

# **Therapeutic window of dopamine D2/3 receptor occupancy to treat psychosis in Alzheimer's disease**

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**Abbreviations:** BBB = Blood brain barrier;  $BP_{ND}$  = Binding Potential non-displaceable;  $BP_{POST}$  = Post-treatment  $BP_{ND}$ ;  $BP_{PRE}$  = Pre-treatment  $BP_{ND}$ ; Cc = Blood drug concentration; D2/3 = Dopamine receptor subtypes 2 and 3; IC50 = Blood drug concentration associated with 50%  $I_{max}$ ; IIV = Interindividual variability;  $I_{max}$  = Maximum inhibitory effect of amisulpride at D2/3 receptors; MMSE = Mini Mental State Examination; NLME = Non-linear mixed effects modelling;  $C_{average}$  = Average steady state exposure across the dosage interval (24 hours); EPS = Extrapyramidal side effects; PK = Pharmacokinetic.

## Abstract

Antipsychotic drugs, originally developed to treat schizophrenia, are used to treat psychosis, agitation and aggression in Alzheimer's disease. In the absence of dopamine D2/3 receptor occupancy data to inform antipsychotic prescribing for psychosis in Alzheimer's disease, the mechanisms underpinning antipsychotic efficacy and side effects are poorly understood. This study used a population approach to investigate the relationship between amisulpride blood concentration and central D2/3 occupancy in older people with Alzheimer's disease by combining (i) Pharmacokinetic data (280 venous samples) from a phase I single (50mg) dose study in healthy older people (n=20, 65-79 years) (ii) Pharmacokinetic, [<sup>18</sup>F]fallypride D2/3 receptor imaging and clinical outcome data on patients with Alzheimer's disease who were prescribed amisulpride (25-75mg daily) to treat psychosis as part of an open study (n=28; 69-92 years; 41 blood samples, five pre-treatment scans, 19 post-treatment scans) (iii) [<sup>18</sup>F]fallypride imaging of an antipsychotic free Alzheimer's disease control group (n=10, 78-92 years), to provide additional pre-treatment data. Non-linear mixed effects modelling was used to describe pharmacokinetic-occupancy curves in caudate, putamen and thalamus. Model outputs were used to estimate threshold steady state blood concentration and occupancy required to elicit a clinically relevant response (>25% reduction in scores on delusions, hallucinations and agitation domains of the Neuropsychiatric Inventory) and extrapyramidal side-effects (Simpson Angus Scale scores >3). Average steady state blood levels were low (71±30 ng/ml), and associated with high D2/3 occupancies (65±8%, caudate; 67±11%, thalamus; 52±11%, putamen). Antipsychotic clinical response occurred at a threshold concentration of 20ng/ml and D2/3 occupancies of 43% (caudate), 25% (putamen), 43% (thalamus). Extrapyramidal side effects (n=7) emerged at a threshold

concentration of 60ng/ml, and D2/3 occupancies of 61% (caudate), 49% (putamen) and 69% (thalamus). This study has established that, as in schizophrenia, there is a therapeutic window of D2/3 receptor occupancy for optimal treatment of psychosis in Alzheimer's disease. We have also shown that occupancies within *and beyond* this window are achieved at very low amisulpride doses in Alzheimer's disease due to higher than anticipated occupancies for a given blood drug concentration. Our findings support a central pharmacokinetic contribution to antipsychotic sensitivity in Alzheimer's disease and implicate the blood brain barrier, which controls central drug access. Whether high D2/3 receptor occupancies are primarily accounted for by age- or disease-specific blood brain barrier disruption is unclear, and this is an important future area of future investigation, as it has implications beyond antipsychotic prescribing.

## Introduction

Establishing that 60-80% occupancy of striatal dopamine D2/3 receptors constitutes a ‘therapeutic window’ of antipsychotic prescribing (Farde *et al.*, 1992, Kapur *et al.*, 1995) was a landmark achievement for PET neuroreceptor imaging, and has had an enduring impact on the pharmacological management of schizophrenia. Optimal occupancy ranges to achieve a clinical response with minimal EPS have since been defined for both first and second generation antipsychotics (Stone *et al.*, 2009, Uchida *et al.*, 2011), and dosage strategies for young adults further refined through statistical modelling of PK and PK-occupancy data (Sparshatt *et al.*, 2009, Uchida *et al.*, 2011, Lako *et al.*, 2013). In stark contrast, there is a relative absence of empirical data in older patients and clinicians are required to adopt a ‘start low, go slow’ approach (Pretorius *et al.*, 2013) to reduce the associated risks. There is an urgent need to redress this balance, particularly in older people with dementia, in whom substantial morbidity and increased mortality have led to a restriction of antipsychotic use (Schneider *et al.*, 2006, Jeste *et al.*, 2008, Jennum *et al.*, 2015, Maust *et al.*, 2015).

The mechanisms underlying antipsychotic sensitivity in older people and Alzheimer’s disease are under-researched and poorly understood (Uchida *et al.*, 2009). Although age-related changes in peripheral PK, which reduce drug clearance, contribute to adverse events by increasing blood concentration for a given dose of drug (Feng *et al.*, 2008), this effect is not consistent across antipsychotics (Bigos *et al.*, 2008). Occupancy studies in older patients with schizophrenia (Uchida *et al.*, 2009, Uchida *et al.*, 2014, Graff-Guerrero *et al.*, 2015) suggest that pharmacodynamic changes, which lower the threshold occupancy for response and EPS, may be important. The D2/3 receptor mediated contribution to clinical outcome has not been investigated within the context of psychosis in Alzheimer’s disease.

