwise differences between genotype groups in local GM volume so it is unlikely that the genotype-associated perfusion effects observed are the consequence of underlying differences in tissue partial volumes. PVE were controlled for in the ROI definition used to obtain the global GM perfusion results. Replication of this study with a pcASL method, with improved spatial resolution, would reduce the impact of PVE and provide more information on the effects of COMT genotype and inhibition in smaller regions of interest as compared to a whole brain analysis. We suggest such replication studies correct for PVE at the single subject level.

We found no effect of COMT genotype on GM maps, in line with findings from the majority of previous studies, which report no associations between COMT and brain structure (e.g. (Barnes et al., 2012; Tunbridge et al., 2013; Zinkstok et al., 2008) although see (Ohnishi et al., 2005; Rowe et al., 2010)). They are also consistent with a

lack of robust associations between COMT Val¹⁵⁸Met and any structural measures in the large-scale ($n=8428$) UK Biobank cohort (Elliott et al., 2018) (Data obtained from big.stats.ox.ac.uk [Version 2] on 28th June 2019). Thus, COMT Val¹⁵⁸Met genotype effects on fMRI measures – whether ASL or BOLD – are unlikely to be confounded by group differences in brain structure.

5. Conclusion

Our data highlight the importance of understanding resting CBF in order to accurately interpret BOLD fMRI data. At present, it is unclear to what extent our findings of pharmacological and genetic effects on regional CBF will generalise beyond COMT, and beyond the healthy young adult male participant group studied here. However, given the increasing availability and sensitivity of ASL, and the simplicity of adding an ASL sequence to BOLD fMRI studies, we would advocate the routine collection of data of this type. Our findings also have relevance for the interpretation of the BOLD fMRI data in large-scale cohorts, which often do not have associated CBF data (Sudlow et al., 2015).

Data and Code availability statement

The data that support the findings of this study are available from the corresponding author (MM), upon reasonable request. The code used for these analyses is publicly available, as detailed in the Methods section.

Declaration of Competing Interest

PJH and EMT are in receipt of an Unrestricted Educational Grant from J&J Innovations for research unrelated to that presented here.

Credit authorship contribution statement

MAG Martens: Investigation, Formal analysis, Writing – original draft, Project administration. **FA Kennedy McConnell:** Formal analysis, Writing – review & editing. **N Filippini:** Investigation, Writing – review & editing. **CE Mackay:** Conceptualization, Resources, Writing – review & editing, Funding acquisition. **PJ Harrison:** Conceptualization, Supervision, Writing – review & editing, Funding acquisition. **EM Tunbridge:** Conceptualization, Supervision, Writing – original draft, Funding acquisition.

