

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

Suzanne Reeves,<sup>1,2</sup> Emma McLachlan,<sup>2</sup> Julie Bertrand,<sup>3</sup> Fabrizia D'Antonio,<sup>1,2</sup> Stuart Brownings,<sup>1</sup> Akshay Nair,<sup>1</sup> Suki Greaves,<sup>4</sup> Alan Smith,<sup>4</sup> David Taylor,<sup>4</sup> Joel Dunn,<sup>5</sup> Paul Marsden,<sup>5</sup> Robert Kessler<sup>6</sup> and Robert Howard<sup>1,2</sup>

See Caravaggio and Graff-Guerrero (doi:10.1093/awx023) for a scientific commentary on this article.

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 (50 mg) 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 (25–75 mg daily) to treat psychosis as part of an open study ( $n = 28$ ; 69–92 years; 41 blood samples, five pretreatment scans, 19 post-treatment scans); and (iii) <sup>18</sup>F-fallypride imaging of an antipsychotic free Alzheimer's disease control group ( $n = 10$ , 78–92 years), to provide additional pretreatment 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 ( $71 \pm 30$  ng/ml), and associated with high D2/3 occupancies ( $65 \pm 8\%$ , caudate;  $67 \pm 11\%$ , thalamus;  $52 \pm 11\%$ , putamen). Antipsychotic clinical response occurred at a threshold concentration of 20 ng/ml and D2/3 occupancies of 43% (caudate), 25% (putamen), 43% (thalamus). Extrapyramidal side effects ( $n = 7$ ) emerged at a threshold concentration of 60 ng/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.

1 Division of Psychiatry, 149 Tottenham Court Road, London W1T 7NF, University College London, UK

2 Department of Old Age Psychiatry, London, SE58AF, Kings College London, UK

3 UMR 1137 IAME INSERM University Paris 7, France; and Genetics Institute, WC1E6BT, University College London, UK

4 South London and Maudsley NHS Foundation Trust, London, SE58AZ, UK

5 Division of Imaging Sciences, St Thomas Hospital, London, SE17EH, UK

6 University of Alabama, Birmingham, AL 35294-4550, USA

Received August 26, 2016. Revised October 20, 2016. Accepted November 30, 2016.

© The Author (2017). Published by Oxford University Press on behalf of the Guarantors of Brain. All rights reserved.

For Permissions, please email: journals.permissions@oup.com

Correspondence to: Dr Suzanne Reeves,  
Clinical Senior Lecturer in Old Age Psychiatry,  
Division of Psychiatry, Faculty of Brain Sciences, University College London,  
6<sup>th</sup> Floor, Maple House, 149 Tottenham Court Road,  
London W1T 7NF, UK

**Keywords:** amisulpride; Alzheimer's; psychosis; D2/3 occupancy; <sup>18</sup>F-fallypride

**Abbreviations:** BP<sub>ND/POST/PRE</sub> = binding potential non-displaceable/post-treatment/pretreatment; C<sub>average</sub> = average steady state exposure across the dosage interval (24 h); EPS = extrapyramidal side effects; IC<sub>50</sub> = blood drug concentration associated with 50% I<sub>max</sub>; I<sub>max</sub> = maximum inhibitory effect of amisulpride at D2/3 receptors

## 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 extrapyramidal side effects (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 pharmacokinetic and pharmacokinetic-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.*, 2009b). Although age-related changes in peripheral pharmacokinetics, 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.*, 2009a, 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 with Alzheimer's disease during low dose amisulpride treatment (50 mg daily); a finding that was difficult to meaningfully interpret in the absence of data on amisulpride blood concentrations. We have since developed a population pharmacokinetic model for amisulpride specifically for older people, by combining data from a phase 1, single dose study in healthy elderly subjects with data on patients with psychosis in Alzheimer's disease who were prescribed open amisulpride (25–75 mg daily). There was wide variability in amisulpride blood concentrations (9–109 ng/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 (100–319 ng/ml) to avoid non-response and EPS in schizophrenia (Hiemke *et al.*, 2011). The current study aimed to combine the pharmacokinetic 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 characterize pharmacokinetic occupancy profiles in caudate, putamen and thalamus; and (ii) to estimate the average steady state exposure across the dosage interval (24 h) (C<sub>average</sub>) and corresponding occupancies across the prescribed dose range, and establish the threshold exposure and occupancy required for clinical response and EPS.

## Material and methods

### Pharmacokinetic data in the healthy elderly subjects

Venous blood samples ( $n = 280$ ) were obtained from 20 healthy older participants (10 males, mean age =  $68.7 \pm 4.1$ ) over 72 h following a single 50 mg 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).

## Pharmacokinetic, imaging and clinical outcome data in patients with 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 'pretreatment' data to inform model development; and (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 2 to 4 weeks during dose titration, included: (i) frequency  $\times$  severity ratings for three Neuropsychiatric Inventory domains (delusions, hallucinations, agitation) (Cummings *et al.*, 1994); and (ii) EPS rating (Simpson Angus Scale scores  $>$  3) (Simpson and Angus, 1970). Patients commenced amisulpride at a dose of 25 mg or 50 mg, (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).  $^{18}\text{F}$ -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 anonymized 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 9 ng/ml.

