5-HT_{2A} receptor blockade in patients with schizophrenia treated with risperidone or clozapine

A SPET study using the novel 5-HT_{2A} ligand ^{123}I-5-I-R-91150

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Background 5-HT_{2A} receptor antagonism may be crucial to the action of atypical antipsychotics. Previous work has related 5-HT_{2A} receptor blockade to clinical efficacy and protection from extrapyramidal side-effects.

Method We developed a SPET imaging protocol for assessing 5-HT_{2A} receptor binding using the selective ligand ^{123}I-5-I-R91150. Six healthy volunteers, five clozapine- and five risperidone-treated subjects with DSM–IV schizophrenia were studied. Multi-slice SPET was performed on each subject.

Results Cortex:cerebellum ratios were significantly lower in both clozapine- and risperidone-treated subjects compared with the healthy volunteers in all cortical regions. There was no difference in occupancy between the two drug-treated groups. No correlation was found between the percentage change in the Global Assessment Scale (GAS) and 5-HT_{2A} receptor binding indices in the drug-treated groups.

Conclusions Clozapine and risperidone potently block 5-HT_{2A} receptors *in vivo*. The lack of relationship between receptor binding indices and change in GAS suggests that 5-HT_{2A} receptor blockade may be unrelated to clinical improvement. Future studies will substantiate this finding by studying 5-HT_{2A} receptor binding in large groups of patients treated with both typical and novel atypical antipsychotics.

Increasing interest in the role of 5-HT_{2A} receptors in the neuropharmacology of schizophrenia has been generated by the high *in vitro* affinity for the 5-HT_{2A} receptor of the majority of the new atypical antipsychotics (Schotte *et al*, 1996). The hypothesis that these atypical antipsychotics effect their actions via 5-HT_{2A} mechanisms (Busatto & Kerwin, 1997) is consistent with evidence of a neuroanatomical and functional interaction of 5-HT and dopaminergic systems (Jenner *et al*, 1983) and a relationship between serotonergic neuroendocrine responses and symptomatic improvement on clozapine (Kahn *et al*, 1993; Curtis *et al*, 1995). Furthermore, preliminary data suggests that allelic variation in the 5-HT_{2A} gene varies with and may predict treatment response to clozapine (Arranz *et al*, 1995).

POSITRON EMISSION TOMOGRAPHY STUDIES

In vivo positron emission tomography (PET) studies, of the atypical antipsychotic drugs clozapine and risperidone, have shown greater than 80% occupancy of 5-HT_{2A} receptors in people with schizophrenia on clinically relevant doses (Nyberg *et al*, 1996). Much effort has been recently directed to the development of more selective 5-HT_{2A} ligands for PET. Iodine-123 labelled 5-HT_{2A} ligands for single photon emission tomography (SPET) are also of considerable interest, as this technique is cheaper and more accessible than PET.

^{123}I-5-I-R91150

^{123}I-5-I-R91150 is a new iodine-123 labelled ligand for SPET that binds reversibly and with high-affinity *in vitro* to 5-HT_{2A} receptors (Mertens *et al*, 1994).

Characterisation of ^{123}I-5-I-R91150 in humans has shown it to be a potentially useful ligand to study 5-HT_{2A} receptors *in vivo*. The cortico:cerebellar ratios at pseudo-equilibrium reflect a distribution in the brain similar to that expected from post-mortem studies (Busatto *et al*, 1997).

In this study we have used ^{123}I-5-I-R91150 SPET in patients on clinically relevant doses of clozapine and risperidone to substantiate earlier data and perform a preliminary test of the hypothesis that 5-HT_{2A} receptor blockade is associated with clinical response to neuroleptics.

METHOD

Subjects and study design

Ethical approval for the study was provided by the Ethics Committees of the Bethlem and Maudsley NHS Trust, City and Hackney Community Health Services, Oxleas and Maidstone NHS Trusts. Permission to administer the radiopharmaceutical dose was obtained from the national UK Administration for Radioactive Substances Advisory Committee (ARSAC). Written informed consent was obtained from all subjects.

Healthy volunteers

Six healthy volunteers were recruited, five males and one female. These subjects had no previous psychiatric or medical history and no family psychiatric history. They were medication-free and were not using illicit substances.