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References

- Alsop, D.C., Detre, J.A., Golay, X., Günther, M., Hendrikse, J., Hernandez-Garcia, L., Lu, H., MacIntosh, B.J., Parkes, L.M., Smits, M., van Osch, M.J., Wang, D.J., Wong, E.C., Zaharchuk, G., 2015. Recommended implementation of arterial spin-labeled perfusion MRI for clinical applications: A consensus of the ISMRM perfusion study group and the European consortium for ASL in dementia. *Magn Reson Med.* 73, 102–116.
- Annerbrink, K., Westberg, L., Nilsson, S., Rosmond, R., Holm, G., Eriksson, E., 2008. Catechol O-methyltransferase val158-met polymorphism is associated with abdominal obesity and blood pressure in men. *Metab. - Clin. Exp.* 57, 708–711.
- Apud, J.A., Mattay, V., Chen, J., Kolachana, B.S., Callicott, J.H., Rasetti, R., Alce, G., Iudicello, J.E., Akbar, N., Egan, M.F., Goldberg, T.E., Weinberger, D.R., 2006. Tolcapone improves cognition and cortical information processing in normal human subjects. *Neuropsychopharmacology* 32, 1011.
- Barkus, C., Korn, C., Stumpfenhorst, K., Laatikainen, L.M., Ballard, D., Lee, S., Sharp, T., Harrison, P.J., Bannerman, D.M., Weinberger, D.R., Chen, J., Tunbridge, E.M., 2016. Genotype-dependent effects of COMT inhibition on cognitive function in a highly specific, novel mouse model of altered COMT activity. *Neuropsychopharmacol.: Off. Publ. Am. Coll. Neuropsychopharmacol.* 41, 3060–3069.
- Barnes, A., Isohanni, M., Barnett, J.H., Pietiläinen, O., Veijola, J., Miettinen, J., Paunio, T., Tanskanen, P., Ridler, K., Suckling, J., Bullmore, E.T., Murray, G.K., Jones, P.B., 2012. No association of COMT (Val158Met) genotype with brain structure differences between men and women. *PLoS One* 7, e33964.
- Brown, G.G., Zorilla, L.T.E., Georgy, B., Kindermann, S.S., Wong, E.C., Buxton, R.B., 2003. BOLD and perfusion response to finger-thumb apposition after acetazolamide administration: differential relationship to global perfusion. *J. Cereb. Blood Flow Metab.* 23, 829–837.
- Ceravolo, R., Piccini, P., Bailey, D.L., Jorga, K.M., Bryson, H., Brooks, D.J., 2002. 18F-dopa PET evidence that tolcapone acts as a central COMT inhibitor in Parkinson's disease. *Synapse* 43, 201–207.
- Chappell, M.A., Groves, A.R., Whitcher, B., Woolrich, M.W., 2009. Variational Bayesian inference for a nonlinear forward model. *Trans. Sig. Proc.* 57, 223–236.
- Cipolla, M.J., 2009. Chapter 5, Control of Cerebral Blood Flow., *The Cerebral Circulation. Morgan & Claypool Life Sciences*, San Rafael (CA).
- Detre, J.A., Rao, H., Wang, D.J., Chen, Y.F., Wang, Z., 2012. Applications of arterial spin labeled MRI in the brain. *J. Magn. Reson. Imaging* 35, 1026–1037.
- Dingemans, J., Jorga, K.M., Schmitt, M., Gieschke, R., Fotteler, B., Zürcher, G., Prada, M., van Brummelen, P., 1995. Integrated pharmacokinetics and pharmacodynamics of the novel catechol-O-methyltransferase inhibitor tolcapone during first administration to humans. *Clin. Pharmacol. Therapeut.* 57, 508–517.
