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ORIGINAL INVESTIGATION

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Cerebral metabolic changes induced by clozapine in schizophrenia and related to clinical improvement

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Abstract *Rationale:* The study of the different effects on brain metabolism between typical and atypical antipsychotics would aid in understanding their mechanisms of action. Clozapine is of special interest, since it is one of the most effective antipsychotic drugs and demonstrates a distinctive mechanism of action in pre-clinical studies with respect to typical neuroleptics. *Objective:* To study the differences in cerebral activity induced by clozapine as compared to those produced by haloperidol. *Methods:* [^{18}F]Fluoro-deoxy-glucose (FDG)-positron emission tomography (PET) scans were obtained in the resting condition before and after 6 months of treatment with clozapine in 22 treatment-resistant patients with schizophrenia. Before inclusion, patients had been chronically treated with classical drugs, and all of them received haloperidol during the last month. Data were analyzed with statistical parametric mapping (SPM′99) methods, comparing pre-treatment and post-treatment conditions. The association between the changes in symptom scores and metabolism was also assessed to corroborate the functional relevance of possible metabolic changes. *Results:* Clozapine decreased prefrontal and basal ganglia activity, and increased occipital metabolism, including primary and association visual areas. The change in negative symptoms was related with the decrease of basal ganglia activity; the improvement in disorganization related to the metabolic decrease in the motor area, and the change in positive symptoms was associated to the increase of activity in the visual area. *Conclusions:* These results show that haloperidol and clozapine produce different patterns of metabolic changes in schizophrenia. Compared to the haloperidol baseline, clozapine inhibited the metabolic activity of the prefrontal and motor cortical regions and basal ganglia and induced a higher activation of the visual cortex. The improvement in disorganization, negative and positive syndromes with clozapìne may be respectively associated with metabolic changes in the motor area, basal ganglia, and visual cortex.

Keywords Brain metabolism · (FDG)-PET · Hypofrontality · Haloperidol · Clozapine · SPM

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Introduction

The study of the changes in brain metabolism induced by neuroleptics provides useful information for understanding the action of these drugs, especially if those changes relate to clinical improvement. Clozapine is one of the most effective antipsychotic drugs and probably its mechanism of action differs from that of typical neuroleptics. In particular, clozapine appears to act more selectively than typical neuroleptics on the prefrontal (PF) region (Robertson and Fibiger 1992), an area of special relevance in higher cognitive functions and schizophrenia.

Few studies have investigated the effect of clozapine on brain metabolism in schizophrenia. A cross-sectional study reported a global decrease of gray matter activity similar for both clozapine and fluphenazine accompanied by a higher decrease of the activity of the inferior PF cortex with clozapine (Cohen et al. 1997). A similar decrease of PF activity with clozapine was reported by Potkin et al. (1994). The same group later found that only patients with a particular allele for the D_1 dopamine

receptor showed both clinical improvement and a cortical metabolic decrease with clozapine (Potkin et al. 2003). Longitudinal data in five patients showed an inhibition in the resting state with clozapine of some PF areas and hippocampus as compared to an haloperidol baseline, while other PF areas were instead activated (Lahti et al. 2003b).

Pre-clinical data also show that clozapine inhibits the metabolic activity in PF and limbic regions in the rat and increases immediate-early gene expression in the same region (Cochran et al. 2002). Along the same lines, Tsai et al. (2001) have reported a decrease of glucose metabolism in thalamic, limbic, and cortical (including PF) regions in rats following acute and chronic clozapine treatment.

These results suggest that the superior therapeutic effects of clozapine in treatment-resistant cases might be related to its inhibitory effects in the PF region and/or its limbic connections. That possibility can be addressed by means of a longitudinal study of the activity changes resulting from switching from typical neuroleptics to clozapine, also considering the extent to which these changes relate to clinical improvement.

Therefore, the objective of this paper is to analyze the effect clozapine has on brain metabolic patterns. ^{18}F-deoxy-glucose positron emission tomography (FDG-PET) images are analyzed using statistical parametric mapping (SPM) methods to carry out pair-wise comparisons between pre-treatment and post-treatment conditions using a paired, longitudinal design (i.e. switching from haloperidol to clozapine) in the same subjects. Furthermore, we assessed the relationship between metabolic and symptom changes to test the functional relevance of metabolic changes with clozapine.