## PET image acquisition and analysis

Participants were scanned between 12.00–2.30 pm, using a GE (GE Healthcare) VCT Discovery PET-CT camera

(full-width at half-maximum, 5 mm), at St Thomas' Hospital PET Centre, London.  $^{18}\text{F}$ -fallypride was administered via a single bolus intravenous injection of 250 MBq. Each scan consisted of three 20-min dynamic scans (3D mode, each preceded by low dose CT for attenuation correction) at baseline (0–20, 70–90, 220–240 min) and post-treatment (0–20, 40–60, 130–150 min) (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  $^{18}\text{F}$ -fallypride to achieve a transient equilibrium (Kegeles *et al.*, 2008; Vernaleken *et al.*, 2011). Image analysis was carried out (using electron microscopy) blind to treatment status.  $^{18}\text{F}$ -fallypride non-displaceable binding potential ( $\text{BP}_{\text{ND}}$ ) (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, 3D iteratively reconstructed PET scans (GE 'VuePoint') 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  $^{18}\text{F}$ -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. Pharmacokinetic-occupancy models were developed using an oral two-compartment pharmacokinetic model (Reeves *et al.*, 2016) (Table 2) and an  $I_{\text{max}}$  model to describe serial  $^{18}\text{F}$ -fallypride  $\text{BP}_{\text{ND}}$  data in each region, as follows:

$$\text{BP}_{\text{POST}} = \text{BP}_{\text{PRE}} \times [(1 - I_{\text{max}} \times C_c)/(C_c + \text{IC}_{50})] \quad (1)$$

where  $\text{BP}_{\text{PRE}}$  and  $\text{BP}_{\text{POST}}$  represent pre- and post-treatment  $\text{BP}_{\text{ND}}$  respectively;  $I_{\text{max}}$ , which represents the maximum inhibitory effect of amisulpride at D2/3 receptors (1 corresponds to 100% occupancy);  $C_c$ , amisulpride blood concentration; and  $\text{IC}_{50}$ , blood concentration associated with 50%  $I_{\text{max}}$ . Non-linear mixed effects modelling (NLME) was implemented using Monolix software (version 4.33). Model parameters were estimated using a stochastic approximation expectation minimization algorithm. NLME simultaneously estimates fixed effects (parameters which describe dose-concentration and concentration-occupancy relationships), and random effects, comprised of interindividual variability (difference between individual and

predicted model parameter values), and residual variability (system noise, dosage history errors and/or model misspecifications) (Ette *et al.*, 2004). Interindividual variability for pharmacokinetic 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  $\varepsilon_{ij}$  was assumed to be normally distributed (mean 0, variance  $\sigma$ ,  $\sigma^2$ ). Covariates included age and weight on drug clearance (using estimates from the previously developed pharmacokinetic model) and were restricted to age, gender and Mini-Mental State Examination (MMSE; Folstein *et al.*, 1975) for the  $I_{max}$  model. Models were evaluated using goodness-of-fit criteria, including diagnostic scatter plots, visual predictive checks, degree of shrinkage, change in interindividual variability, model precision, and approximate likelihood ratio tests, based on log-likelihood and standard error (SE) of the Monto Carlo estimate. A change in log likelihood estimate was considered significant if  $\geq 4$  (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 pretreatment imaging data, using the equation [ $(BP_{PRE} - BP_{POST}) / BP_{PRE} \times 100$ ]; (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 pharmacokinetic-occupancy data in young adults (Vernaleken *et al.*, 2004), blood concentrations required for 60% occupancy in caudate and putamen; and (iv) blood drug concentration and corresponding occupancies in caudate and putamen at peak (4 h post-dose) and trough (24 h post-dose).

## Results

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

There were 28 patients in the treated group [mean age  $82.1 \pm 6.6$  years; 11 (39%) males; mean 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] (Table 1); and 10 antipsychotic-free patients [mean age  $83.6 \pm 3.8$  years, 4 (40%) males, mean MMSE  $20.3 \pm 6.1$ ]. A total of 41 blood samples ( $2 \pm 0.78$  per person; blood concentration

**Table 1 Demographic and clinical characteristics of treated patients (n = 28)**

Characteristic	Values
Age, mean $\pm$ SD (range)	82.1 $\pm$ 6.6 (69–92)
Male, n (%)	11 (39)
MMSE, mean $\pm$ SD (range)	17.7 $\pm$ 5.4 (7–26)
Ethnicity, n (%)	
White British or other	16 (57)
Black African, Caribbean or other	12 (43)
Living status, n (%)	
Living alone <sup>a</sup>	8 (29)
Living with family	17 (61)
Care home	3 (10)
Prescribed medication, n (%)	
Donepezil	9 (32)
Memantine	4 (14)
Antidepressant	3 (10)
Benzodiazepines	4 (14)
Symptom rating at baseline, mean $\pm$ SD (range)	
Delusions <sup>b</sup>	8.5 $\pm$ 4 (0–12)
Hallucinations <sup>b</sup>	5.8 $\pm$ 4.8 (0–12)
Agitation <sup>b</sup>	5.5 $\pm$ 4.4 (0–12)
Simpson Angus Scale	0.25 $\pm$ 0.6 (0–2)

<sup>a</sup>Compliance aided by dosette box and/or informal and formal carers.

<sup>b</sup>Frequency  $\times$  severity scores for the relevant Neuropsychiatric Inventory domain (maximum score of 12).

$40.9 \pm 27.1$  ng/ml) were taken during steady state treatment (25–75 mg daily);  $16.2 \pm 3.1$  h post-dose; and after  $56.9 \pm 58$  days treatment. During dose titration, seven (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 39 ng/ml and 88 ng/ml at the time of imaging). All patients who completed the study ( $n = 21$ ) were prescribed 50 mg daily, and had achieved a mean reduction in symptom scores of  $80 \pm 27\%$  (delusions),  $95 \pm 15\%$  (hallucinations) and  $84 \pm 28\%$  (agitation).