- Douaud, G., Smith, S., Jenkinson, M., Behrens, T., Johansen-Berg, H., Vickers, J., James, S., Voets, N., Watkins, K., Matthews, P.M., James, A., 2007. Anatomically related grey and white matter abnormalities in adolescent-onset schizophrenia. *Brain* 130, 2375–2386.
- 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.
- Egan, M.F., Goldberg, T.E., Kolachana, B.S., Callicott, J.H., Mazzanti, C.M., Straub, R.E., Goldman, D., Weinberger, D.R., 2001. Effect of COMT Val108/158 Met genotype on frontal lobe function and risk for schizophrenia. *Proc. Natl. Acad. Sci.* 98, 6917–6922.
- Eisenberg, D.P., Sarpal, D., Kohn, P.D., Meyer-Lindenberg, A., Wint, D., Kolachana, B., Apud, J., Weinberger, D.R., Berman, K.F., 2010. Catechol-O-methyltransferase valine158methionine genotype and resting regional cerebral blood flow in medication-free patients with schizophrenia. *Biol. Psychiatry* 67, 287–290.
- Elliott, L.T., Sharp, K., Alfaro-Almagro, F., Shi, S., Miller, K.L., Douaud, G., Marchini, J., Smith, S.M., 2018. Genome-wide association studies of brain imaging phenotypes in UK Biobank. *Nature* 562, 210–216.
- Farrell, S.M., Tunbridge, E.M., Braeutigam, S., Harrison, P.J., 2012. COMT Val158Met genotype determines the direction of cognitive effects produced by catechol-O-methyltransferase inhibition. *Biol. Psychiatry* 71, 538–544.
- 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.
- Giakoumaki, S.G., Roussos, P., Bitsios, P., 2008. Improvement of prepulse inhibition and executive function by the COMT inhibitor tolcapone depends on COMT Val158Met polymorphism. *Neuropsychopharmacology* 33, 3058–3068.
- Gogos, J.A., Morgan, M., Luine, V., Santha, M., Ogawa, S., Pfaff, D., Karayiorgou, M., 1998. Catechol-O-methyltransferase-deficient mice exhibit sexually dimorphic changes in catecholamine levels and behavior. *Proc. Natl. Acad. Sci.* 95, 9991–9996.
- Good, C.D., Johnsrude, I.S., Ashburner, J., Henson, R.N.A., Friston, K.J., Frackowiak, R.S.J., 2001. A voxel-based morphometric study of ageing in 465 normal adult human brains. *NeuroImage* 14, 21–36.
- GTEx Consortium, Aguet, F., Brown, A.A., Castel, S.E., Davis, J.R., He, Y., Jo, B., Mohammadi, P., Park, Y., Parsana, P., Segrè, A.V., Strober, B.J., Zappala, Z., Cummings, B.B., Gelfand, E.T., Hadley, K., Huang, K.H., Lek, M., Li, X., Nedzel, J.L., Nguyen, D.Y., Noble, M.S., Sullivan, T.J., Tukiainen, T., MacArthur, D.G., Getz, G., Addington, A., Guan, P., Koester, S., Little, A.R., Lockhart, N.C., Moore, H.M., Rao, A., Struwing, J.P., Volpi, S., Brigham, L.E., Hasz, R., Hunter, M., Johns, C., Johnson, M., Kopen, G., Leinweber, W.F., Lonsdale, J.T., McDonald, A., Mestichelli, B., Myer, K., Roe, B., Salvatore, M., Shad, S., Thomas, J.A., Walters, G., Washington, M., Wheeler, J., Bridge, J., Foster, B.A., Gillard, B.M., Karasik, E., Kumar, R., Miklos, M., Moser, M.T., Jewell, S.D., Montroy, R.G., Rohrer, D.C., Valley, D., Mash, D.C., Davis, D.A., Sobin, L., Barcus, M.E., Branton, P.A., Abell, N.S., Bal-liu, B., Delaneau, O., Frésard, L., Gamazon, E.R., Garrido-Martín, D., Gewirtz, A.D.H., Gliner, G., Gloude-mans, M.J., Han, B., He, A.Z., Hormozdiari, F., Li, X., Liu, B., Kang, E.Y., McDowell, I.C., Ongen, H., Palowitch, J.J., Peterson, C.B., Quon, G., Ripke, S., Saha, A., Shabalin, A.A., Shimko, T.C., Sul, J.H., Teran, N.A., Tsang, E.K., Zhang, H., Zhou, Y.