Materials and methods

Subjects

Our sample included 22 cases (16 males; 19 paranoid and 3 undifferentiated patients, according to DSM-IV schizophrenia criteria). Diagnosis was confirmed using the SCID (patients version) and data obtained from clinical interviews and information from families and clinical staff. The SANS (Andreasen 1983a), SAPS (Andreasen 1983b), and UKU scale were used to evaluate symptoms and parkinsonian side effects, respectively, immediately prior to initiating treatment with clozapine and 6 months later. Symptoms were grouped into the following syndromes according to the usual convention: positive (delusions and hallucinations), disorganization (formal thought disorder, bizarre behavior, and attention disorder), and negative (alogia, affective blunting, apathy, and anhedonia). The corresponding scores were calculated as the sum of the symptoms included in each syndrome, according to SAPS and SANS, not including the global scores (Table 1). Exclusion criteria were: any other axis I diagnosis, any neurological illness, history of cranial trauma with loss of consciousness, past substance dependence, excluding nicotine or caffeine, drug abuse during the past 3 months (current consumption being ruled out by urinalysis), and any current treatment with known CNS action. No patient had received mood-stabilizers, antidepressants, or depot neuroleptics during the 6 months preceding the study. All patients and controls underwent a cautionary MRI scan to exclude any abnormality of neurological relevance as judged by an expert radiologist. After full description of the study to the subjects, written informed consent was obtained from each patient and from a first-degree relative. The research and ethics boards of the participating institutions endorsed the study.

Table 1 Patient demographic data. Values expressed as mean (SD)

	Mean (SD) *N*=22
Duration (years)	7.40 (8.50)
Age at onset (years)	24.03 (6.39)
Age (years)	31.31 (10.22)
Education (years)	12.90 (5.51)
Parental socioeconomic status	2.1 (0.9)

Medication status

Patients evinced a poor response to at least two different classical treatments during the previous year, each one lasting for more than 1 month, at doses above 800 mg/day in CPZ equivalents (Table 2). At the time of the basal scan,

Table 2 Treatment status of patients. The two last drugs to which each case was resistant are shown. Doses are expressed in mg/day, in chlorpromazine equivalents. Every case was only treated with haloperidol during the month preceding the first PET scan

Patient	Gender (M/F)	Treatments	Dose mg/day
1	F	Haloperidol; thioridazine	850
2	M	Haloperidol; thioridazine	900
3	M	Pimozide; thioridazine	800
4	M	Trifluoperazine; haloperidol	840
5	F	Haloperidol; chlorpromazine	900
6	M	Triflouperazine; thioridazine	950
7	M	Sulpiride; haloperidol	1000
8	F	Haloperidol; thioridazine	900
9	F	Pimozide; trifluoperazine	1200
10	M	Haloperidol; thioridazine	920
11	M	Haloperidol; chlorpromazine	800
12	M	Haloperidol; thioridazine	870
13	M	Sulpiride; chlorpromazine	970
14	F	Pimozide; thioridazine	1000
16	M	Haloperidol; thioridazine	1200
17	M	Pimozide; thioridazine	1100
18	F	Haloperidol; thioridazine	900
19	M	Haloperidol; chlorpromazine	870
20	M	Haloperidol; thioridazine	860
21	M	Sulpiride; trifluoperazine	1100
22	M	Haloperidol; thioridazine	900

all patients were receiving haloperidol at least for the 4 weeks preceding the scan. Eleven subjects had abandoned their medication during the months prior to the study and as a result, were hospitalized during a psychotic break; they received haloperidol (10–15 mg/day) during 4 weeks to corroborate treatment resistance. The other 11 patients had been continuously treated with classical antipsychotics during the previous year, also receiving haloperidol during the 4 weeks prior to the basal scan as prescribed by their treating psychiatrist.

The follow-up scan was made after 6 months of treatment with clozapine, titrated up to an effective dose (on clinical grounds). Minimum and maximum doses were respectively 300 mg/day and 600 mg/day. The mean final clozapine dose was 477.56 mg/day (SD, 109.25). The patients received no other medication. Compliance with clozapine was monitored by weekly clinical interviews during the study period, taking into account clinical examination (psychiatric status and side effects) and information collected from patients and relatives. Compliance was deemed to have been good during this period in all cases, according to the same sources of information. Blood cell counting was performed according to the protocol for clozapine prescription in Spain (weekly during the initial 18 weeks and then monthly).

Scanning procedure

Both PET scans were obtained for each patient with the same scanner and according to identical protocols. PET studies were obtained with a Posicam EZL PET tomograph, 20 min after injection of 370 MBq 18-FDG. Matrix size was 256×256×61, and slices were 2.6 mm thick. Subjects were instructed to lie in a supine position in a dark, quiet room with eyes open and ears unplugged starting 10 min before FDG administration and for another 20 min before image acquisition. They did not receive any other special instruction but to try to remain as relaxed as possible. PET study was performed after a fasting period of more than 6 h. Coffee and psychoactive beverages were prohibited.

Image analysis

PET images were analyzed with the SPM99 software package (from the Wellcome Dept. of Cognitive Neurology, London, UK) (Frackowiak et al. 1997). Studies were transformed into a Talairach stereotactic space (Talairach and Tournoux 1988) ,warping each scan to a reference template that already conforms to the standard space. Instead of using the standard SPM template based on O^{15}-PET, we used our own FDG-template image, which was created using FDG-PET scans from a series of control subjects, following the procedure described in Gispert et al. (2003). Use of a reference scan based on the same radio-tracer as the scans studied provides a higher sensitivity in the posterior statistical analysis than the standard PET template included in SPM99 (Gispert et al. 2003). Images were reformatted to a final voxel size of 2×2×2 mm and smoothed using an isotropic Gaussian kernel of 12×12×12 mm FWHM. Gray-level threshold was set to 0.8, i.e. only voxels with an intensity level above 0.8 of the mean level for that scan were included in the statistical analysis. Intensity normalization was carried out using proportional scaling, thus assuming that global brain metabolism was equal for every scan.