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

Baseline <sup>18</sup>F-fallypride imaging ( $BP_{PRE}$ ) data were obtained in 15 patients (10 'antipsychotic free', and five 'treated'). Post-treatment imaging ( $BP_{POST}$ ) data were obtained from 19 patients, at  $80 \pm 58$  days of treatment,  $16 \pm 2$  h post dose. Amisulpride concentration was  $42.4 \pm 31.9$  ng/ml, apart from two samples, which were below the limit of quantification. Mean administered <sup>18</sup>F-fallypride dose was  $246 \pm 4.7$  MBq, with no difference between pre- and post-treatment groups ( $t = -0.66$ ,  $df = 32$ ,  $P = 0.51$ ). Mean  $BP_{PRE}$  was  $17.12 \pm 3.96$  in the caudate,  $22.68 \pm 4.83$  in the putamen and  $1.50 \pm 0.36$  in the thalamus. Age was correlated with  $BP_{PRE}$  in the caudate ( $r = -0.56$ ,  $P = 0.03$ ) and putamen ( $r = -0.54$ ,  $P = 0.03$ ), but not thalamus



**Table 2 Pharmacokinetic D2/3 receptor occupancy models**

Parameter	Estimate (RSE%)	Interindividual variability % (RSE%)	Residual variability % (RSE%)
<b>PK model (oral 2 compartment)</b>			
Absorption constant, $k_a$ (1/l)	0.84 (18)	46.8 (23)	17.6 (5)
Clearance from central compartment, $Cl$ (l/h) <sup>a,b</sup>	49.2 (7)	49.5 (10)	
Central volume of distribution, $V_1$ (l)	452 (15)	46.2 (22)	
Inter-compartmental clearance, $Q$ (l/h)	112 (16)	65.2 (19)	
Peripheral volume of distribution, $V_2$ (l)	759 (11)	48.7 (17)	
<b>PK- Caudate Imax model</b>			
$BP_{PRE}$	17.3 (5)	13.5 (40)	15.3 (58)
$I_{max}$ (%)	83.4 (11)	8.9 (36)	
IC50 (ng/ml)	19.1 (40)	Not estimated	
$-2 \times \log$ likelihood = -2104			
Bayesian Information Criteria = -1999			
<b>PK- Putamen Imax model</b>			
$BP_{PRE}$	23.0 (5)	12.1 (41)	14.4 (50)
$I_{max}$ (%)	98.7 (33)	9.6 (67)	
IC50 (ng/ml)	61.3 (70)	Not estimated	
$-2 \times \log$ likelihood = -2081			
Bayesian Information Criteria = -1975			
<b>PK- Thalamus Imax model</b>			
$BP_{PRE}$	1.54 (6)	17.5 (33)	17.2 (48)
$I_{max}$ %	100 (13)	Not estimated	
IC50 (ng/ml)	29.5 (42)	Not estimated	
$-2 \times \log$ likelihood = -2262			
Bayesian Information Criteria = -2161			

<sup>a</sup>Allometric scaling (power 0.75) fixed for weight on clearance (CL).

<sup>b</sup>Power effect of -3.21 estimated for age on clearance (CL).

$BP_{PRE}$  = baseline <sup>18</sup>F-fallypride binding potential;  $I_{max}$  = maximum inhibitory effect, expressed as a percentage D2/3 receptor occupancy; IC50 = amisulpride concentration needed to obtain 50%  $I_{max}$ ; RSE = relative standard error; PK = pharmacokinetic.

( $r = -0.22$ ,  $P = 0.43$ ). Mean  $BP_{POST}$  was  $8.18 \pm 2.70$  in the caudate,  $14.52 \pm 4.40$  in the putamen and  $0.74 \pm 0.33$  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). Figure 1 shows <sup>18</sup>F-fallypride uptake before and after amisulpride treatment.