Patients

Eleven patients fulfilled the inclusion criteria which were: DSM–IV diagnosis of schizophrenia and treatment with medication for at least six weeks. Treatment was initiated and sustained by the clinical team. Six clozapine-treated patients were recruited (five males and one female) and five risperidone-treated patients (five male).

Exclusion criteria were as follows: prominent or recent alcohol or drug dependency; concomitant use of prescribed psychoactive medication except anti-cholinergic side-effect medication; or presence of major physical or neurological illness. Fertile women not using a reliable method of contraception or who thought they might be pregnant were also excluded. One male patient from the clozapine group was excluded from the final analysis as he had been referred to a neurologist for investigation of an idiopathic movement disorder.

Psychiatric symptoms were rated within one week of the scan by one of the investigators (M.J.T.) and further assessment of psychiatric symptoms prior to treatment were obtained from a search of case note records and interviews with the patients' carers and families.

Clinical rating of symptom presence and severity were conducted using a combination of a modified Schedule for Affective Disorders and Schizophrenia – Lifetime Version (SADS–L; Endicott & Spitzer, 1978), the Structured Clinical Interview for the Positive and Negative Syndrome Scale (SCI–PANSS; Kay *et al*, 1987) and the Scale for the Assessment of Negative Symptoms (SANS; Andreasen, 1989). The presence and degree of severity of extrapyramidal side-effects were assessed by the administration of the Simpson–Angus rating scale for extrapyramidal side-effects (Simpson & Angus, 1970), subjective and objective akathisia by the Barnes Akathisia Rating Scale (Barnes, 1989) and dyskinesia by the Abnormal Involuntary Movement Scale (AIMS; Lane *et al*, 1985). Global function and clinical improvement were rated by obtaining a current Global Assessment Scale (GAS; Endicott *et al*, 1976) score over one week prior to the study and an estimate of GAS over the month prior to commencing medication treatment, taken from the clinicians' notes and interviews with carers where available. The GAS rates global symptoms from 0–100 where higher scores imply improvement. It is anchored every 10 units by clear behavioural measures and has high validity and reliability (Endicott *et al*, 1976). The change in GAS scores before and after treatment is expressed as a percentage improvement (change in score/initial score × 100). The patients also answered a standard drug and alcohol screening questionnaire and a general health screening questionnaire.

Image acquisition

SPET scanning was performed at the Institute of Nuclear Medicine, UCL Medical School using a brain dedicated SME 810 multi-detector scanner. This is a 12 detector, single slice, high-sensitivity tomograph, which acquires images with an in-plane resolution of 7–9 mm full-width half maximum (FWHM) and an average slice thickness of 12.5 mm.

Subjects lay in the supine position with the head aligned in a plane parallel to the orbitomeatal line. One whole brain acquisition of eight slices was obtained from each subject. Multi-slice SPET was performed starting from a slice at the level of the cerebellum and acquiring five minute slices at 1 cm intervals. Total acquisition time was 45–50 minutes. Acquisition was commenced two hours after injection of 180 MBq ^{123}I-5-I-R91150, a time when specific binding appears to be maximal and stable for up to eight hours (Busatto *et al*, 1997).

Scan analysis

Analysis of the scans was performed blind to subject status using Nuclear Diagnostics Software. Signal density (mean counts/pixel) in several cortical areas and the cerebellum were determined using a region of interest (ROI) approach. ROI templates were derived from an averaged high resolution 99mTc-hexamethylpropyleneamine-oxime SPET scan data set from age-matched volunteers. The study images were resized and overlaid on the template to provide a standardised semi-automated region of interest analysis.

A standard reference region approach was used to obtain measures of specific binding in different cortical regions (Pilowsky *et al*, 1992; Busatto *et al*, 1997). Radioactivity estimates in the cortex were assumed to represent 'total' ligand binding ((specific+non-specific binding)+free ligand). The uptake in the cerebellum, presumed free from 5-HT$_{2A}$ receptors (Pazos *et al*, 1987), was used as reference for background radioactivity (non-specific binding+free ligand). Relative indices of 'specific' binding (RSB) are therefore calculated as:

$$RSB = \text{'total' ROI binding} / \text{cerebellar uptake} = 5\text{-}HT_{2A} \text{ binding index}$$

Occupancy of cortical 5-HT$_{2A}$ receptors by cold antipsychotic drugs decreases the amount of receptors available for specific binding to the tracer, ^{123}I-5-I-R91150. The RSB is reduced in proportion to the degree of occupancy by the antipsychotic drugs. Thus, a low 5-HT$_{2A}$ receptor RSB implies high 5-HT$_{2A}$ receptor occupancy by the drug (Pilowsky *et al*, 1992).