The metabolic effect of treatment switching from haloperidol to clozapine was assessed by means of longitudinal comparisons using one-tailed paired Student's *t*-tests to check separately for hyperactivations and hypoactivations between both treatment conditions. Association of metabolic changes with the three symptom scores (positive, negative, and disorganization) was assessed by calculating the correlation between metabolic activity values and rank transformed symptom scores. One-tailed significance threshold was set to P=0.001, resulting in an overall significance level of P=0.002 for the two-tailed model.

P-values must be corrected in order to overcome the problem of multiple comparisons. SPM provides two correction criteria: (1) peak height-corrected *P*-value and (2) extent-corrected *P*-value (Poline et al. 1997). Establishing the proper thresholds to consider an observed *P*-value as significant is critical in this type of study (Andreasen 1996). For this reason, we carried out a validation procedure based on bootstrap techniques (Efron and Tibshirani 1986) to determine the adequate significance threshold for our data, as previously described (Desco et al. 2003; Molina et al. 2003). This procedure provided an empirical validation for our data of the significance threshold used throughout the study (uncorrected P<0.001).

Clinical significance of the changes in metabolism is graded into three different levels of evidence: (L1) peak height-corrected *P*-value below 0.05; (L2) extent-corrected *P*-value below 0.05; and (L3) uncorrected *P*-value below 0.001 and the area has already been previously reported as relevant in schizophrenia. These levels of significance allow to label those clusters that also meet more restrictive criteria beyond the uncorrected *P*-level of (P<0.001), thus highlighting stronger effects and facilitating the comparison with other studies that make use of alternative thresholds equivalent to our L1 and L2.

Results

Clinical changes

The three clinical dimensions significantly improved and the extrapyramidal side effects significantly decreased with clozapine (Table 3). No patient showed hematological side effects. There was a significant correlation between the changes in the positive and disorganization dimensions (r=0.45, df=21, P=0.04). Negative symptom

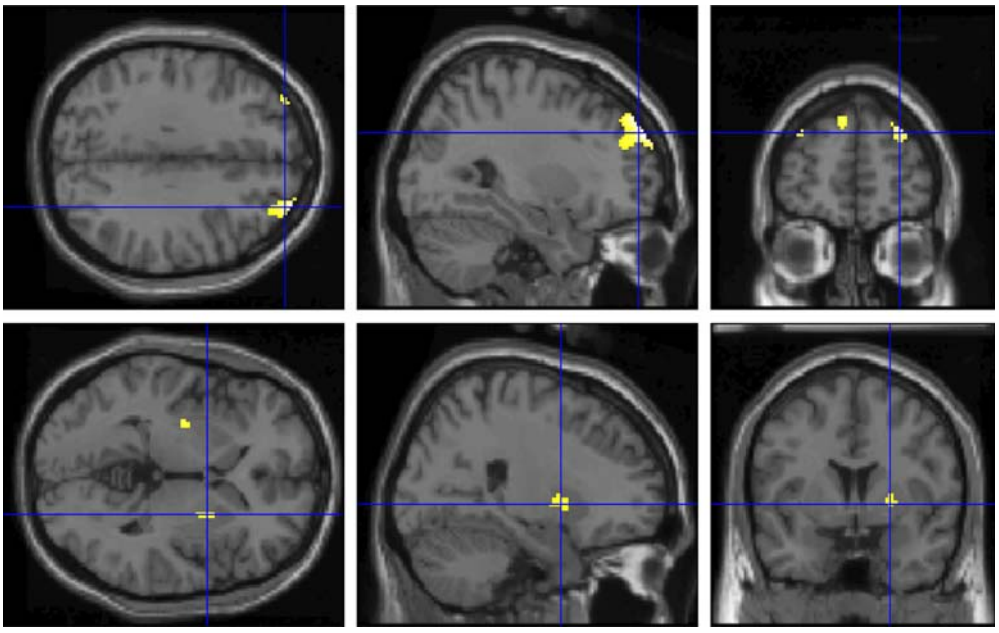

Fig. 1 Regions showing higher activity in the haloperidol condition (*HC*) than in the clozapine condition (*CC*). Images are in radiological convention: the left side of the image is the right side of the patient. To help the anatomical interpretation, regions are superimposed to the SPM MR template. See Table 4 for details about significance values and coordinates of each region

Table 3 Clinical data before and after clozapine conditions, (N=22). Values are expressed as mean (SD). Clinical scores correspond to SAPS and SANS, and extrapyramidal scores to the UKU scale. Statistical significance of clinical changes is assessed with paired t-tests. *P<0.05, ***P<0.001

	Before clozapine	After clozapine
Positive***	27.3 (13.9)	12.2 (9.8)
Disorganization***	18.2 (11.7)	10.6 (10.0)
Negative*	(7.2 (19.5)	(1.8 (18.9)
Extrapyramidal***	8.6 (8.2)	2.3 (1.9)

change was not associated with the change in any other dimension.