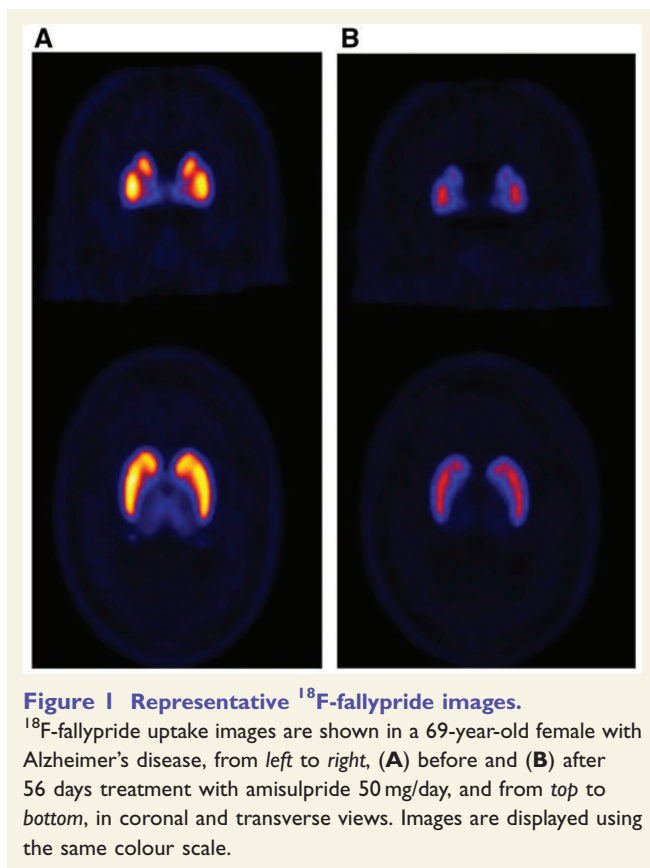
## Pharmacokinetic-occupancy model

Pharmacokinetic-occupancy parameters are shown in Table 2.  $I_{max}$  was 84.3% for the caudate (IC50 19.1 ng/ml), 98.7% for the putamen (IC50 61.3 ng/ml), and 100% for the thalamus (IC50 29.5 ng/ml). Fixed effects were estimated with good precision, apart from IC50 for the putamen (relative SE 70%). Random effects were estimated on all parameters apart from IC50 (not estimated in any model), and  $I_{max}$  (only estimated with precision for the caudate); with residual variability being estimated with better precision for pharmacokinetic (relative SE 5%) than  $I_{max}$  (relative SE 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

$56 \pm 13\%$  in the caudate,  $39 \pm 17\%$  in the putamen, and  $59 \pm 12\%$  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  $BP_{PRE}$  and  $BP_{POST}$  data, visual predictive (percentile) plots and estimated occupancy at the time of imaging are shown in Fig. 2A–C for caudate, putamen and thalamus. Amisulpride concentrations required to achieve 60% occupancy were estimated as 48 ng/ml for the caudate and 94 ng/ml for the putamen.

## Model predicted $C_{average}$ , occupancy and clinical outcome

Outcome was evaluated across an estimated  $C_{average}$   $71 \pm 30$  ng/ml (range 20–137 ng/ml), and occupancies of  $65 \pm 8\%$  (range 43–84%) in the caudate,  $52 \pm 11\%$



(range 25–74%) in the putamen, and  $67 \pm 11\%$  (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 60 ng/ml and corresponding occupancies of 61%, 49% and 69% for caudate, putamen and thalamus, respectively.

Figure 3A–C shows  $C_{\text{average}}$  plotted against predicted occupancy, and separated on the basis of EPS for caudate, putamen, and thalamus. Model predicted  $C_{\text{average}}$ , estimated blood concentrations at 4 and 24 h 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 ( $P > 0.34$ ) across all comparisons.