In an imaging pilot study (Clark-Papasavas *et al.*, 2014), we observed high (40-70%) striatal and thalamic D2/3 occupancy in three patients Alzheimer’s disease during low dose

amisulpride (50mg daily) treatment; a finding which was difficult to meaningfully interpret in the absence of data on amisulpride blood concentrations. We have since developed a population PK model for amisulpride specifically for older people, by combining data from a phase one, single dose study in healthy elderly with data on patients with psychosis in Alzheimer's disease who were prescribed open amisulpride (25-75mg daily). There was wide variability in amisulpride blood concentrations (9-109ng/ml), which was largely accounted for by an effect of age on drug clearance (Reeves *et al.*, 2016). However, only one patient achieved amisulpride blood concentrations within the currently recommended therapeutic range (100-319ng/ml) to avoid non-response and EPS in schizophrenia (Hiemke *et al.*, 2011). The current study aimed to combine the PK model with [<sup>18</sup>F]fallypride D2/3 receptor imaging and clinical outcome data from the observational study and preceding imaging pilot, with the following objectives:

- (i) To use a population approach to characterise PK- occupancy profiles in caudate, putamen and thalamus
- (ii) To estimate  $C_{\text{average}}$  and corresponding occupancies across the prescribed dose range, and establish the threshold exposure and occupancy required for clinical response and EPS.

## **Material and Methods**

### **Data sources**

#### **PK data (healthy elderly)**

Venous blood samples (n=280) were obtained from 20 healthy older participants (10 men, mean age =  $68.7 \pm 4.1$ ) over 72 hours following a single 50mg amisulpride tablet (Hamon-Vilcot *et al.*, 1998). Amisulpride racemate concentrations were determined using a validated HPLC (high performance liquid chromatography) method (detection limit 0.5 ng/ml).

## **PK, imaging and clinical outcome data (Alzheimer's disease)**

Data from 38 patients with probable Alzheimer's disease (McKhann *et al.*, 1984) included (i) Pharmacokinetic, imaging and clinical outcome data on 25 patients with psychotic symptoms, prescribed amisulpride off label as part of an open study (Research ethics committee reference 11/SC/0486) (ii) Imaging data on 10 antipsychotic-free patients, recruited to provide additional 'pre-treatment' data to inform model development (iii) Paired imaging and clinical outcome data on three patients who participated in the pilot (Research ethics committee reference 10/H0807/75). Patients were recruited from the South London and Maudsley NHS Foundation Trust. All were antipsychotic naïve, and included on the basis of having no history of psychiatric illness, traumatic brain injury, epilepsy, significant cardiorespiratory disease, needle phobia, any contraindication to amisulpride, or features suggestive of Lewy Body Dementia (McKeith *et al.*, 1996). Verbal and written informed consent was obtained from participants, or appropriate carers where capacity was lacking. Clinical assessment, carried out at baseline and every two to four weeks during dose titration, included (i) Frequency x severity ratings for three Neuropsychiatric Inventory domains (delusions, hallucinations, agitation) (Cummings *et al.*, 1994) (ii) EPS rating (Simpson Angus Scale scores > 3) (Simpson and Angus, 1970). Patients commenced amisulpride at a dose of 25mg or 50mg, (based on the preference of the prescribing clinician), which was administered as a single evening dose, and increased to an optimum dose (25% or greater reduction in symptoms and minimal EPS). [<sup>18</sup>F]fallypride imaging was carried out at baseline (all patients) and at an 'optimum' dose (treated patients). Flexibility was built into the design around the timing of follow-up assessments, to account for variability in the dose titration regimen across prescribers. Compliance with medication (pill counts and discussion with carer), concomitant medication and clinical outcome (symptom ratings, side effects) were recorded at each visit. The timing of blood collection reflected convenience samples, which

coincided with follow-up assessments and/or imaging. Date, time of sample and hours since last dose (confirmed by a carer where possible) were recorded on the anonymised assay request form. Blood samples were analysed in a secure, CPA accredited laboratory (Clinical Toxicology Unit, Kings College Hospital). Amisulpride (racemate) blood concentrations were determined using validated LC-MS/MS (liquid chromatography with tandem mass spectrometry) method, with a detection limit of 9ng/ml.

### **PET image acquisition and analysis**

Participants were scanned between 12-2.30pm, using a GE (GE Healthcare, Hatfield, UK) VCT Discovery PET-CT camera (full width half maximum 5mm), at St Thomas' Hospital PET Centre, London. [<sup>18</sup>F]fallypride was administered via a single bolus intravenous injection of 250MBq. Each scan consisted of three 20 minute dynamic scans (three dimensional mode, each preceded by low dose CT for attenuation correction) at baseline (0--20, 70-90, 220-240 minutes) and post-treatment (0-20, 40-60, 130-150 minutes) (Dunn *et al.*, 2013, Clark-Papasavas *et al.*, 2014); shorter post-treatment scan duration reflecting the fact that occupancy of D2/3 receptor sites reduces the time for [<sup>18</sup>F]fallypride to achieve a transient equilibrium (Kegeles *et al.*, 2008, Vernaleken *et al.*, 2011). Image analysis was carried out (by EM) blind to treatment status. [<sup>18</sup>F]fallypride BP<sub>ND</sub> (Innis *et al.*, 2007) was quantified using a simplified reference tissue model with cerebellar reference region (Lammertsma and Hume, 1996). Preprocessing was performed using statistical parametric mapping version 8 ([www.fil.ion.ucl.ac.uk/spm](http://www.fil.ion.ucl.ac.uk/spm)) and all other analyses using Matlab ([www.mathworks.com](http://www.mathworks.com)). Non-attenuation corrected, three dimensional iteratively reconstructed PET scans (VuePoint<sup>®</sup>) were used for frame-by-frame realignment. Transformations were applied to attenuation corrected, filtered back projected PET images which were used for quantification, and attenuation corrected-VuePoint PET images used for

warping atlases. The cerebellum was defined using the Automated Anatomical Labeling atlas (Tzourio-Mazoyer *et al.*, 2002) and regions of interest (caudate, putamen, thalamus) were defined using the Tziortzi atlas (Tziortzi *et al.*, 2011). Atlases were warped to subject space via a [<sup>18</sup>F]fallypride template in standard space (Dunn *et al.*, 2013).