-H., Bustamante, C.D., Cox, N.J., Guigó, R., Kellis, M., McCarthy, M.I., Conrad, D.F., Eskin, E., Li, G., Nobel, A.B., Sabatti, C., Stranger, B.E., Wen, X., Wright, F.A., Ardlie, K.G., Dermitzakis, E.T., Lappalainen, T., Aguet, F., Ardlie, K.G., Cummings, B.B., Gelfand, E.T., Getz, G., Hadley, K., Handsaker, R.E., Huang, K.H., Kashin, S., Karczewski, K.J., Lek, M., Li, X., MacArthur, D.G., Nedzel, J.L., Nguyen, D.T., Noble, M.S., Segrè, A.V., Trowbridge, C.A., Tukiainen, T., Abell, N.S., Balliu, B., Barshir, R., Basha, O., Battle, A., Bogu, G.K., Brown, A., Brown, C.D., Castel, S.E., Chen, L.S., Chiang, C., Conrad, D.F., Cox, N.J., Damani, F.N., Davis, J.R., Delaneau, O., Dermitzakis, E.T., Engelhardt, B.E., Eskin, E., Ferreira, P.G., Frésard, L., Gamazon, E.R., Garrido-Martín, D., Gewirtz, A.D.H., Gliner, G., Gloude-mans, M.J., Guigo, R., Hall, I.M., Han, B., He, Y., Hormozdiari, F., Howald, C., Kyung-Im, H., Jo, B., Yong-Kang, E., Kim, Y., Kim-Hellmuth, S., Lappalainen, T., Li, G., Li, X., Liu, B., Mangul, S., McCarthy, M.I., McDowell, I.C., Mohammadi, P., Monlong, J., Montgomery, S.B., Muñoz-Aguirre, M., Ndungu, A.W., Nicolae, D.L., Nobel, A.B., Oliva, M., Ongen, H., Palowitch, J.J., Panousis, N., Papasaiaks, P., Park, Y., Parsana, P., Payne, A.J., Peterson, C.B., Quan, J., Reverter, F., Sabatti, C., Saha, A., Sammeth, M., Scott, A.J., Shabalin, A.A., Sodaie, R., Stephens, M., Stranger, B.E., Strober, B.J., Sul, J.H., Tsang, E.K., Urbut, S., van-de-Bunt, M., Wang, G., Wen, X., Wright, F.A., Xi, H.S., Yeger-Lotem, E., Zappala, Z., Zaugg, J.B., Zhou, Y.-H., Akey, J.M., Bates, D., Chan, J., Chen, L.S., Claussnitzer, M., Demanelis, K., Diegel, M., Doherty, J.A., Feinberg, A.P., Fernando, M.S., Halow, J., Hansen, K.D., Haugen, E., Hickey, P.F., Hou, L., Jasmine, F., Jian, R., Jiang, L., Johnson, A., Kaul, R., Kellis, M., Kibriya, M.G., Lee, K., Billy Li, J., Li, Q., Li, X., Lin, J., Lin, S., Linder, S., Linke, C., Liu, Y., Maurano, M.T., Molin, B., Montgomery, S.B., Nelson, J., Neri, F.J., Oliva, M., Park, Y., Pierce, B.L., Rinaldi, N.J., Rizzardi, L.F., Sandstrom, R., Skol, A., Smith, K.S., Snyder, M.P., Stamatiouanopoulos, J., Stranger, B.E., Tang, H., Tsang, E.K., Wang, L., Wang, M., Van