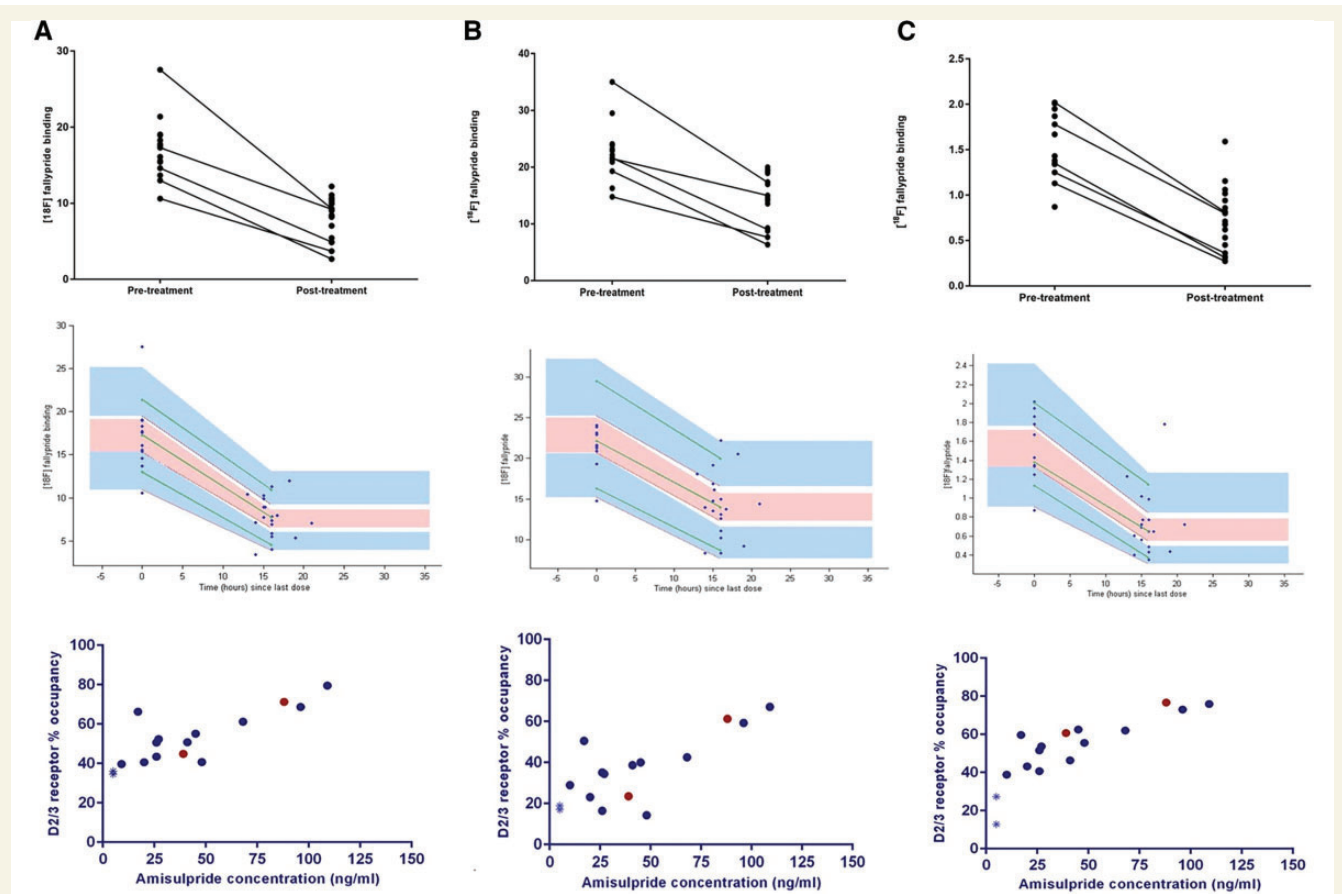
## Discussion

Amisulpride is a highly D2/3 receptor selective antipsychotic drug for which optimal effective dose (400–800 mg), blood concentration (100–319 ng/ml), and striatal occupancy (40–70%) have been established in schizophrenia (Sparshatt et al., 2009; Hiemke et al., 2011), but for which there are limited data in patients aged over 65

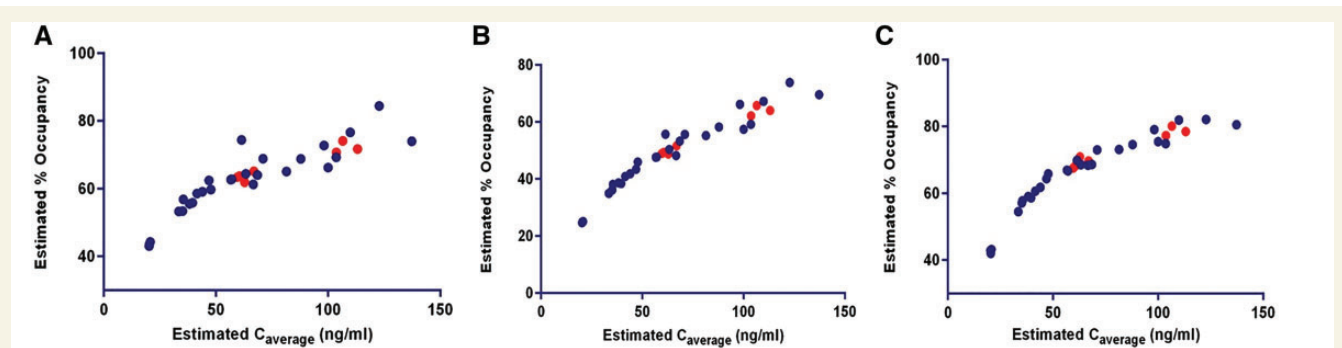
years. This study is the first to characterize the relationship between blood levels, central D2/3 occupancy and clinical outcome in older patients with psychosis in the context of Alzheimer's disease. Low blood concentrations were associated with high central occupancies, which overlapped with the reported 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 (50–100 mg) amisulpride are 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 ( $K_d$  0.03 nM) require higher concentrations of competing drug to displace them from the receptor site than lower affinity tracers such as  $^{11}\text{C}$ -raclopride ( $K_d$  1.9 nM), resulting in lower apparent occupancies (Seeman and Van Tol, 1995; Seeman, 2011, 2014). *In vivo*, underestimation 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. (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 (119 ng/ml) and putamen (241 ng/ml) are higher than the current study (48 ng/ml and 94 ng/ml for caudate and putamen, respectively). Taking dissociation constants for the two tracers into account ( $^{18}\text{F}$ -fallypride, 0.03 nM;  $^{18}\text{F}$ -desmethoxyfallypride, 0.34 nM) (Mukherjee et al., 1996), if anything we may be underestimating 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



**Figure 2 Pharmacokinetic-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 50th (pink) and 90th (blue) percentiles, overlaid to observed  $^{18}\text{F}$ -fallypride binding; and D2/3 receptor occupancies.



**Figure 3 Model estimated  $C_{\text{average}}$  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 based on EPS, present (red) or absent (blue).

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

blood-brain barrier, 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.*, 2009a; Graff-Guerrero *et al.*, 2015), in whom the relationship between blood concentration and occupancy was found to be similar to that observed in young

**Table 3** Model predicted blood drug concentration and striatal D2/3 receptor occupancy

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

\*Estimated by independent samples t-tests.

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 blood–brain barrier 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 blood–brain barrier penetration relative to other antipsychotics (Natesan *et al.*, 2008) and has been identified as a substrate for several blood–brain barrier 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 *et al.*, 2016). These properties may mean that amisulpride is a more sensitive tool with which to explore blood–brain barrier 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 CNS (Uchida *et al.*, 2009b), 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 pretreatment imaging in a proportion of patients. It was thus important to combine the dataset with a richly sampled phase 1 study, to fully parameterize the pharmacokinetic model and, with the addition of paired scans from the imaging pilot, more precisely model pharmacokinetic–occupancy profiles. Model-based estimation of BP<sub>PRE</sub> 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.*, 2009a, 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 the relationship of pharmacokinetic (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<sub>POST</sub> was not correlated with treatment duration. Non-adherence is a potential confound when modelling the relationship between pharmacokinetic, 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 9 ng/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 individualization without further population modelling of clinical outcome data (Assawasuwannakit *et al.*, 2016). However, the terminal half-life of amisulpride (which varies between 12.4 h in those aged 65 years and 22.7 h 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 pharmacokinetic-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 <sup>11</sup>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 blood-brain barrier 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 pharmacokinetic 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.