## Statistical analysis

Demographic data were analysed using statistical package for social sciences version 22.0. Correlations were expressed as Spearman's correlation coefficient  $r$ , and independent samples t-tests were used for group comparisons. PK-occupancy models were developed using an oral two compartment PK model (Reeves *et al.*, 2016) (see Table 2) and an Imax model to describe serial [<sup>18</sup>F]fallypride  $BP_{ND}$  data in each region, as follows:  $BP_{POST} = BP_{PRE} * [(1 - Imax * Cc) / (Cc + IC50)]$ , where  $BP_{PRE}$  and  $BP_{POST}$  represent pre- and post-treatment  $BP_{ND}$  respectively; Imax, which represents the maximum inhibitory effect of amisulpride at D2/3 receptors (1 corresponds to 100% occupancy); Cc, amisulpride blood concentration; and IC50, blood concentration associated with 50% Imax. NLME was implemented using Monolix software (version 4.33). Model parameters were estimated using a Stochastic Approximation Expectation Minimisation algorithm. NLME simultaneously estimates fixed effects (parameters which describe dose-concentration and concentration-occupancy relationships), and random effects, comprised of IIV (difference between individual and predicted model parameter values), and residual variability (system noise, dosage history errors and/or model misspecifications) (Ette *et al.*, 2004). IIV for PK and occupancy parameters was estimated using an exponential model  $P_i = P_{TV} \times e^{\eta_p}$  where  $P_i$  is the parameter estimate for the  $i$ th individual, and  $P_{TV}$  is the typical value for the parameter at the population level. Variability between  $i$ th individual and population parameter values ( $\eta$ ,  $\eta_p$ ), was assumed to be normally distributed (mean of 0, variance of  $\omega$ ,  $\omega_{\eta}^2$ ). Residual variability was described using a proportional residual error model ( $y_{ij} = \hat{y}_{ij} (1 + \varepsilon_{ij})$ ), where  $y_{ij}$  and  $\hat{y}_{ij}$

represents the  $j$ th observed  $BP_{ND}$  and corresponding model-predicted  $BP_{ND}$ ; and  $\epsilon_{ij}$  was assumed to be normally distributed (mean 0, variance  $\sigma^2$ ). Covariates included age and weight on drug clearance (using estimates from the previously developed PK model) and were restricted to age, gender and MMSE (Folstein *et al.*, 1975) for the Imax model. Models were evaluated using goodness-of-fit criteria, including diagnostic scatter plots, visual predictive checks, degree of shrinkage, change in IIV, model precision, and approximate likelihood ratio tests, based on log-likelihood and standard error of the Monte Carlo estimate. A change in log likelihood estimate was considered significant if equal to or greater than four (equivalent to  $P < 0.05$ , one degree of freedom), and accompanied by no change or a decrease in BIC (Bayesian Information Criteria).

Model predictions were used to estimate the following:

- i) Regional occupancy for individuals with no pre-treatment imaging data, using the equation  $[(BP_{PRE}-BP_{POST})/BP_{PRE}*100]$ .
- ii) Average steady state blood drug concentration across the dosage interval ( $C_{average}$ ) and corresponding regional occupancies across the dose titration range.
- iii) For the purposes of direct comparison with PK-occupancy data in young adults (Vernaleken *et al.*, 2004), blood concentrations required for 60% occupancy in caudate and putamen.
- iv) Blood drug concentration and corresponding occupancies in caudate and putamen at peak (four hours post dose) and trough (24 hours post dose).

## **Results**

### **Demographic and clinical characteristics of Alzheimer's disease patients**

There were 28 patients in the treated group (mean age 82.1  $\pm$  6.6 years; 11 (39%) men; mean Mini Mental State (MMSE) 17.7  $\pm$  5.4), all of whom experienced psychotic symptoms at baseline (26 (92.8%) delusions, 19 (67.8%) hallucinations), 20 (71.4%) associated agitation)

(described in Table 1); and 10 antipsychotic free patients (mean age 83.6±3.8 years, 4 (40%) men, mean MMSE 20.3±6.1). A total of 41 blood samples (2±0.78 per person; blood concentration 40.9±27.1ng/ml) were taken during steady state treatment (25-75mg daily); 16.2 ± 3.1 hours post dose; and after 56.9 ± 58 days treatment. During dose titration, 7 (25%) patients were withdrawn, due to EPS ( $n=5$ ), falls ( $n=1$ ), and unrelated health problems ( $n=1$ ). Subclinical EPS, which did not lead to treatment cessation, emerged in two patients (blood concentrations of 39ng/ml and 88 ng/ml at the time of imaging). All patients who completed the study ( $n=21$ ) were prescribed 50mg daily, and had achieved a mean reduction in symptom scores of 80 ± 27% (delusions), 95 ± 15% (hallucinations) and 84 ± 28% (agitation).