Metabolic results

These results are presented as statistical maps showing pixels with an uncorrected P-value lower than P<0.001 superimposed on an MRI template (the T1-weighted anatomical template of SPM99). Tables 4 and 5 summarize the results of the different comparisons, indicating regions with significant differences or correlation with metabolic changes, the SPM coordinates of their statistical maximum, the z-value, the number of voxels and the evidence criteria as defined above.

Changes between haloperidol and clozapine conditions

This longitudinal comparison revealed a significant metabolic decrease from haloperidol to clozapine conditions in the cortex in dorsolateral prefrontal, medial prefrontal and left inferior temporal regions, and bilaterally in basal ganglia (all L3 criteria). A significant increase in activity from haloperidol to clozapine conditions was detected in the occipital cortex, including the primary visual area (L1 and L2 criteria). These results are shown in Figs. 1 and 2 and Table 4.

Table 4 Areas that showed significant differences of activity in the different comparisons (*HC* haloperidol condition, *CC* clozapine condition, *L* left, *R* right). Figures showing the corresponding SPM maps are indicated in square brackets. The table presents the SPM coordinates of the maximum, its z-value (Z_{max}), and number of voxels (N_{vox}) and level of evidence (*LOE*) for each area. Levels of evidence are L1: peak height-corrected P-value lower than P<0.05; L2: extent-corrected P-value lower than P<0.05; L3: uncorrected P-value below P<0.001 in an area that has been previously reported as relevant in schizophrenia (see Materials and methods for details). Regions with identical number of voxels indicate that they belong to the same cluster

Comparison of regions	x, y, z (mm)	Z_{max}	N_{vox}	LOE
HC (n=22) > CC (n=22) paired t-test (Fig. 1)				
Dorsolateral cortex (L)	30, 58, 32	4.10	232	L3
Pallidum, putamen (R)	−30, −12, 2	3.62	37	L3
Medial prefrontal	−6, 54, 40	3.53	64	L3
Dorsolateral cortex (R)	−34, 52, 34	3.51	13	L3
Pallidum, putamen (L)	26, 4, 2	3.32	42	L3
HC (n=22) < CC (n=22) paired t-test (Fig. 2)				
Occipital cortex (L)	34, −78, 4	4.98	3,427	L1
Primary visual (R)	14, −70, −4	3.92	3,427	L2
Occipital cortex (R)	−30, −82, 20	4.13	280	L3
Primary visual (L)	14, −48, 16	4.10	161	L3
Occipital cortex (M)	4, −96, 22	4.00	426	L2

Correlation between metabolic and clinical changes

The decrease of activity in the basal ganglia was significantly associated with the degree of improvement of negative symptoms (L2 criteria). On the other hand, the

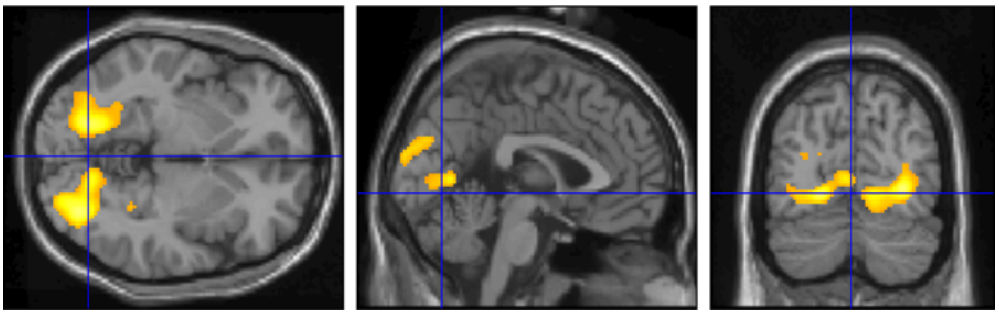

Fig. 2 Regions showing lower activity in the haloperidol condition (*HC*) than in the clozapine condition (*CC*). Images are in radiological convention: the left side of the image is the right side of the patient. To help the anatomical interpretation, regions are superimposed to the SPM MR template. See Table 4 for details about significance values and coordinates of each region

Table 5 Coordinates, significance values, and the level of evidence (*LOE*) for the regions that showed an association between changes in clinical dimensions and metabolic activity changes. (*L* left, *R* right, *M* medial). Figures showing the corresponding SPM maps are indicated in square brackets. The table presents the SPM coordinates of the maximum, its *z*-value (Z_{max}), the number of voxels (N_{vox}), and level of evidence (*LOE*) for each area. Levels of evidence are L1: peak height-corrected *P*-value lower than $P<0.05$; L2: extent-corrected *P*-value lower than $P<0.05$; L3: uncorrected *P*-value below $P<0.001$ in an area that has been previously reported as relevant in schizophrenia (see Materials and methods for details). Regions with identical number of voxels indicate that they belong to the same cluster

Syndrome	*x*, *y*, *z* (mm)	Z_{max}	N_{vox}	LOE
Disorganization				
Motor (L and R)	0, −12, 54	5.21	22105	L1
Negative				
Pallidum/putamen (L)	−24, 0, 10	3.74	18	L2
Pallidum/putamen/caudate head (L)	16, 2, 14	3.26	5	L2
Positive				
Visual (M)	2, −70, 32	4.67	230	L1

decrease of activity in the motor area was related to the improvement in the disorganization dimension (L1 criteria). Improvement in positive symptom scores was significantly related to the increase of activity in the primary visual area (L1 criteria). These data are shown in Figs. 3 and 4 and Table 5. There was no association between the changes in extrapyramidal scores and activity changes in any area.