Statistical analysis

Statistical analyses were performed with the Statistical Package for Social Sciences (SPSS for Windows version 6.0) using an IBM compatible PC. Between group differences of regional specific binding were assessed using analysis of variance with Student–Newman–Keuls *post hoc* tests and Mann–Whitney *U*-tests (two-tailed). Mann–Whitney *U*-tests (two-tailed) for independent samples were also used to compare clinical ratings between clozapine- and risperidone-treated subjects. The relationship between 5-HT$_{2A}$ receptor binding and clinical indices was investigated using Spearman correlation coefficients.

RESULTS

Demographic and clinical data

There was no significant difference in age between the healthy volunteer and patient groups (see Table 1).

Table 1 Mean (s.d.) cortical binding indices (RSB) and clinical characteristics of patients and healthy volunteers

	Mean age (years)	Mean drug dose (mg/day)	Mean treatment duration (months)	Mean duration of illness (years)	Mean total PANSS scores	Mean total SANS scores	Mean % change in GAS score	Mean frontal cortex RSB	Mean temporal cortex RSB	Mean parietal cortex RSB
Healthy volunteers	29.7 (3.4)	–	–	–	–	–	–	1.33 (0.16)	1.30 (0.22)	1.38 (0.07)
Clozapine treated	26.8 (3.7)	470 (110)	17 (8.1)	7.2 (4.6)	78.6 (21)**	60 (17)**	245 (160)	0.87 (0.09)*	0.9 (0.07)*	0.89 (0.05)*
Risperidone treated	29.8 (10)	7.4 (5)	13.8 (15)	7.8 (9.2)	56 (15)	30 (23)	165 (125)	0.88 (0.9)*	0.91 (0.04)*	0.91 (0.09)*

PANSS, Positive and Negative Syndrome Scales; SANS, Scale for the Assessment of Negative Symptoms; GAS, Global Assessment Scale.
*$P < 0.05$ *v.* risperidone-treated group (Mann–Whitney *U*-test).
**$P < 0.01$ *v.* healthy volunteers (Mann–Whitney *U*-test).

Table 2 Clinical characteristics of clozapine- and risperidone-treated groups of patients with a DSM–IV diagnosis of schizophrenia

Subject	Age (years)	Drug dose (per day)	Treatment duration (months)	Duration of illness (years)	Total PANSS scores	Total SANS scores	% Change in GAS score	Simpson–Angus score at SPET study	Barnes Akathisia Scale score at SPET scan	AIMS score at SPET study	Procyclidine treatment Yes/No?
Clozapine-treated											
1	23	300	17	3	66	56	364	3	0	0	No
2	32	600	27	15	112	86	200	0	2	5	No
3	26	500	5	5	66	58	64	0	0	0	No
4	24	450	21	6	63	38	455	4	2	0	No
5	29	500	15	7	86	61	143	4	3	0	No
Risperidone-treated											
1	25	16	23	6	53	25	233	1	0	0	Yes
2	30	6	3	4	45	18	329	0	9	0	Yes
3	46	3	3	24	63	51	0	0	5	0	No
4	29	6	36	4	40	2	161	0	0	0	No
5	19	6	4	1	77	56	100	6	0	0	Yes

PANSS, Positive and Negative Syndrome Scale; SANS, Scale for the Assessment of Negative Symptoms; GAS, Global Assessment Scale; SPET, Single Positron Emission Tomography; AIMS, Abnormal Involuntary Movement Scale.

Within the patient group the clozapine-treated group had significantly higher total SCI-PANSS and SANS scores than the risperidone-treated group. However, there were no significant differences in the duration of treatment between the two groups and no significant difference in the percentage change in GAS with treatment.