Wittenberghe, N., Wu, F., Zhang, R., Nierras, C.R., Branton, P.A., Carithers, L.J., Guan, P., Moore, H.M., Rao, A., Vaught, J.B., Gould, S.E., Lockart, N.C., Martin, C., Struwing, J.P., Volpi, S., Addington, A.M., Koester, S.E., Little, A.R., Brigham, L.E., Hasz, R., Hunter, M., Johns, C., Johnson, M., Kopen, G., Leinweber, W.F., Lonsdale, J.T., McDonald, A., Mestichelli, B., Myer, K., Roe, B., Salvatore, M., Shad, S., Thomas, J.A., Walters, G., Washington, M., Wheeler, J., Bridge, J., Foster, B.A., Gillard, B.M., Karasik, E., Kumar, R., Miklos, M., Moser, M.T., Jewell, S.D., Montroy, R.G., Rohrer, D.C., Valley, D.R., Davis, D.A., Mash, D.C., Undale, A.H., Smith, A.M., Tabor, D.E., Roche, N.V., McLean, J.A., Vatanian, N., Robinson, K.L., Sobin, L., Barcus, M.E., Valentino, K.M., Qi, L., Hunter, S., Hariharan, P., Singh, S., Um, K.S., Matose, T., Tomaszewski, M.M., Barker, L.K., Mosavel, M., Siminoff, L.A., Traino, H.M., Flicek, P., Juettemann, T., Ruffier, M., Sheppard, D., Taylor, K., Trevanion, S.J., Zerbino, D.R., Craft, B., Goldman, M., Haessler, M., Kent, W.J., Lee, C.M., Paten, B., Rosenbloom, K.R., Vivian, J., Zhu, J., 2017. Genetic effects on gene expression across human tissues. *Nature* 550, 204.
- Hagen, K., Pettersen, E., Stovner, L.J., Skorpén, F., Holmen, J., Zwart, J.-A., 2007. High systolic blood pressure is associated with Val/Val genotype in the catechol-O-methyltransferase gene: the nord-trøndelag health study (HUNT). *Am. J. Hypertens.* 20, 21–26.
- 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.
- Htun, N.C., Miyaki, K., Song, Y., Ikeda, S., Shimbo, T., Muramatsu, M., 2011. Association of the catechol-O-methyl transferase gene Val158Met polymorphism with blood pressure and prevalence of hypertension: interaction with dietary energy intake. *Am. J. Hypertens.* 24, 1022–1026.
- Karhunen, T., Tilgmann, C., Ulmanen, I., Julkunen, I., Panula, P., 1994. Distribution of catechol-O-methyltransferase enzyme in rat tissues. *J. Histochem. Cytochem.* 42, 1079–1090.
- Karhunen, T., Tilgmann, C., Ulmanen, I., Panul, P., 1995. Catechol-O-methyltransferase (COMT) in rat brain: immunoelectron microscopic study with an antiserum against rat recombinant COMT protein. *Neurosci. Lett.* 187, 57–60.
- Karoum, F., Chrapusta, S.J., Egan, M.F., 1994. 3-Methoxytyramine is the major metabolite of released dopamine in the rat frontal cortex: reassessment of the effects of antipsychotics on the dynamics of dopamine release and metabolism in the frontal cortex, nucleus accumbens, and striatum by a simple two pool model. *J. Neurochem.* 63, 972–979.
- 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.
- Krimer, L.S., Muly, E.C., Williams, G.V., Goldman-Rakic, P.S., 1998. Dopaminergic regulation of cerebral cortical microcirculation. *Nat. Neurosci.* 1, 286–289.
- Laatikainen, L.M., Sharp, T., Harrison, P.J., Tunbridge, E.M., 2013. Sexually dimorphic effects of catechol-O-methyltransferase (COMT) inhibition on dopamine metabolism in multiple brain regions. *PLoS One* 8, e61839.
- Lewis, D., Campbell, M., Foote, S., Goldstein, M., Morrison, J., 1987. The distribution of tyrosine hydroxylase-immunoreactive fibers in primate neocortex is widespread but regionally specific. *J. Neurosci.* 7, 279–290.
- Lewis, D.A., 1997. Development of the prefrontal cortex during adolescence: insights into vulnerable neural circuits in schizophrenia. *Neuropsychopharmacology* 16, 385–398.