### **[<sup>18</sup>F] fallypride imaging pre- and post-treatment**

Baseline [<sup>18</sup>F]fallypride imaging (BP<sub>PRE</sub>) data was obtained in 15 patients (10 ‘antipsychotic free’, and five ‘treated’). Post-treatment imaging (BP<sub>POST</sub>) data was obtained from 19 patients, at 80 ± 58 days of treatment, 16±2 hours post dose. Amisulpride concentration was 42.4±31.9 ng/ml, apart from two samples which were below the limit of quantification. Mean administered [<sup>18</sup>F]fallypride dose was 246 ±4.7 MBq, with no difference between pre- and post-treatment groups ( $t = -0.66$ ,  $df = 32$ ,  $P = 0.51$ ). Mean BP<sub>PRE</sub> was 17.12±3.96 in the caudate, 22.68±4.83 in the putamen and 1.50±0.36 in the thalamus. Age was correlated with BP<sub>PRE</sub> in the caudate ( $r = -0.56$ ,  $P = 0.03$ ) and putamen ( $r = -0.54$ ,  $P = 0.03$ ), but not thalamus ( $r = -0.22$ ,  $P = 0.43$ ). Mean BP<sub>POST</sub> was 8.18±2.70 in the caudate, 14.52±4.40 in the putamen and 0.74±0.33 in the thalamus, and showed no relationship with treatment duration, after adjusting for dose (partial correlation coefficient,  $r < 0.2$ ,  $P > 0.43$  for each region). Fig 1 shows [<sup>18</sup>F]fallypride uptake A) before and B) after amisulpride treatment.

### **PK-occupancy model**

PK-occupancy parameters are shown in Table 2. Imax was 84.3% for the caudate (IC<sub>50</sub> 19.1 ng/ml), 98.7% for the putamen (IC<sub>50</sub> 61.3 ng/ml), and 100% for the thalamus (IC<sub>50</sub> 29.5

ng/ml). Fixed effects were estimated with good precision, apart from IC50 for the putamen (relative standard error 70%). Random effects were estimated on all parameters apart from IC50 (not estimated in any model), and I<sub>max</sub> (only estimated with precision for the caudate); with residual variability being estimated with better precision for PK (relative standard error 5%) than I<sub>max</sub> (relative standard error 48-58%) parameters. Base models were chosen as the best fit for the data, as covariate testing did not improve the model fit or precision. Occupancy estimates at the time of imaging (excluding patients with amisulpride concentrations below the limit of quantification) were 56±13% in the caudate, 39±17% in the putamen, and 59 ±12 % in the thalamus. EPS was present at a lower threshold of 45%, 24% and 61% in caudate, putamen and thalamus respectively (Fig 2). Occupancies were correlated across all regions ( $P < 0.0001$ ; correlation coefficient  $r$  ranged from 0.77 between thalamus and putamen, to 0.96 between caudate and putamen); and with amisulpride concentration ( $P < 0.0001$ ;  $r = 0.82$  for caudate and thalamus,  $r = 0.77$  for putamen), but not age ( $r < 0.2$ ,  $P > 0.4$  for all regions). Occupancy did not differ in those prescribed donepezil compared to those who were not ( $P > 0.25$  all regions). Scatterplots of BP<sub>PRE</sub> and BP<sub>POST</sub> data, visual predictive (percentile) plots and estimated occupancy at the time of imaging are shown in Fig 2 for A) caudate B) putamen and C) thalamus. Amisulpride concentrations required to achieve 60% occupancy were estimated as 48ng/ml for the caudate and 94ng/ml for the putamen.

### **Model predicted C<sub>average</sub>, occupancy and clinical outcome**

Outcome was evaluated across an estimated C<sub>average</sub> 71±30ng/ml (range 20-137ng/ml), and occupancies of 65±8% (range 43-84%) in the caudate, 52±11% (range 25-74%) in the putamen, and 67±11% (range 43-82%) in the thalamus. Clinically meaningful responses (25% or more reduction in symptoms) were achieved at a lower threshold of 20.1 ng/ml,

corresponding to occupancies of 43% in caudate and thalamus and 26% in the putamen. EPS emerged at a threshold  $C_{\text{average}}$  of 60ng/ml and corresponding occupancies of 61%, 49% and 69% for caudate, putamen and thalamus respectively. Figure 3 shows  $C_{\text{average}}$  plotted against predicted occupancy, and separated on the basis of EPS for A) caudate B) putamen and C) thalamus. Model estimates for  $C_{\text{average}}$ , blood concentrations at four and 24 hours post dose, and corresponding occupancies in caudate and putamen, are detailed in Table 3, separated on the basis of EPS.  $C_{\text{average}}$  was higher in those with emergent EPS (77.7  $\pm$  24.0 ng/ml versus 64.8  $\pm$  32.6 ng/ml), but did not achieve significance ( $P = 0.37$ ) and showed a similar pattern ( $P > 0.18$ ) across all comparisons.

## Discussion

Amisulpride is a highly D2/3 receptor selective antipsychotic drug for which optimal effective dose (400-800mg), blood concentration (100-319ng/ml), and striatal occupancy (40-70%) have been established in schizophrenia (Sparshatt *et al.*, 2009, Hiemke *et al.*, 2011), but for which there is limited data in patients aged over 65 years. This study is the first to characterise the relationship between blood levels, central D2/3 occupancy and clinical outcome in older patients with psychosis in the context of Alzheimer's disease. Low blood concentrations were associated with high central occupancies which overlapped with the reported therapeutic window described for young patients with schizophrenia (Bressan *et al.*, 2003, Sparshatt *et al.*, 2009, Lako *et al.*, 2013), and were associated with clinical response (greater than 25% reduction in psychotic symptoms) and EPS.