## Acknowledgements

We would like to acknowledge participants, carers and clinical teams in Mental Health for Older Adult services in the South London and Maudsley (SLaM) NHS Foundation Trust who made this possible; the research team; the central research network who facilitated recruitment; radiographers and radiochemists at St Thomas' Hospital PET Centre (KCL); the London Pharmacometric Interest Group (UCL); and Hiroyuki Uchida for generously sharing his ideas on antipsychotic drug sensitivity in later life. Demographic and pharmacokinetic data obtained in 20 elderly healthy volunteers were provided by SANOFI-AVENTIS R&D, from the report entitled "Amisulpride: study of pharmacokinetics and safety following a single oral administration (50 mg tablets) in the elderly" (SYNTHELABO report n° 95-00596-EN-00). These data were supplied by SANOFI-AVENTIS R&D in the interest of supporting medical research in very elderly patients. Sanofi does not support any use of the medicine outside the locally approved labelling.

## Funding

The project and salary of S.R. was funded by the National Institute for Health Research (NIHR) (PCDKBRA) during the course of the study. R.H. is also supported by the NIHR University College London Hospital Biomedical Research Centre (UCLH BRC).

## References

- Assawasuwannakit P, Braund R, Duffull SB. A framework for quantifying the influence of adherence and dose individualization. *Clin Pharmacol Ther* 2016; 99: 452–9.
- Bigos KL, Pollock BG, Coley KC, Miller del D, Marder SR, Aravagiri M, *et al.* Sex, race, and smoking impact olanzapine exposure. *J Clin Pharmacol* 2008; 48: 157–65.
- Blaschke TF, Osterberg L, Vrijens B, Urquhart J. Adherence to medications: insights arising from studies on the unreliable link between prescribed and actual drug dosing histories. *Annu Rev Pharmacol Toxicol* 2012; 52: 275–301.