With regard to medication side-effects, several of the clozapine-treated patients scored on the Simpson–Angus scale. This was mainly due to high scores on the excess salivation sub-scale. None were receiving prophylactic anticholinergic medication (see Table 2). In the risperidone-treated group the number of patients scoring on the Simpson–Angus scale was lower. Three of the people in this group were receiving procyclidine. There were no significant differences in side-effects between the groups.

Cortical 5-HT$_{2A}$ receptor binding indices

Table 1 shows the mean RSB indices for the frontal, parietal and temporal cortices. Individual values for the frontal cortex are shown in the scatter plot (see Fig. 1), scatter plots from the parietal and temporal cortices are similar. Analysis of variance showed significant differences in RSB, in all cortical regions, between both drug-treated groups and the healthy volunteers at the $P<0.05$ level. ANOVA revealed no significant differences in RSB between the two drug-treated groups.

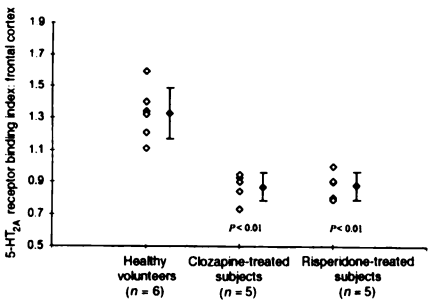

Fig. 1 5-HT$_{2A}$ receptor binding index (RSB) in the frontal cortex: healthy volunteers *versus* patients with schizophrenia treated with risperidone or clozapine.

Confirming this, Mann–Whitney *U*-tests indicated that the 5-HT$_{2A}$ receptor RSB index was significantly lower in the frontal, temporal and parietal cortices, in both the clozapine- and risperidone-treated groups ($P<0.01$). The extent of receptor blockade is qualitatively obvious and shown in Fig. 2. It will be noted that the cortical distribution of receptor binding evident in the study from the healthy volunteers cannot be seen in the studies from the drug-treated cases.

Relationship of clinical variables and 5-HT$_{2A}$ binding indices

There was no relationship between any of the ratings of current symptomatology and 5-HT$_{2A}$ receptor binding. There was generally a low level of side-effects among our subjects and there was no indication of a relationship between side-effect ratings and receptor binding.

There were no correlations between percentage change in GAS scores and the 5-HT$_{2A}$ receptor binding index in any of the three cortical areas. A representative scatter plot for the frontal RSB and percentage change in GAS is shown in Fig. 3, temporal and parietal regions showed a similar lack of correlation.

DISCUSSION

5-HT$_{2A}$ binding: clozapine *versus* risperidone

We have performed the first *in vivo* study, with the novel highly selective 5-HT$_{2A}$ ligand ^{123}I-5-I-R91150 and SPET, that estimates cortical 5-HT$_{2A}$ receptor binding in patients with schizophrenia treated with the different atypical antipsychotic drugs clozapine and risperidone.

These data are consistent with *in vitro* findings of high 5-HT$_{2A}$ receptor affinity for clozapine and risperidone and *in vivo* findings of high occupancy for these drugs (Schotte *et al*, 1996, Nyberg *et al*, 1996).

The lack of difference in 5-HT$_{2A}$ receptor RSB between the two drug-treated groups indicates that they have equal propensity to block this receptor at therapeutically efficacious doses. Risperidone