Discussion

In this work we found significant changes in regional activity when the treatment of patients was switched from haloperidol to clozapine. Moreover, we found that regions associated with clinical changes partially overlapped with those showing changes between the two treatment conditions. We will discuss the findings in each of these areas.

Clozapine increased occipital cortex activity (including primary and association visual cortex), and the improvement in positive symptoms was related to the metabolic increase in the primary visual area. Other results support the relevance of the metabolic increase with clozapine at this level in schizophrenia. First, we reported that risperidone produced a similar effect in the visual area in schizophrenic patients of recent onset (Molina et al. 2003). Second, a decrease of activity in the visual area has been reported in untreated schizophrenic patients in resting state (Andreasen et al. 1997), and such hypoactivity was found to be a feature common to minimally treated and chronic patients (Desco et al. 2003). Nevertheless, a primary involvement of the visual area in schizophrenia or in the action of clozapine seems unlikely; thus, other explanations must be sought to justify the association of metabolic changes in the occipital region with clinical improvement in the present report. One of these possibilities has to do with a possible thalamic dysfunction in schizophrenia. The occipital cortex is activated when the individual is at rest with eyes open and that region receives excitatory projections from the thalamus (Mufson and Mesulam 1984). Therefore, a deficit of visual cortex activation might derive from a thalamic dysfunction, which has been replicated in schizophrenia (Pakkenberg 1992; Andreasen et al. 1994; Buchsbaum et al. 1996; Young et al. 2000). Indeed, a decreased volume of the pulvinar nucleus of the thalamus, a key component of the visual system, has been recently reported in schizophrenia using both MRI (Kemether et al. 2003) and post-mortem (Byne et al. 2002) assessments. Thus, the increased activation of the occipital cortex with clozapine may reflect an improvement in thalamic function, in line with a report of clozapine-induced Fos in the midline thalamic nuclei (Cohen and Wan 1996). This interpretation should be viewed cautiously since most of the previous literature rather supports the alteration of the mediodorsal thalamic nucleus in schizophrenia (Pakkenberg 1992; Hazlett et al. 1999; Popken et al. 2000; Young et al. 2000). Nevertheless, an involvement in schizophrenia of the mediodorsal nucleus would not be incompatible with a role in that disease for other nuclei, such as the pulvinar, whose dysfunction might disrupt the activity of posterior areas.

It could be argued that the increase in occipital activity in our group was related to haloperidol withdrawal, as suggested by the decrease of occipital activity with haloperidol from an off-medication baseline reported by Lahti et al. (2003b). However, other authors failed to find