Occupancy data on low dose (50-100mg) amisulpride is limited and highly variable in young adults, with occupancy estimates ranging from below 10% (Martinot *et al.*, 1996, Xiberas *et al.*, 2001) to 40% (la Fougere *et al.*, 2005, Meisenzahl *et al.*, 2008) in the striatum and 30-40% in the thalamus (Xiberas *et al.*, 2001). These discrepancies are partly accounted for by

methodological differences - imaging modality, scan duration, and timing of dose relative to scan (Meisenzahl *et al.*, 2008) - but also reflect the steeper gradient of the occupancy slope at low blood concentrations, which means that small changes in concentration produce larger changes in occupancy (Vernaleken *et al.*, 2004). Choice of tracer is an additional consideration, as in vitro data have shown that tracers with higher affinity (low dissociation constant,  $K_d$ ) such as [ $^{18}\text{F}$ ]fallypride ( $K_d$  0.03nM) require higher concentrations of competing drug to displace them from the receptor site than lower affinity tracers such as [ $^{11}\text{C}$ ]raclopride ( $K_d$  1.9nM), resulting in lower apparent occupancies (Seeman and Van Tol, 1995, Seeman, 2011, Seeman, 2014). In vivo, under estimation of occupancy is more pronounced if D2/3 receptor availability is calculated prior to the onset of a transient equilibrium, and is particularly relevant for the striatum, as time to equilibrium is dependent on the density of receptor sites (Olsson and Farde, 2001, Xiberas *et al.*, 2001, Vernaleken *et al.*, 2011). Sampling times for the current study were therefore informed both by previous post-treatment [ $^{18}\text{F}$ ]fallypride protocols (Kessler *et al.*, 2006, Kegeles *et al.*, 2008) and the above studies.

Occupancies in the current study are at least as high as those previously reported, and up to twice as high (80% occupancy) in a proportion of patients. This is perhaps most clearly demonstrated by comparing our findings with those of Vernaleken *et al.* (Vernaleken *et al.*, 2004), who modelled the non-linear occupancy curve for [ $^{18}\text{F}$ ]desmethoxyfallypride, and whose estimates for amisulpride blood concentration required to achieve 60% occupancy in caudate (119ng/ml) and putamen (241 ng/ml) are higher than the current study (48ng/ml and 94 ng/ml for caudate and putamen respectively). Taking dissociation constants for the two tracers into account ([ $^{18}\text{F}$ ]fallypride, 0.03nM; [ $^{18}\text{F}$ ]desmethoxyfallypride, 0.34nM) (Mukherjee *et al.*, 1996), if anything we may be under-estimating the true extent of the differences between young adults and older patients with Alzheimer's disease, and it will be

important to directly compare older and younger adults in future studies, using comparable methodology (tracer, sampling times and modelling approach)

The finding of higher than anticipated occupancies for a given blood level provides the first *in vivo* evidence of a central pharmacokinetic contribution to antipsychotic sensitivity in Alzheimer's disease and strongly implicates the BBB, which controls central drug access (Zeevi *et al.*, 2010). Our findings are at odds with occupancy studies in older patients with schizophrenia (Uchida *et al.*, 2009, Graff-Guerrero *et al.*, 2015), in whom the relationship between blood concentration and occupancy was found to be similar to that observed in young people. Whether these differences are explained by the extreme age of participants in the current study, their diagnosis, or possibly both, is unclear, but there is certainly evidence that BBB integrity is disrupted (increased permeability and reduced expression of efflux transporters) with age and more profoundly affected by Alzheimer's disease (Zeevi *et al.*, 2010). A further consideration is our choice of amisulpride, which has low lipophilicity, shows poor BBB penetration relative to other antipsychotics (Natesan *et al.*, 2008) and has been identified as a substrate for several BBB transporters, including the efflux transporter p-glycoprotein (Schmitt *et al.*, 2012), and the widely expressed (brain, biliary, renal) organic cation transport system (Dos Santos Pereira *et al.*, 2014, Sekhar G, 2016). These properties may mean that amisulpride is a more sensitive tool with which to explore BBB disruption than antipsychotics previously studied.

We cannot rule out the possibility that age and/or disease-specific changes in distribution and clearance of amisulpride *within* the central nervous system (Uchida *et al.*, 2009), which could potentially increase synaptic levels of amisulpride, may have contributed to the high observed occupancies. Reduced competition with amisulpride at D2/3 receptor sites, due to age-related reductions in endogenous dopamine release (Volkow *et al.*, 1994), also needs to be

considered, although the magnitude of such changes would not be sufficient to account for the extent of the differences observed between our findings and those of previous studies.

The clinical dataset was limited by small sample size, sparse blood sampling and absence of pre-treatment imaging in a proportion of patients. It was thus important to combine the dataset with a richly sampled phase 1 study, to fully parameterise the PK model and, with the addition of paired scans from the imaging pilot, more precisely model PK-occupancy profiles. Model-based estimation of  $BP_{PRE}$  is an established method to calculate occupancy (Kim *et al.*, 2011), and one which produced the anticipated occupancy gradients for amisulpride - thalamus > striatum (Xiberas *et al.*, 2001, Bressan *et al.*, 2003) and caudate > putamen (Vernaleken *et al.*, 2004) - in the current study.