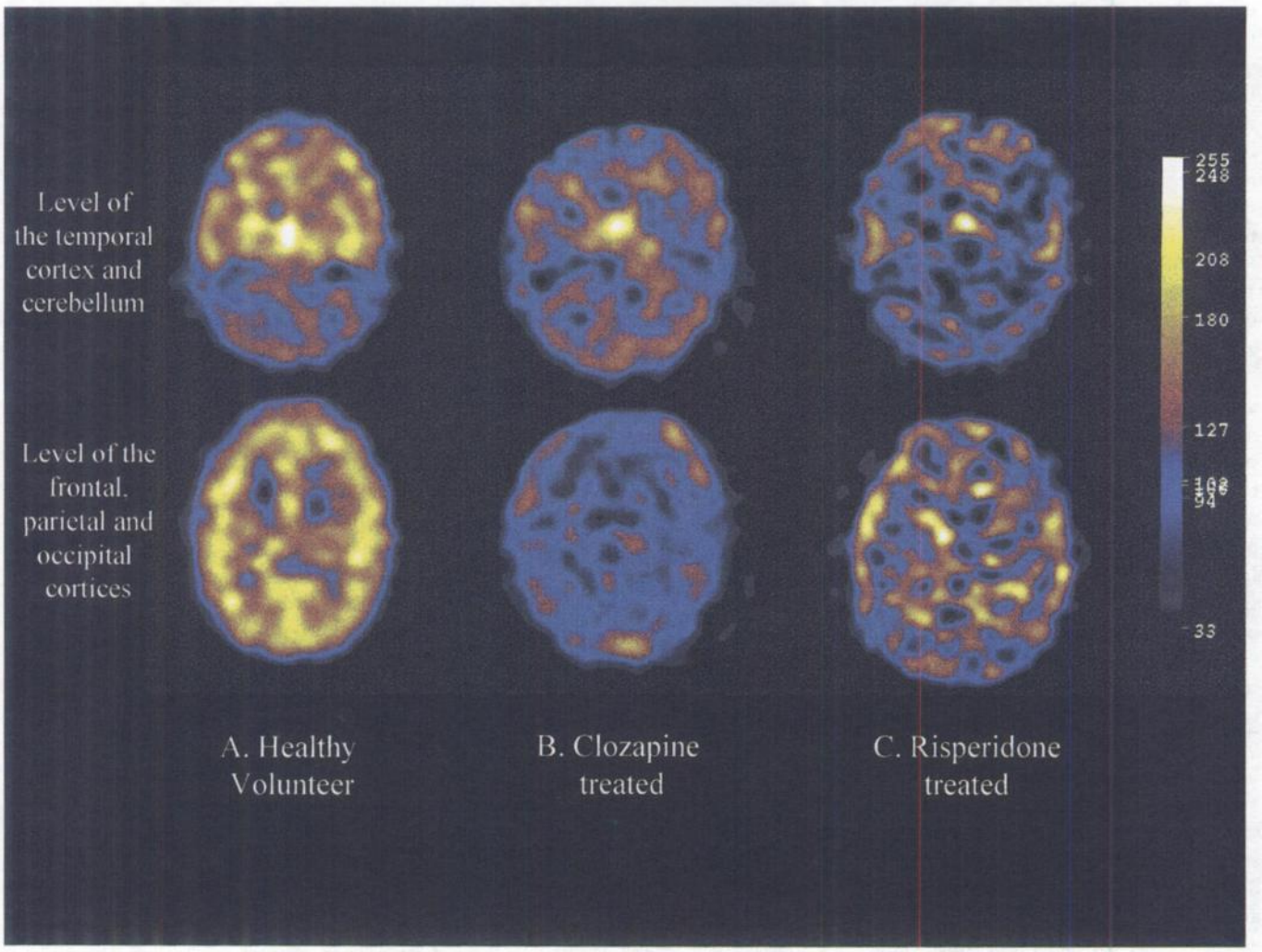

Fig. 2 ^{123}I-5-I-R91150 SPET brain scan of 5-HT$_{2A}$ receptors in a healthy volunteer on no medication and volunteers with schizophrenia, treated with clozapine 450 mg per day or risperidone 6 mg per day (note the total activity in the study from the risperidone-treated patient is higher than that in the clozapine-treated subject. The cortico-cerebellar ratios are similar).

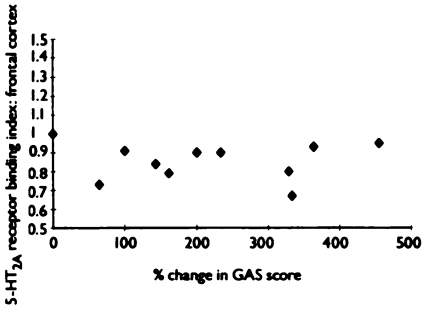

Fig. 3 Relationship between frontal cortex 5-HT$_{2A}$ binding index (RSB) and percentage change in Global Assessment Scale (GAS) score; $r=-0.05$; $P=0.85$