- Bressan RA, Erlandsson K, Jones HM, Mulligan R, Flanagan RJ, Ell PJ, et al. Is regionally selective D2/D3 dopamine occupancy sufficient for atypical antipsychotic effect? An *in vivo* quantitative [123I]epidepride SPET study of amisulpride-treated patients. *Am J Psychiatry* 2003; 160: 1413–20.
- Clark-Papasavas C, Dunn JT, Greaves S, Mogg A, Gomes R, Brownings S, et al. Towards a therapeutic window of D2/3 occupancy for treatment of psychosis in Alzheimer's disease, with [18F]fallypride positron emission tomography. *Int J Geriatr Psychiatry* 2014; 29: 1001–9.
- Cummings JL, Mega M, Gray K, Rosenberg-Thompson S, Carusi DA, Gornbein J. The neuropsychiatric inventory: comprehensive assessment of psychopathology in dementia. *Neurology* 1994; 44: 2308–14.
- Dos Santos Pereira JN, Tadjerpisheh S, Abu Abed M, Saadatmand AR, Weksler B, Romero IA, et al. The poorly membrane permeable antipsychotic drugs amisulpride and sulpiride are substrates of the organic cation transporters from the SLC22 family. *AAPS J* 2014; 16: 1247–58.
- Dunn JT, Clark-Papasavas C, Marsden P, Baker S, Cleij M, Kapur S, et al. Establishing test-retest reliability of an adapted [(18)F]fallypride imaging protocol in older people. *J Cereb Blood Flow Metab* 2013; 33: 1098–103.
- Ette EI, Williams PJ, Lane JR. Population pharmacokinetics III: design, analysis, and application of population pharmacokinetic studies. *Ann Pharmacother* 2004; 38: 2136–44.
- Farde L, Nordstrom AL, Wiesel FA, Pauli S, Halldin C, Sedvall G. Positron emission tomographic analysis of central D1 and D2 dopamine receptor occupancy in patients treated with classical neuroleptics and clozapine. Relation to extrapyramidal side effects. *Arch Gen Psychiatry* 1992; 49: 538–44.
- Feng Y, Pollock BG, Coley K, Marder S, Miller D, Kirshner M, et al. Population pharmacokinetic analysis for risperidone using highly sparse sampling measurements from the CATIE study. *Br J Clin Pharmacol* 2008; 66: 629–39.
- Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* 1975; 12: 189–98.
- Graff-Guerrero A, Rajji TK, Mulsant BH, Nakajima S, Caravaggio F, Suzuki T, et al. Evaluation of antipsychotic dose reduction in late-life Schizophrenia: a prospective dopamine D2/3 receptor occupancy study. *JAMA Psychiatry* 2015; 72: 927–34.
- Hamon-Vilcot B, Chaufour S, Deschamps C, Canal M, Zieleniuk I, Ahtoy P, et al. Safety and pharmacokinetics of a single oral dose of amisulpride in healthy elderly volunteers. *Eur J Clin Pharmacol* 1998; 54: 405–9.
- Hiemke C, Baumann P, Bergemann N, Conca A, Dietmaier O, Egberts K, et al. AGNP consensus guidelines for therapeutic drug monitoring in psychiatry: update 2011. *Pharmacopsychiatry* 2011; 44: 195–235.
- Innis RB, Cunningham VJ, Delforge J, Fujita M, Gjedde A, Gunn RN, et al. Consensus nomenclature for *in vivo* imaging of reversibly binding radioligands. *J Cereb Blood Flow Metab* 2007; 27: 1533–9.
- Jankowska-Polanska B, Katarzyna L, Lidia A, Joanna J, Dudek K, Izabella U. Cognitive function and adherence to anticoagulation treatment in patients with atrial fibrillation. *J Geriatr Cardiol* 2016; 13: 559–65.
- Jennum P, Baandrup L, Ibsen R, Kjellberg J. Increased all-cause mortality with use of psychotropic medication in dementia patients and controls: a population-based register study. *Eur Neuropsychopharmacol* 2015; 25: 1906–13.
- Jeste DV, Blazer D, Casey D, Meeks T, Salzman C, Schneider L, et al. ACNP White Paper: update on use of antipsychotic drugs in elderly persons with dementia. *Neuropsychopharmacology* 2008; 33: 957–70.
- Kapur S, Remington G, Zipursky RB, Wilson AA, Houle S. The D2 dopamine receptor occupancy of PET studies. *Life Sci* 1995; 57: PL103–7.
- Kegeles LS, Slifstein M, Frankle WG, Xu X, Hackett E, Bae SA, et al. Dose-occupancy study of striatal and extrastriatal dopamine D2 receptors by aripiprazole in schizophrenia with PET and [18F]fallypride. *Neuropsychopharmacology* 2008; 33: 3111–25.
- Kessler RM, Ansari MS, Riccardi P, Li R, Jayathilake K, Dawant B, et al. Occupancy of striatal and extrastriatal dopamine D2 receptors by clozapine and quetiapine. *Neuropsychopharmacology* 2006; 31: 1991–2001.
- Kim E, Howes OD, Yu KS, Jeong JM, Lee JS, Jang IJ, et al. Calculating occupancy when one does not have baseline: a comparison of different options. *J Cereb Blood Flow Metab* 2011; 31: 1760–7.
- la Fougere C, Meisenzahl E, Schmitt G, Stauss J, Frodl T, Tatsch K, et al. D2 receptor occupancy during high- and low-dose therapy with the atypical antipsychotic amisulpride: a 123I-iodobenzamide SPECT study. *J Nucl Med* 2005; 46: 1028–33.
- Lako IM, van den Heuvel ER, Kneegtering H, Bruggeman R, Taxis K. Estimating dopamine D(2) receptor occupancy for doses of 8 antipsychotics: a meta-analysis. *J Clin Psychopharmacol* 2013; 33: 675–81.
- Lammertsma AA, Hume SP. Simplified reference tissue model for PET receptor studies. *Neuroimage* 1996; 4 (3 Pt 1): 153–8.
- Martinot JL, Paillere-Martinot ML, Poirier MF, Dao-Castellana MH, Loc'h C, Maziere B. *In vivo* characteristics of dopamine D2 receptor occupancy by amisulpride in schizophrenia. *Psychopharmacology* 1996; 124: 154–8.
- Maust DT, Kim HM, Seyfried LS, Chiang C, Kavanagh J, Schneider LS, et al. Antipsychotics, other psychotropics, and the risk of death in patients with dementia: number needed to harm. *JAMA Psychiatry* 2015; 72: 438–45.
- McKeith IG, Galasko D, Kosaka K, Perry EK, Dickson DW, Hansen LA, et al. Consensus guidelines for the clinical and pathologic diagnosis of dementia with Lewy bodies (DLB): report of the consortium on DLB international workshop. *Neurology* 1996; 47: 1113–24.
- McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's disease. *Neurology* 1984; 34: 939–44.
- Meisenzahl EM, Schmitt G, Grunder G, Dresel S, Frodl T, la Fougere C, et al. Striatal D2/D3 receptor occupancy, clinical response and side effects with amisulpride: an iodine-123-iodobenzamide SPECT study. *Pharmacopsychiatry* 2008; 41: 169–75.
- Morris ED, Chefer SI, Lane MA, Muzic RF Jr, Wong DF, Dannals RF, et al. Loss of D2 receptor binding with age in rhesus monkeys: importance of correction for differences in striatal size. *J Cereb Blood Flow Metab* 1999; 19: 218–29.
- Mukherjee J, Yang ZY, Brown T, Roemer J, Cooper M. 18F-desmethoxyfallypride: a fluorine-18 labeled radiotracer with properties similar to carbon-11 raclopride for PET imaging studies of dopamine D2 receptors. *Life Sci* 1996; 59: 669–78.
- Natesan S, Reckless GE, Barlow KB, Nobrega JN, Kapur S. Amisulpride the 'atypical' atypical antipsychotic—comparison to haloperidol, risperidone and clozapine. *Schizophr Res* 2008; 105: 224–35.
- Olsson H, Farde L. Potentials and pitfalls using high affinity radioligands in PET and SPET determinations on regional drug induced D2 receptor occupancy—a simulation study based on experimental data. *Neuroimage* 2001; 14: 936–45.
- Pretorius RW, Gataric G, Swedlund SK, Miller JR. Reducing the risk of adverse drug events in older adults. *Am Fam Physician* 2013; 87: 331–6.
- Reeves S, Bertrand J, D'Antonio F, McLachlan E, Nair A, Brownings S, et al. A population approach to characterise amisulpride pharmacokinetics in older people and Alzheimer's disease. *Psychopharmacology* 2016; 233: 3371–81.
- Reeves S, Brown R, Matthews D, Howard R, Grasby P. No effect of donepezil on striatal dopamine release in mild to moderate