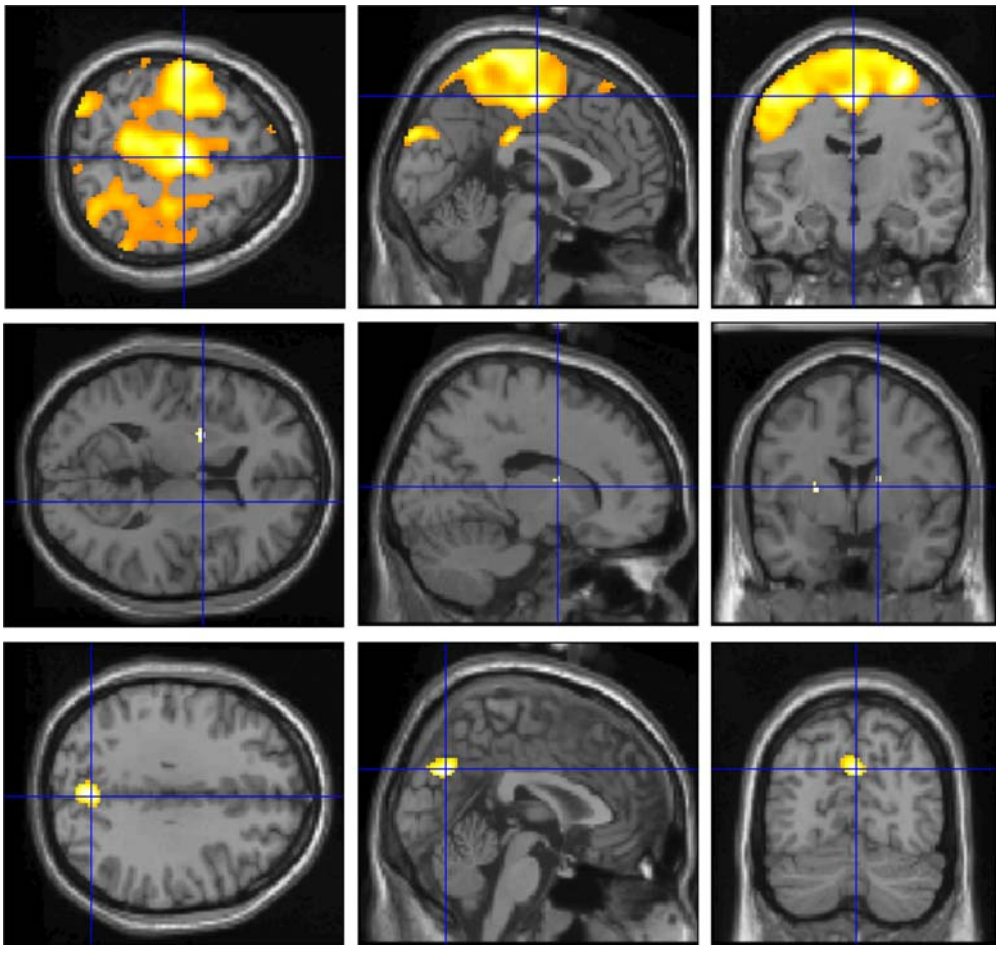

Fig. 3 Regions showing significant correlation between changes in metabolic activity and syndrome scores. *Top row*, correlation between the motor cortex and disorganization; *middle row*, correlation between basal ganglia and negative symptoms; *bottom row*, correlation between the visual cortex and positive symptoms. See Table 5 for details about significance values and coordinates of each region

a significant metabolic effect of haloperidol withdrawal at occipital level (Holcomb et al. 1996). Besides, the biological relevance of the metabolic change at occipital level was supported by the significant correlation with symptom improvement in our patients

It would be also possible that the increase of occipital activity with clozapine in our patients was due to a global activity decrease in other parts of the brain, which, after normalization of the PET scans, could yield an apparent occipital increase. This is theoretically supported by the higher D_1 blockade ratio induced by clozapine that might inhibit cortical activity in other cortical regions to a greater degree than in the occipital lobe, as D_1 receptors activate adenylyl-cyclase and are specially distributed in the anterior regions (Fuster 1997b). However, if the increase of occipital metabolism were due only to the inhibition of other regions we would expect significant changes of activity in the corresponding area(s). On the other hand, the use of an FDG template for spatial normalization in our patients may control against bias derived from the normalization procedure better than the use of the standard SPM template, based on O^{15}-PET scans (Gispert et al. 2003).

Basal ganglia activity was lower in the haloperidol than in the clozapine condition. The significant association between changes in negative symptom scores and striatal metabolic decrease suggests that basal ganglia inhibition may be relevant to the therapeutic action of clozapine. Two mechanisms may explain that association: a decrease in extrapyramidal side effects, and thus of secondary negative symptoms, or a specific action of clozapine on negative symptoms. These possibilities are not mutually exclusive. The first possibility is supported by the association between extrapyramidal symptoms and the occupancy of striatal D_2 receptors (Broich et al. 1998; Kapur et al. 2000), the D_2 occupancy rate being lower with clozapine than with classical neuroleptics (Farde et al. 1994; Tauscher et al. 1999). In addition, extrapyramidal scores in treated patients also directly relate to dorsal putamen metabolism (Shihabuddin et al. 1998), and classical antipsychotics increase striatal activity more intensely than clozapine (Lahti et al. 2003b). Therefore, less D_2 occupation with clozapine would produce both milder extrapyramidal effects (and subsequently, milder secondary negative symptoms) and lower activity rates in basal ganglia. In this case, the association between striatal inhibition with clozapine and improvement in negative symptoms would simply reflect the lesser parkinsonism with this drug, in turn consistent with the lack of efficacy of clozapine in the deficit syndrome (Buchanan et al. 1998).