has a high *in vitro* affinity for the 5-HT$_{2A}$ receptor (K_i=0.52 nM). Clozapine has a lower affinity with a K_i for the 5-HT$_{2A}$ receptor of 9.6 nM (haloperidol, K_i=200 nM; Schotte *et al*, 1996). Our results suggest that at clinically relevant doses the degree of receptor blockade is independent of the absolute affinity of risperidone and clozapine for the 5-HT$_{2A}$ receptor. This finding would tend to indicate that, in terms of receptor blockade, clinician-directed prescription of antipsychotic medication should be guided more by the findings from *in vivo* imaging studies and less by the *in vitro* receptor affinity data.

5-HT$_{2A}$ binding and clinical improvement

The evidence from clinical trials shows that clozapine clearly has enhanced efficacy compared with typical antipsychotics in treatment-resistant schizophrenia (Travis, 1997). Interestingly, risperidone has been shown both to have slightly better clinical efficacy than typical antipsychotics in the treatment of chronic schizophrenia, and to be better tolerated (Peuskens, 1995). It has been postulated that this enhanced efficacy may be due to 5-HT$_{2A}$ receptor blockade. For clozapine this is supported by neuroendocrine studies of 5-HT function (Busatto & Kerwin, 1997). However, the data from this preliminary study does not support this notion. There was no relationship between treatment response and 5-HT$_{2A}$ receptor occupancy in any cortical region. Nevertheless, because of the small sample sizes a lack of correlation cannot be said to be a definitive finding.

Relevance of the findings to extrapyramidal side-effects

Several authors have proposed that the benefit of 5-HT$_{2A}$ blockade by atypical antipsychotics may lie in reducing side-

effects (Meltzer, 1989; Kerwin, 1994), and the results from this study are compatible with this hypothesis. All of the patients in this study had a low rate of extrapyramidal side-effects, and a high degree of receptor blockade (as indicated by low 5-HT_{2A} receptor RSBs). Risperidone, compared with clozapine, has high *in vitro* affinity for D_2 receptors, (K_i for risperidone=5.9 nM, clozapine=190 nM; Schotte *et al*, 1996). Kapur & Remington (1996) hypothesised that 5-HT_{2A} receptor blockade has a protective effect against extrapyramidal side-effects that is lost at D_2 occupancies above a certain threshold. One group have demonstrated that greater than 70% of D_2 occupancy by risperidone is associated with the need for prophylactic anticholinergic medication and the appearance of extrapyramidal side-effects (Knable *et al*, 1997). Indeed over half the risperidone-treated subjects in our sample were receiving procyclidine, suggesting they experienced extrapyramidal side-effects. It may be that the risperidone-treated subjects in our group who required procyclidine had D_2 occupancy levels that exceeded the threshold for the development of extrapyramidal side-effects. Interestingly, our results indicate that significant 5-HT_{2A} occupancy is seen with doses of risperidone as low as 3 mg per day suggesting that the use of atypicals in low doses may lead to favourable 5-HT_{2A} occupancy ratio, which could optimise clinical response and minimise extrapyramidal side-effects.

CLINICAL IMPLICATIONS

- Despite different *in vitro* affinity for the 5-HT_{2A} receptor, both clozapine and risperidone appear to produce similar significant levels of *in vivo* 5-HT_{2A} receptor RSB at therapeutic doses. This suggests that *in vivo* receptor imaging data may be more useful in guiding clinical prescribing than *in vitro* receptor affinities.
- ^{123}I-5-I-R91150 SPET is a useful method and accessible for studying the *in vivo* occupancy of 5-HT_{2A} receptors caused by psychotropic medication.
- There does not appear to be a correlation between change in GAS scores and the degree of 5-HT_{2A} receptor occupancy.

LIMITATIONS

- The sample sizes are small, therefore, the lack of correlation of receptor occupancy with clinical change is not a definitive result.
- Clinician-determined doses of medication were used rather than fixed doses. Therefore the minimum degree of 5-HT_{2A} receptor binding associated with therapeutic response cannot be determined from these results.
- Healthy volunteers were studied rather than drug-naive subjects with schizophrenia. Therefore illness effects on changes in 5-HT_{2A} binding cannot be ruled out. However, the results are in accordance with other studies using different ligands.