Use of a population approach allowed estimation of average, peak and trough blood drug concentrations, and corresponding occupancies across the dose titration range in all individuals, including those who were withdrawn due to EPS. The fact that the threshold occupancy for EPS in the striatum was lower than that observed in young people is broadly consistent with data on older patients with schizophrenia (Uchida *et al.*, 2009, Uchida *et al.*, 2014, Graff-Guerrero *et al.*, 2015) and suggests there may be an additional pharmacodynamic contribution to EPS. The absence of significant differences between maximal and minimal blood drug concentration and occupancy when the group was separated on the basis of EPS does not necessarily rule out a contribution to emergent side effects, as the study was not sufficiently powered to detect such differences. The population approach will be used to model and further explore the relationship of PK (average, peak and trough) and occupancy with EPS in future analyses.

Other limitations need to be considered, including variable length of follow-up, due to technical (cyclotron, radiochemistry) issues, which delayed post-treatment imaging in a proportion of patients. Although this could have potentially impacted on post-treatment D2/3

receptor availability, this is unlikely to have affected our findings, as  $BP_{POST}$  was not correlated with treatment duration. Non-adherence is a potential confound when modelling the relationship between PK, occupancy, response and toxicity and is a common issue in people with cognitive impairment (Jankowska-Polanska *et al.*, 2016). Although compliance was closely monitored and facilitated both by carers and clinical teams, tablet counts and self-report are not wholly reliable (Blaschke *et al.*, 2012) and it is likely that ‘undetectable’ amisulpride concentrations (below the 9ng/ml lower limit of the assay sensitivity) in two patients at the time of imaging reflect imperfect compliance. It is not possible to fully quantify the influence of non-adherence on amisulpride dose individualisation without further population modelling of clinical outcome data (Assawasuwannakit *et al.*, 2016). However, the terminal half life of amisulpride (which varies between 12.4 hours in those aged 65 years and 22.7 hours in those aged 85 years) (Reeves *et al.*, 2016), and the fact that occupancy decreases at a slower rate than blood drug concentration (Tauscher *et al.*, 2002, Takano *et al.*, 2004, Sekine *et al.*, 2011), would suggest that amisulpride is relatively ‘forgiving’ of imperfect adherence (Urquhart, 1997), as the duration of effect is likely to exceed the dosage interval.

The decision not to obtain volumetric MRI for co-registration with PET data was both pragmatic and scientific, based on our previous experience of imaging older and cognitively impaired older patients, in whom close confinement is poorly tolerated (Clark-Papasavas *et al.*, 2014), and the fact that D2/3 receptor binding can be reliably quantified using an atlas-based approach (Dunn *et al.*, 2013, Clark-Papasavas *et al.*, 2014). However, the fact that templates originated from young adults could have impacted upon the warping process, particularly in smaller, noisier regions (Dunn *et al.*, 2013); and partial volume effects (Morris *et al.*, 1999) may have further affected the accuracy of tracer quantification. The absence of volumetric MRI meant that we were unable to investigate the relationship between occupancy

and disease stage or vascular pathology and this should be investigated in future studies. Neither was the study sufficiently powered to examine the impact of concomitant medication, including donepezil, on PK-occupancy models. However, we have previously shown no significant effect of donepezil on striatal D2/3 receptor availability in patients with mild to moderate Alzheimer's disease, using [ $^{11}\text{C}$ ] raclopride PET (Reeves *et al.*, 2010) and similarly observed no differences in occupancy in relation to donepezil use in the current study. The issue of diagnosis is important when psychosis is present in the context of a dementia, and careful screening was thus carried out to exclude patients with suspected Lewy Body Dementia, including case note review, clinical assessment and, where appropriate, referral for dopamine transporter imaging (McKeith *et al.*, 1996). While we cannot completely rule out the possibility that Lewy body pathology may have contributed to the observed drug sensitivity, EPS are sufficiently explained by the higher than anticipated occupancies.

In summary, this study has shown that, similar to schizophrenia, there is a therapeutic window of D2/3 receptor occupancy for the optimal treatment of psychosis in Alzheimer's disease. Furthermore, we have clearly demonstrated that occupancies within and beyond this window are achieved at very low amisulpride doses and correspondingly low blood concentrations, due to higher than anticipated occupancies. These data provide the first *in vivo* evidence to support a central pharmacokinetic contribution to antipsychotic drug sensitivity, in a representative group of older people with Alzheimer's disease. Whether these findings are primarily explained by age or disease-specific BBB disruption, or a combination of both is unclear and this is an important avenue for future investigation. The population approach will be extended to clinical outcome data in future analyses, with the aim of establishing the relationship of PK and regional D2/3 receptor occupancy with response across individual symptom domains (delusions, hallucinations, agitation) and EPS, establish the target amisulpride concentration and D2/3 occupancy range required to optimally treat

psychosis in Alzheimer's disease, and provide clear guidance on age and weight based dose adjustments.