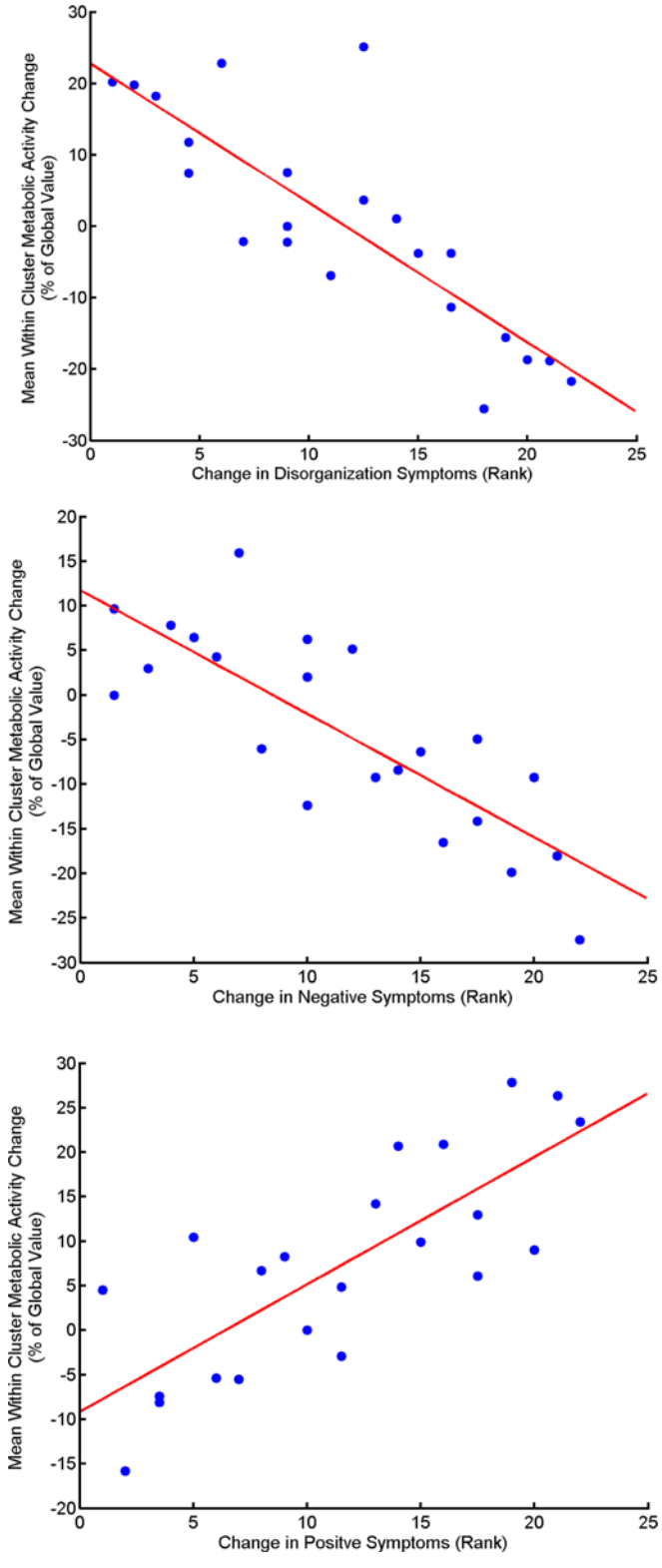

Fig. 4 Scatterplots showing the association between changes in symptoms (rank-transformed) and metabolic changes (mean within-cluster metabolic activity) from haloperidol to clozapine conditions. *Top*: motor cortex and disorganization. *Middle*: basal ganglia and negative symptoms. *Bottom*: visual cortex and positive symptoms. See Table 5 for details about significance values and coordinates of each region

The second possibility is a primary role for basal ganglia in the improvement of negative symptoms with clozapine. Several factors suggest that this may be the case. First, in our patients, no association was found between changes in extrapyramidal symptom scores and metabolic changes. Second, Pickar et al. (1996) reported that negative symptom worsening from standard to low clozapine doses was unrelated to the change in D_2 occupancy. Third, a reduction in basal ganglia metabolism may be primarily caused by clozapine, as demonstrated by studies in animals not previously exposed to classical neuroleptics (Tsai et al. 2001; Cochran et al. 2002). Fourth, we have reported elsewhere that responders to clozapine (whose negative symptoms improved) showed a significant decrease of basal ganglia perfusion with this drug (Molina Rodriguez et al. 1996). This effect was not found in non-responders to clozapine, whose negative symptom scores did not change. Finally, a recent study showed that clozapine but not haloperidol restores task-related anterior cingulate activity in patients with schizophrenia to the same levels as in normal volunteers (Lahti et al. 2003a). This result is consistent with the view that clozapine might regulate the activity of corticostriatal loops, yielding the striatal decrease observed in our study, in turn perhaps related to the improvement of the negative dimension.

According to the initial hypothesis of a prefrontal involvement in the action of clozapine, the switch from haloperidol to this drug decreased prefrontal metabolism. That change is not attributable to haloperidol withdrawal, since the effect of the withdrawal of this drug points towards an increase in PF activity (Holcomb et al. 1996). Our patients were studied under a "resting" cognitive condition, but other authors using a standard cognitive activation paradigm also reported lower prefrontal activity following clozapine treatment with respect to fluphenazine-treated (Cohen et al. 1997) or placebo-treated (Potkin et al. 2003) patients. The metabolic decrease in the PF region agrees with observed effects in animal pre-clinical studies after acute (Tsai et al. 2001; Cochran et al. 2002) and chronic (Tsai et al. 2001) clozapine administration.