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Methodological caveats

This study was performed using small samples. Nevertheless, the 5-HT_{2A} receptor RSB values are unequivocal and these data do provide useful preliminary evidence of the relationship between clinical variables and 5-HT_{2A} receptor occupancy as revealed by SPET. Ratings of clinical effectiveness were retrospective, however, high quality case notes were available and were reviewed thoroughly with a detailed checklist. Carers gave information about the clinical and psychosocial state of the patients when they began taking their medication. The GAS is particularly robust to this approach as it is anchored in highly specific behavioural indicators and may be applied to case notes retrospectively. The use of clinician-determined doses of medication rather than fixed dose regime provides a potential confounder to the link between receptor blockade and clinical efficacy. Patients were all optimised on their medication by treating clinicians. It is accepted that clinical response and initial baseline function will vary between individuals in a naturalistic sample. Nevertheless, regardless of their initial baseline, good response was documented for all patients in the study excluding the third risperidone-treated subject who was relatively high functioning prior to switching and required risperidone because of intolerance to typical antipsychotic treatment. Although healthy volunteers were used as controls rather than drug-naive patients with schizophrenia, the changes seen in this study are wholly consistent with studies involving drug-naive control subjects (Trichard *et al*, 1998) and where patients are used as their own controls (Nyberg *et al*, 1996). The lack of anatomical landmarks in the scans of patients on medication made image analysis problematic because of the anatomic disruption. The use of the extra-cortical rim of activity seen in the studies from medicated subjects (see Fig. 2), allowed a template to be placed on the scans. This analysis was performed on the clozapine scans by M.J.T. and G.F.B. with an interrater reliability of r=0.82. The application of fixed template regions of interest is a well described methodology in PET and SPET and provides highly reliable estimates of activity (and change in activity following activation) in anatomical areas (Ebmeier *et al*, 1991).

SUMMARY

We have confirmed earlier findings of high 5-HT_{2A} occupancy by the atypical antipsychotic drugs clozapine and risperidone, with ^{123}I-5-I-R91150, a novel highly selective ligand for SPET.

Preliminary evidence suggests that 5-HT_{2A} blockade may not be related to clinical response. However, most broad spectrum typical antipsychotics have some affinity for 5-HT_{2A} receptors, therefore future studies should include patients on typical antipsychotics and other atypical antipsychotics. Defining this therapeutic target is critical for novel drug development. The relationship between clinical parameters and the extent of receptor occupancy *in vivo* at therapeutically beneficial doses still requires further clarification.

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REFERENCES

Andreasen N. C. (1989) The Scale for the Assessment of Negative Symptoms (SANS): conceptual and theoretical foundations. *British Journal of Psychiatry*, **155**, (suppl. 7), 49–58.

Arranz, M., Collier, D., Sodhi, M., *et al* (1995) Association between clozapine response and allelic variation in 5-HT_{2A} receptor gene. *Lancet*, **346**, 281–282.

Barnes, T. R. E. (1989) A rating scale for drug-induced akathisia. *British Journal of Psychiatry*, **154**, 672–676.

Busatto, G. F., Pilowsky, L. S., Costa, D. C., *et al* (1997) Initial evaluation of 123-I-5-I-R91150, a selective 5-HT_{2A} ligand for single photon emission tomography (SPET), in healthy human subjects. *European Journal of Nuclear Medicine*, **24**, 119–124.

— & Kerwin, R. W. (1997) Perspectives on the role of serotonergic mechanisms in the pharmacology of schizophrenia. *Journal of Psychopharmacology*, **11**, 3–12.

Curtis, V. A., Wright, P., Reveley, A., *et al* (1995) Effect of clozapine on *d*-fenfluramine evoked neuroendocrine responses in schizophrenia and its relationship to clinical improvement. *British Journal of Psychiatry*, **166**, 642–664.

Ebmeier, K. P., Dougall, N. J., Austin, M. V., *et al* (1991) The split-dose technique for the study of psychological and pharmacological activation with the cerebral blood flow marker exametazime and single photon emission tomography: reproducibility and rater reliability. *International Journal of Methods in Psychiatric Research*, **1**, 27–38.