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## **Financial Disclosures**

The authors report no competing interests

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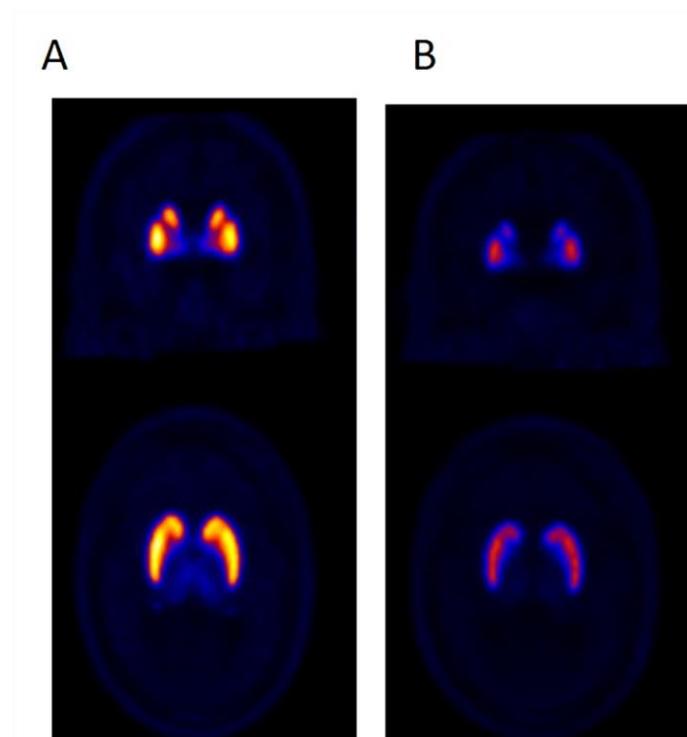
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## Figure Legends

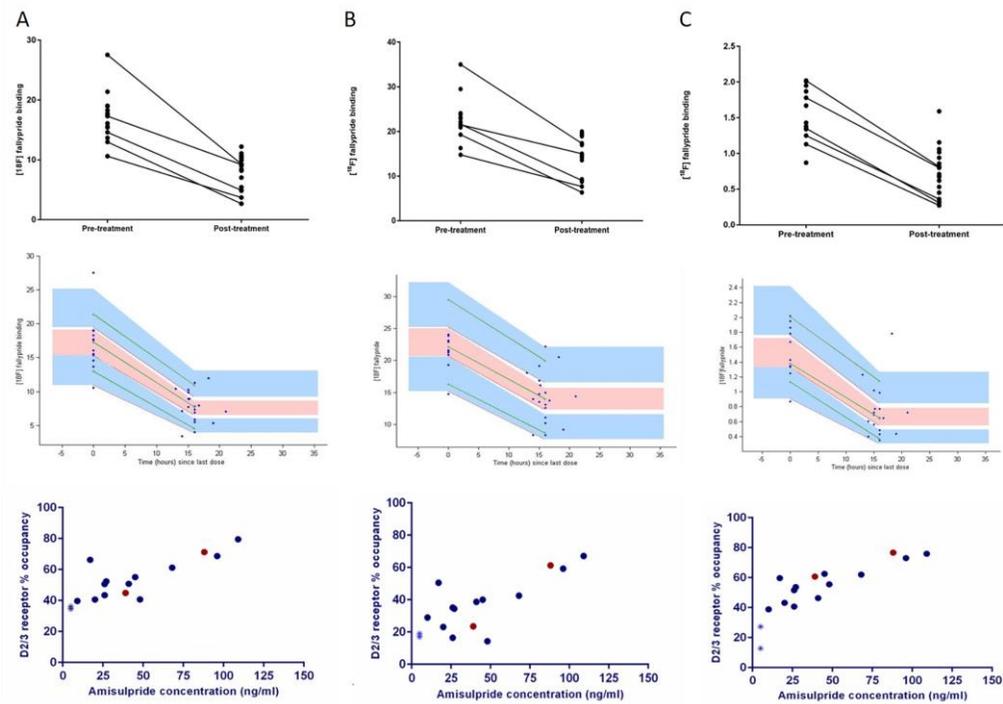
### Figure 1. Representative [<sup>18</sup>F] Fallypride images

[<sup>18</sup>F]Fallypride uptake images are shown in a 69 year old woman with Alzheimer's disease, from left to right, A) before B) after 56 days treatment with amisulpride 50mg/day, and from top to bottom, in coronal and transverse views. Images are displayed using the same colour scale.



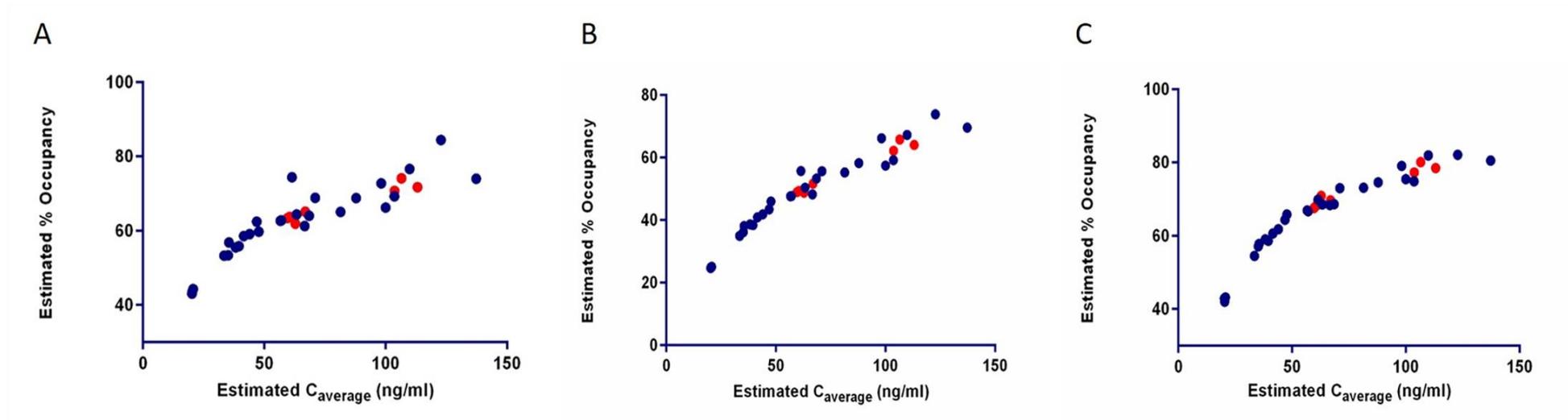
## Figure 2. PK-occupancy model and predictions

From left to right, in A) caudate B) putamen C) thalamus and from top to bottom: [ $^{18}\text{F}$ ]fallypride uptake pre and post treatment (lines connecting paired scans); visual predictive checks (VPC), 95% prediction intervals around 50<sup>th</sup> (pink) and 90<sup>th</sup> (blue) percentiles, overlaid to observed [ $^{18}\text{F}$ ]fallypride binding; and D2/3 receptor occupancies.