A greater decrease in PF (dorsolateral and medial) metabolism may therefore be a distinctive feature of clozapine versus classical neuroleptics. This decrease is also probably larger than that produced by risperidone, since risperidone does not appear to decrease PF activity to a greater degree than haloperidol (Miller et al. 2001; Molina et al. 2003). The greater metabolic decrease in the PF region observed with clozapine, one of the most effective antipsychotics, challenges the concept of the decrease of frontal activity as simply a side effect of antipsychotic drugs. Indeed, in previous studies using single photon emission tomography, our group found that only responders to clozapine showed a significant decrease of PF and thalamic activity compared to a haloperidol baseline (Molina Rodriguez et al. 1996; Rodríguez et al. 1997). This seems coherent with the report from Potkin et al. (2003), who described that only responders to clozapine showed a significant metabolic decrease in the cortex.

Despite being unrelated to symptom improvement, it can be speculated that the observed PF metabolical decrease could relate to the clozapine-induced improvement of neuropsychological PF dysfunction in schizophrenia (Galletly et al. 1997; Manschreck et al. 1999). This possibility can be tested using an adequate design.

The metabolic decrease in the PF region with clozapine is consistent with other pre-clinical data. Clozapine increases extracellular dopamine in the prefrontal cortex (Moghaddam and Bunney 1990; Yamamoto et al. 1994) and dopamine increases GABA release in the same region (Grobin and Deutch 1998), this GABA release being coherent with a lower metabolic rate. From another perspective, the higher ratio of D_1 to D_2 blockade with clozapine than with haloperidol (Meltzer et al. 1989) may also be relevant to the metabolic inhibition with clozapine. D_1-type receptors are more densely distributed in the PF cortex, and their activation stimulates neuronal adenylcyclase (Fuster 1997b). It is therefore plausible that a higher degree of blockade of these receptors may contribute to the PF metabolic decrease observed with clozapine. That possibility would be consistent with an improvement of neuropsychological performance in schizophrenia, since D_1 antagonists may potentiate working memory (Williams and Goldman-Rakic 1995). From this perspective it is interesting to note that both metabolic changes with clozapine and clinical changes with this drug were found to be associated with a D_1 receptor allele (Potkin et al. 2003).

A previous study reported increases of the activity in some subregions of the PF lobe, while metabolism decreased in other PF regions (Lahti et al. 2003b). This discrepancy may suggest a complex pattern of changes within this region with clozapine or, instead, be related to methodological differences. For example, in that study patients scored low in psychopathological scales, and an interaction might be suspected between clinical state and the metabolic actions of clozapine.

Regarding the motor region, a significant association between changes at this level and improvement in disorganization was found. Although this finding is quite unusual, and clearly needs replication, it suggests a metabolic decrease in the motor region limited to the patients with a greater improvement on the disorganization dimension. This idea is consistent with the direct association between disorganization scores and motor area activity reported by Schroder et al. (1996). A similar association was reported by Kircher et al. (2001), who described using functional MRI that the severity of formal thought disorder (a key feature of the disorganization syndrome) was directly related to the activity of the right pre-central region. Functional correlates of the disorganization syndrome are still incompletely understood, since thought disorder has been positively (Sabri et al. 1997) and negatively (McGuire et al. 1998) related with frontal and temporal activity and with activity in the inferior parietal lobe (Kaplan et al. 1993). In this context, our results may imply that changes induced by clozapine at pre-central level could compensate for abnormalities in other regions related to the disorganization syndrome.

On the other hand, a role for previous treatment with haloperidol might have influenced the association between changes in motor area and improvement in disorganization. This is suggested by the increase seen in motor area activity in patients chronically treated, when compared with recent-onset patients (Desco et al. 2003). Even so, it seems unlikely that this association would explain the greater metabolic decrease in patients showing a greater improvement in disorganization. Motor area activity correlated with the parkinsonian side effects of haloperidol (Molina Rodriguez et al. 1997), but disorganized thinking and behavior are not parkinsonian effects.

We could not find a decrease in the limbic region with clozapine as recently reported (Lahti et al. 2003b; Potkin et al. 2003). Nevertheless, the decrease of PF activity in our patients seems coherent with a downstream limbic hypoactivation, as hippocampus receives a strong excitatory projection from the PF lobe (Barbas 1992; Huntley et al. 1994; Fuster 1997a).

The main limitation of our study is that patients had been treated with haloperidol prior to clozapine, as previously discussed for each region showing significant changes. This limitation is shared by other similar studies (Lahti et al. 2003a, 2003b). The confounding effect of the previous treatment could only be avoided by administering primary treatment with clozapine, precluded in light of current clinical recommendations. Another limitation is the lack of plasma clozapine levels, unavailable to us. This would be the best way to ensure that patients had been compliant with treatment. However, compliance was judged to be good on a clinical basis, and the symptom improvement shown by our treatment-resistant patients is consistent with good compliance with clozapine.

The strengths of our study include longitudinal comparisons, a larger sample than previous reports, and the assessment of the relationship between clinical improvement and changes in metabolic activity. We can conclude that prominent effects of clozapine are a greater PF and basal ganglia metabolic decrease than observed with haloperidol, as well as an increase in visual cortex activity. The improvement in clinical dimensions observed with clozapine appears to relate to the increased activity in the visual cortex (positive dimension) and decreased activity in the basal ganglia (negative dimension) and motor cortex (disorganization dimension).

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