Endicott, J., Spitzer, R., Fleiss, J. L., *et al* (1976) The Global Assessment Scale. *Archives of General Psychiatry*, **33**, 766–771.

— & — (1978) A diagnostic interview. The schedule for affective disorders and schizophrenia. *Archives of General Psychiatry*, **35**, 837–844.

Jenner, P., Sheehy, M. & Marsden, C. D. (1983) Noradrenaline and 5-hydroxytryptamine modulation of brain dopamine function: implications for the treatment of Parkinson's disease. *British Journal of Clinical Pharmacology*, **15** (suppl. 2), 277s–289s.

Kahn, R. S., Davidson, M., Siever, L., *et al* (1993) Serotonin function and treatment response to clozapine in schizophrenic patients. *American Journal of Psychiatry*, **150**, 1337–1342.

Kapur, S. & Remington, G. (1996) Serotonin–dopamine interaction and its relevance to schizophrenia. *American Journal of Psychiatry*, **153**, 466–476.

Kay, S. R., Fiszbein, A. & Opler, L. A. (1987) The positive and negative syndrome scale (PANSS) for schizophrenia. *Schizophrenia Bulletin*, **13**, 261–276.

Kerwin, R. W. (1994) The new atypical antipsychotics. *British Journal of Psychiatry*, **164**, 141–148.

Knable, M. B., Heinz, A., Raedler, T., *et al* (1997) Extrapyramidal side effects with risperidone and haloperidol at comparable D2 receptor occupancy levels. *Psychiatry Research*, **75**, 91–101.

Lane, R. D., Glazer, W. M., Hansen, T. E., *et al* (1985) Assessment of tardive dyskinesia using the abnormal involuntary movement scale. *Journal of Nervous and Mental Disorders*, **173**, 353–357.

Meltzer, H. Y. (1989) Clinical studies on the mechanism of action of clozapine: the dopamine–serotonin hypothesis of schizophrenia. *Psychopharmacology*, **99** (suppl.), 18–27.

Mertens, J., Terrière, D., Sipido, V., *et al* (1994) Radiosynthesis of a new radioiodinated ligand for serotonin-5-HT_{2A}-receptors, a promising tracer for γ-emission tomography. *Journal of Labelled Compounds and Radiopharmaceuticals*, **34**, 795–806.

Nyberg, S., Nakashima, Y., Nordström, A.-L., *et al* (1996) Positron emission tomography of *in-vivo* binding characteristics of atypical antipsychotic drugs. Review of D_2 and 5-HT_{2A} receptor occupancy studies and clinical response. *British Journal of Psychiatry*, **168** (suppl. 29), 40–44.

Pazos, A., Probst, A. & Palacios, J. M. (1987) Serotonin receptors in the human brain. IV. Autoradiographic mapping of serotonin-2 receptors. *Neuroscience*, **21**, 123–139.

Peuskens, J. (1995) Risperidone in the treatment of patients with chronic schizophrenia: A multi-national, multi-centre, double-blind parallel-group study *versus* haloperidol. *British Journal of Psychiatry*, **166**, 712–726.

Pilowsky, L. S., Costa, D. C., Ell, P. J., *et al* (1992) Clozapine, single photon emission tomography, and the D_2 dopamine receptor blockade hypothesis of schizophrenia, *Lancet*, **240**, 199–202.

Schotte, A., Janssen, P. F., Gommeren, W., *et al* (1996) Risperidone compared with new and reference antipsychotic drugs: *in vitro* and *in vivo* receptor binding. *Psychopharmacology*, **124**, 57–73.

Simpson, G. M. & Angus, J. W. S. (1970) A rating scale for extrapyramidal side-effects. *Acta Psychiatrica Scandinavica*, **212** (suppl. 44), 11–19.

Trichard, C., Paillière-Martinot, M. L., Attar-Levy, D., *et al* (1998) Binding of antipsychotic drugs to cortical 5-HT_{2A} receptors: A PET study of chlorpromazine, clozapine, and amisulpiride in schizophrenic patients. *American Journal of Psychiatry*, **155**, 505–508.

Travis, M. J. (1997) Clozapine: A review. *Journal of Serotonin Research*, **4**, 125–144.