### Figure 3. Model estimated Coverage and corresponding occupancy

From left to right, in A) caudate B) putamen C) thalamus, model estimates for  $C_{\text{average}}$  across the dose titration range are plotted against corresponding D2/3 receptor occupancies, separated on the basis of EPS, present (red) or absent (blue).



<b>Characteristic</b>	<b>Values</b>
Age, Mean +-SD (range)	82.1+-6.6 (69-92)
Men, Number (%)	11 (39)
Mini Mental State Examination, Mean +- SD (range)	17.7+-5.4 (7-26)
Ethnicity, Number (%)	
White British or Other	16 (57)
Black African, Caribbean or Other	12 (43)
Living status, Number (%)	
Living alone *	8 (29)
Living with family	17 (61)
Care Home	3 (10)
Prescribed medication, Number (%)	
Donepezil	9 (32)
Memantine	4 (14)
Antidepressant	3 (10)
Benzodiazepines	4 (14)
Symptom rating at baseline, Mean +-SD (range)	
Delusions †	8.5+-4 (0-12)
Hallucinations †	5.8+-4.8 (0-12)
Agitation †	5.5+-4.4 (0-12)
Simpson Angus Scale	0.25+-0.6 (0-2)

\* compliance aided by dosette box and/or informal and formal carers

† frequency x severity scores for the relevant Neuropsychiatric Inventory domain (maximum score of 12)

<b>Parameter</b>	<b>Estimate (RSE%)</b>	<b>Interindividual variability % (RSE%)</b>	<b>Residual variability % (RSE%)</b>
<b>PK model (oral 2 compartment)</b>			17.6 (5)
Absorption constant, $k_a$ (/L)	0.84 (18)	46.8 (23)	
Clearance from central compartment, $Cl$ (L/hr) *†	49.2 (7)	49.5 (10)	
Central volume of distribution, $V_1$ (L)	452 (15)	46.2 (22)	
Inter-compartmental clearance, $Q$ (L/hr)	112 (16)	65.2 (19)	
Peripheral volume of distribution, $V_2$ (L)	759 (11)	48.7 (17)	
<b>PK- Caudate Imax model</b>			15.3 (58)
$BP_{PRE}$	17.3 (5)	13.5 (40)	
$I_{max}$ (%)	83.4 (11)	8.9 (36)	
$IC_{50}$ (ng/ml)	19.1 (40)	not estimated	
<i>-2 x log likelihood = -2104</i> <i>Bayesian Information Criteria = - 1999</i>			
<b>PK- Putamen Imax model</b>			14.4 (50)
$BP_{PRE}$	23.0 (5)	12.1 (41)	
$I_{max}$ (%)	98.7 (33)	9.6 (67)	
$IC_{50}$ (ng/ml)	61.3 (70)	not estimated	
<i>-2x log likelihood = - 2081</i> <i>Bayesian Information Criteria = - 1975</i>			
<b>PK-Thalamus Imax model</b>			17.2 (48)
$BP_{PRE}$	1.54 (6)	17.5 (33)	
$I_{max}$ %	100 (13)	not estimated	
$IC_{50}$ (ng/ml)	29.5 (42)	not estimated	
<i>-2x log likelihood =- 2262</i> <i>Bayesian Information Criteria =- 2161</i>			

\* Allometric scaling (power 0.75) fixed for weight on  $CL$ ; † Power effect of -3.21 estimated for age on  $CL$

$BP_{PRE}$  - baseline [ $^3F$ ] fallypride binding potential

$I_{max}$  - maximum inhibitory effect, expressed as a percentage D2/3 receptor occupancy

$IC_{50}$  - amisulpride concentration needed to obtain 50%  $I_{max}$

RSE- relative standard error

<b>Model estimate (mean +-SD)</b>	<b>No EPS (n=21)</b>	<b>EPS (n=7)</b>	<b>P*</b>
<b>Average:</b>			
Blood drug concentration (ng/ml)	64.8+-32.6	77.7+-24.0	0.37
Occupancy, caudate (%)	62.7+-10.1	66.1+-4.1	0.43
Occupancy, putamen (%)	48.7+-13.6	54.1+-7.0	0.34
<b>Peak (4 hours post dose)</b>			
Blood drug concentration (ng/ml)	306.7+-175.8	361.4+-138.9	0.48
Occupancy, caudate (%)	78.3+-6.14	78.7+-2.2	0.91
Occupancy, putamen (%)	79.2+-82.8	82.8+-4.9	0.45
<b>Trough (24 hours post dose)</b>			
Blood drug concentration (ng/ml)	36.0+-24.0	41.1+-19.6	0.63
Occupancy, caudate (%)	50.2+-15.2	54.7+-8.7	0.49
Occupancy, putamen (%)	33.9+-15.9	37.9+-10.9	0.57

*EPS, extrapyramidal side effects*

*\* estimated by independent samples t-tests*