- 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.
- Luh, W.-M., Wong, E.C., Bandettini, P.A., Hyde, J.S., 1999. QUIPSS II with thin-slice T1 periodic saturation: a method for improving accuracy of quantitative perfusion imaging using pulsed arterial spin labeling. *Magn. Reson. Med.* 41, 1246–1254.
- Magalona, S.C., Rasetti, R., Chen, J., Chen, Q., Gold, I., Decot, H., Callicott, J.H., Berman, K.F., Apud, J.A., Weinberger, D.R., Mattay, V.S., 2013. Effect of tolcapone on brain activity during a variable attentional control task: a double-blind, placebo-controlled, counter-balanced trial in healthy volunteers. *CNS Drugs* 27, 663–673.
- Mier, D., Kirsch, P., Meyer-Lindenberg, A., 2009. Neural substrates of pleiotropic action of genetic variation in COMT: a meta-analysis. *Mol. Psychiatry* 15, 918.
- Miller, K.L., Luh, W.M., Liu, T.T., Martinez, A., Obata, T., Wong, E.C., Frank, L.R., Buxton, R.B., 2001. Nonlinear temporal dynamics of the cerebral blood flow response. *Hum. Brain Mapp.* 13, 1–12.
- Murphy, S.E., Mackay, C.E., 2011. Using MRI to measure drug action: caveats and new directions. *J. Psychopharmacol.* 25, 1168–1174.
- Nickl-Jockschat, T., Janouschek, H., Eickhoff, S.B., Eickhoff, C.R., 2015. Lack of meta-analytic evidence for an impact of COMT Val158Met genotype on brain activation during working memory tasks. *Biol. Psychiatry* 78, e43–e46.
- Ogawa, S., Lee, T.-M., 1990. Magnetic resonance imaging of blood vessels at high fields: In vivo and in vitro measurements and image simulation. *Magn. Reson. Med.* 16, 9–18.
- Ohnishi, T., Hashimoto, R., Mori, T., Nemoto, K., Moriguchi, Y., Iida, H., Noguchi, H., Nakabayashi, T., Hori, H., Ohmori, M., Tsukue, R., Anami, K., Hirabayashi, N., Harada, S., Arima, K., Saitoh, O., Kunugi, H., 2005. The association between the Val158Met polymorphism of the catechol-O-methyl transferase gene and morphological abnormalities of the brain in chronic schizophrenia. *Brain* 129, 399–410.
- Pycoc, C.J., Kerwin, R.W., Carter, C.J., 1980. Effect of lesion of cortical dopamine terminals on subcortical dopamine receptors in rats. *Nature* 286, 74–77.
- Raz, N., Daugherty, A.M., Sethi, S.K., Arshad, M., Haacke, E.M., 2017. Age differences in arterial and venous extra-cerebral blood flow in healthy adults: contributions of vascular risk factors and genetic variants. *Brain Struct. Funct.* 222, 2641–2653.
- Risbrough, V., Ji, B., Hauger, R., Zhou, X., 2014. Generation and characterization of humanized mice carrying COMT158 Met/Val alleles. *Neuropsychopharmacol.: Off. Publ. Am. Coll. Neuropsychopharmacol.* 39, 1823–1832.
- Rowe, J.B., Hughes, L., Williams-Gray, C.H., Bishop, S., Fallon, S., Barker, R.A., Owen, A.M., 2010. The val158met COMT polymorphism's effect on atrophy in healthy aging and Parkinson's disease. *Neurobiol. Aging* 31, 1064–1068.
- Schouw, M.L.J., Kaag, A.M., Caan, M.W.A., Heijtel, D.F.R., Majoie, C.B.L.M., Nederveen, A.J., Booi, J., Reneman, L., 2013. Mapping the hemodynamic response in human subjects to a dopaminergic challenge with dextroamphetamine using ASL-based pharmacological MRI. *NeuroImage* 72, 1–9.
- Smith, S.M., Jenkinson, M., Woolrich, M.W., Beckmann, C.F., Behrens, T.E.J., Johansen-Berg, H., Bannister, P.R., De Luca, M., Drobnjak, I., Flitney, D.E., Niazy, R.K., Saunders, J., Vickers, J., Zhang, Y., De Stefano, N., Brady, J.M., Matthews, P.M., 2004. Advances in functional and structural MR image analysis and implementation as FSL. *NeuroImage* 23, S208–S219.