- Alzheimer's disease. *J Neurol Neurosurg Psychiatry* 2010; 81: 119–21.
- Schmitt U, Kirschbaum KM, Poller B, Kusch-Poddar M, Drewe J, Hiemke C, et al. *In vitro* P-glycoprotein efflux inhibition by atypical antipsychotics is *in vivo* nicely reflected by pharmacodynamic but less by pharmacokinetic changes. *Pharmacol Biochem Behav* 2012; 102: 312–20.
- Schneider LS, Tariot PN, Dagerman KS, Davis SM, Hsiao JK, Ismail MS, et al. Effectiveness of atypical antipsychotic drugs in patients with Alzheimer's disease. *N Engl J Med* 2006; 355: 1525–38.
- Seeman P. All roads to schizophrenia lead to dopamine supersensitivity and elevated dopamine D2(high) receptors. *CNS Neurosci Ther* 2011; 17: 118–32.
- Seeman P. Therapeutic occupation of dopamine D2 antipsychotic receptors in Alzheimer's disease. *Int J Geriatr Psychiatry* 2014; 29: 1096.
- Seeman P, Van Tol HH. Deriving the therapeutic concentrations for clozapine and haloperidol: the apparent dissociation constant of a neuroleptic at the dopamine D2 or D4 receptor varies with the affinity of the competing radioligand. *Eur J Pharmacol* 1995; 291: 59–66.
- Sekhar G, Reeves S, Thomas S. Exploring the interaction of amisulpride with influx and efflux transporters expressed at the blood-brain barrier. *Proc Br Pharmacol Soc Abstract* 2016; 13: 173P.
- Sekine M, Maeda J, Shimada H, Nogami T, Arakawa R, Takano H, et al. Central nervous system drug evaluation using positron emission tomography. *Clin Psychopharmacol Neurosci* 2011; 9: 9–16.
- Simpson GM, Angus JW. A rating scale for extrapyramidal side effects. *Acta Psychiatr Scand Suppl* 1970; 212: 11–19.
- Sparshatt A, Taylor D, Patel MX, Kapur S. Amisulpride—dose, plasma concentration, occupancy and response: implications for therapeutic drug monitoring. *Acta Psychiatr Scand* 2009; 120: 416–28.
- Stone JM, Davis JM, Leucht S, Pilowsky LS. Cortical dopamine D2/D3 receptors are a common site of action for antipsychotic drugs—an original patient data meta-analysis of the SPECT and PET *in vivo* receptor imaging literature. *Schizophr Bull* 2009; 35: 789–97.
- Takano A, Suhara T, Ikoma Y, Yasuno F, Maeda J, Ichimiya T, et al. Estimation of the time-course of dopamine D2 receptor occupancy in living human brain from plasma pharmacokinetics of antipsychotics. *Int J Neuropsychopharmacol* 2004; 7: 19–26.
- Tauscher J, Jones C, Remington G, Zipursky RB, Kapur S. Significant dissociation of brain and plasma kinetics with antipsychotics. *Mol Psychiatry* 2002; 7: 317–21.
- Tziortzi AC, Searle GE, Tzimopoulou S, Salinas C, Beaver JD, Jenkinson M, et al. Imaging dopamine receptors in humans with [<sup>11</sup>C]-(+)-PHNO: dissection of D3 signal and anatomy. *Neuroimage* 2011; 54: 264–77.
- Tzourio-Mazoyer N, Landeau B, Papathanassiou D, Crivello F, Etard O, Delcroix N, et al. Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *Neuroimage* 2002; 15: 273–89.
- Uchida H, Kapur S, Mulsant BH, Graff-Guerrero A, Pollock BG, Mamo DC. Sensitivity of older patients to antipsychotic motor side effects: a PET study examining potential mechanisms. *Am J Geriatr Psychiatry* 2009a; 17: 255–63.
- Uchida H, Mamo DC, Mulsant BH, Pollock BG, Kapur S. Increased antipsychotic sensitivity in elderly patients: evidence and mechanisms. *J Clin Psychiatry* 2009b; 70: 397–405.
- Uchida H, Suzuki T, Graff-Guerrero A, Mulsant BH, Pollock BG, Arenovich T, et al. Therapeutic window for striatal dopamine D(2/3) receptor occupancy in older patients with schizophrenia: a pilot PET study. *Am J Geriatr Psychiatry* 2014; 22: 1007–16.
- Uchida H, Takeuchi H, Graff-Guerrero A, Suzuki T, Watanabe K, Mamo DC. Dopamine D2 receptor occupancy and clinical effects: a systematic review and pooled analysis. *J Clin Psychopharmacol* 2011; 31: 497–502.
- Urquhart J. The electronic medication event monitor. Lessons for pharmacotherapy. *Clin Pharmacokinet* 1997; 32: 345–56.
- Vernaleken I, Peters L, Raptis M, Lin R, Buchholz HG, Zhou Y, et al. The applicability of SRTM in [(18)F]fallypride PET investigations: impact of scan durations. *J Cereb Blood Flow Metab* 2011; 31: 1958–66.
- Vernaleken I, Siessmeier T, Buchholz HG, Hartter S, Hiemke C, Stoeter P, et al. High striatal occupancy of D2-like dopamine receptors by amisulpride in the brain of patients with schizophrenia. *Int J Neuropsychopharmacol* 2004; 7: 421–30.
- Volkow ND, Wang GJ, Fowler JS, Logan J, Schlyer D, Hitzemann R, et al. Imaging endogenous dopamine competition with [<sup>11</sup>C]raclopride in the human brain. *Synapse* 1994; 16: 255–62.
- Xiberas X, Martinot JL, Mallet L, Artiges E, Canal M, Loc'h C, et al. *In vivo* extrastriatal and striatal D2 dopamine receptor blockade by amisulpride in schizophrenia. *J Clin Psychopharmacol* 2001; 21: 207–14.
- Zeevi N, Pachter J, McCullough LD, Wolfson L, Kuchel GA. The blood-brain barrier: geriatric relevance of a critical brain-body interface. *J Am Geriatr Soc* 2010; 58: 1749–57.