- Stefanovic, B., Warnking, J.M., Rylander, K.M., Pike, G.B., 2006. The effect of global cerebral vasodilation on focal activation hemodynamics. *NeuroImage* 30, 726–734.
- Sudlow, C., Gallacher, J., Allen, N., Beral, V., Burton, P., Danesh, J., Downey, P., Elliott, P., Green, J., Landray, M., Liu, B., Matthews, P., Ong, G., Pell, J., Silman, A., Young, A., Sprosen, T., Peakman, T., Collins, R., 2015. UK Biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. *PLOS Med.* 12, e1001779.
- Thomason, M.E., Waugh, C.E., Glover, G.H., Gotlib, I.H., 2009. COMT genotype and resting brain perfusion in children. *NeuroImage* 48, 217–222.
- Tunbridge, E., Weickert, C., Kleinman, J., Herman, M., Chen, J., Kolachana, B., Harrison, P., Weinberger, D., 2006a. Catechol-o-methyltransferase enzyme activity and protein expression in human prefrontal cortex across the postnatal lifespan. *Cerebr. Cortex* 17, 1206–1212.
- Tunbridge, E.M., Bannerman, D.M., Sharp, T., Harrison, P.J., 2004. Catechol-O-methyltransferase inhibition improves set-shifting performance and elevates stimulated dopamine release in the rat prefrontal cortex. *J. Neurosci.* 24, 5331–5335.
- Tunbridge, E.M., Farrell, S.M., Harrison, P.J., Mackay, C.E., 2013. Catechol-O-methyltransferase (COMT) influences the connectivity of the prefrontal cortex at rest. *NeuroImage* 68, 49–54.
- Tunbridge, E.M., Harrison, P.J., 2011. Importance of the COMT gene for sex differences in brain function and predisposition to psychiatric disorders. *Curr. Top. Behav. Neurosci.* 8, 119–140.
- Tunbridge, E.M., Harrison, P.J., Weinberger, D.R., 2006b. Catechol-o-methyltransferase, cognition, and psychosis: Val158Met and beyond. *Biol. Psychiatry* 60, 141–151.
- Tunbridge, E.M., Huber, A., Farrell, S.M., Stumpenhorst, K., Harrison, P.J., Walton, M.E., 2012. The role of catechol-O-methyltransferase in reward processing and addiction. *CNS Neurol. Disord. Drug Targets* 11, 306–323.
- Tunbridge, E.M., Narajos, M., Harrison, C.H., Beresford, C., Cipriani, A., Harrison, P.J., 2019. Which dopamine polymorphisms are functional? Systematic review and meta-analysis of COMT, DAT, DBH, DDC, DRD1–5, MAOA, MAOB, TH, VMAT1, and VMAT2. *Biol. Psychiatry* 86, 608–620.
- Wang, D.J.J., Chen, Y., Fernández-Seara, M.A., Detre, J.A., 2011a. Potentials and challenges for arterial spin labeling in pharmacological magnetic resonance imaging. *J. Pharmacol. Exp. Ther.* 337, 359–366.
- Wang, Y., Saykin, A.J., Pfeuffer, J., Lin, C., Mosier, K.M., Shen, L., Kim, S., Hutchins, G.D., 2011b. Regional reproducibility of pulsed arterial spin labeling perfusion imaging at 3T. *NeuroImage* 54, 1188–1195.
- Yavich, L., Forsberg, M.M., Karayiorgou, M., Gogos, J.A., Männistö, P.T., 2007. Site-specific role of catechol-O-methyltransferase in dopamine overflow within prefrontal cortex and dorsal striatum. *J. Neurosci.* 27, 10196–10209.
- Zinkstok, J., Schmitz, N., Van Amelsvoort, T., Moeton, M., Baas, F., Linszen, D., 2008. Genetic variation in COMT and PRODH is associated with brain anatomy in patients with schizophrenia. *Genes, Brain Behav.* 7, 61–69.
- Zurcher, G., Keller, H.H., Kettler, R., Borgulya, J., Bonetti, E.P., Eigenmann, R., Da Prada, M., 1990. Ro 40-7592, a novel, very potent, and orally active inhibitor of catechol-O-methyltransferase: a pharmacological study in rats. *Adv. Neurol.* 53, 497–503.