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Regional cerebral blood flow SPECT study, at rest and during Wisconsin Card Sorting Test (WCST) performance, in schizophrenia naive patients or treated with atypical neuroleptics

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Introduction. To corroborate the hypothesis of hypofrontality in schizophrenia and to study the relationship between positive/negative symptoms (measured by the positive and negative syndrome scale [PANSS]) and regional cortical blood flow (rCBF), both at rest and during the Wisconsin Card Sorting Test (WCST) performance (activation).

Methods. We compared a control group (n = 18) to a group of patients with schizophrenia (n = 21) in terms of rCBF, measured by single photon emission computed tomography (SPECT).

Results. We found significantly higher left-frontal-CBF (during the WCST performance and at rest) and right-frontal-CBF (only at rest) in control subjects. Only the control group showed a right-frontal-CBF increase during activation. Only the patients group showed a significant right-occipital-CBF increase during the activation. We observed a positive significant correlation between the PANSS-P score and the left-frontal index at rest. Some negative symptoms such as difficulty in abstract thinking (N5) and lack of spontaneity and flow of conversation (N6) are associated to low frontal blood flow at rest. Affective blunting (N1) is associated to low left-frontal blood flow during activation.

Conclusions. Our data support the hypothesis of hypofrontality, at rest and during activation, which means the incapacity of schizophrenic patients to increase the frontal CBF while performing the WCST (activation). Schizophrenia positive symptoms are associated to high left-frontal blood flow.

Key words:
Schizophrenia. SPECT. PANSS. Frontal cortex.

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Estudio del flujo sanguíneo cerebral regional (FSCr) mediante SPECT, en reposo y activación con el Test de Clasificación de Tarjetas de Wisconsin (WCST), en pacientes con esquizofrenia sin tratamiento o tratados con neurolépticos atípicos

Introducción. Corroborar la hipótesis de la hipofrontalidad en la esquizofrenia y estudiar la posible relación existente entre los síntomas positivos y negativos (medidos con la escala de los síndromes positivos y negativos [PANSS]) y el flujo sanguíneo cerebral regional (FSCr), tanto en reposo como en activación mediante el Wisconsin Card Sorting Test (WCST).

Métodos. Comparamos el FSCr de un grupo de controles (n = 18) con el de un grupo de pacientes con esquizofrenia (n = 21) mediante tomografía computerizada por emisión de fotón único (SPECT).

Resultados. En el grupo control los índices de FSC frontal izquierdo en reposo y activación y derecho en activación son significativamente superiores a los de los pacientes. Únicamente en los controles el índice frontal derecho experimenta un incremento significativo como resultado de la activación. El FSC occipital derecho en activación se incrementa significativamente sólo en los pacientes. Observamos una correlación positiva significativa entre las puntuaciones de la PANSS-P y el índice frontal izquierdo en reposo. Síntomas aislados de la PANSS-N como dificultad en el pensamiento abstracto (N5) y falta de espontaneidad y fluidez en la conversación (N6) se asocian a hipoperfusión frontal en reposo. El embotamiento afectivo (N1) se asocia a hipoperfusión frontal izquierda en activación.

Conclusiones. Nuestros datos apoyan la hipótesis de la hipofrontalidad, tanto en reposo como en activación, es decir, entendida como la incapacidad de los pacientes con esquizofrenia para incrementar el FSC frontal durante la ejecución del WCST (activación). Los síntomas positivos de la esquizofrenia se asocian a hiperperfusión frontal izquierda.

Palabras clave:
Esquizofrenia. SPECT. PANSS. Corteza frontal.

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INTRODUCTION

In recent years, a large part of the advances in pathophysiological knowledge on schizophrenia comes from investigation with functional neuroimaging techniques. The first studies performed with the Xenon inhalation technique and cortical probes suggest a decrease of the frontal CBF in the brains of patients with schizophrenia, in at rest conditions. This hypothesis was confirmed years later by positron emission tomography (PET) and SPECT and then by these techniques, in activation conditions. In other words, this was done during the performance of experimental tasks that hypothetically stimulate the frontal cortex, such as the Wisconsin Card Sorting Test (WCST)[1], Continuous Performance Test[2], or London Tower Test[3]. Some of the studies were conducted in young patients with a first psychotic episode who had never been treated with neuroleptics. Thus, the findings mentioned probably were not due to chronicity of the picture or to pharmacological treatment[2,4]. At that time, the so-called «hypofrontality hypothesis» in schizophrenia was postulated.

During the decades of the nineties, the existing relationship between psychopathology of schizophrenia and the neuroimaging findings found up to then were studied in more depth. Several studies related the previously mentioned frontal hypoperfusion at rest and negative symptoms of schizophrenia[5-7]. Other studies suggest the association of certain hyperfrontality patterns with positive symptoms of an acute psychotic episode in the context of schizophrenia[8].

Parallelly, use of O^{15} labeled water in PET studies or application of functional imaging analysis techniques, such as the Statistical Parametric Mapping, permitted the location of brain areas involved in neuropsychological test performance such as WCST. Frontal regions were mainly involved in controls and it was postulated that other cerebral regions such as the occipital, parietal and temporal ones[9], that could be dysfunctional in schizophrenia, could be involved.

In the present paper, we use SPECT to study the rCBF of patients with schizophrenia and we compare it with that of healthy subjects. We try to examine if there are differences at rest. This would support the hypothesis of hypofrontality defined as a decrease of blood flow of the frontal regions of patients with schizophrenia[10-12]. At the same time, we propose to corroborate the incapacity of patients with schizophrenia to significantly increase the rCBF of the frontal regions in activation, that is, when they perform the WCST[13]. On the other hand, we analyze the role that may be played by other cerebral regions in the performance of complex tasks such as those required during the performance of the WCST. This may also involve temporal, parietal and occipital areas. Finally, we intend to study the possible relationship existing between positive and negative symptoms of schizophrenia and regional brain blood flow, not only in at rest conditions, but also during WCST performance.

OBJECTIVES

- Verify the hypofrontality hypothesis, studying the rCBF variations (decreases and increases) occurring between at rest and activation, in control subjects and schizophrenia patients. Thus, not only the frontal regions were analyzed as classically done, but rather the different areas of the cerebral cortex that could be involved in the performance of executive functions, whose alteration has been stressed so centrally in schizophrenia (frontal, temporal, parietal and occipital regions).
- In the second place, study the relationship that may exist between flow metric variations and schizophrenia symptoms. Some previous studies showed the association between negative symptoms and frontal at rest hypoperfusion. Thus, the new aspect of this objective is that it hypothesizes if the symptoms and symptomatic groups could be predicted by the analysis of the flow variations occurring during the WCST test since all the previous studies had been conducted in at rest conditions.

In short, to know what cortical regions were really involved in the WCST performance in both controls and patients, trying to find the possible differences existing between both groups and if there are symptom groups that could have a correlate in functional neuroimaging.

MATERIAL AND METHODS

Subjects

The study was conducted in two groups: one control of volunteers without psychiatric disease (n = 18; age: 26.61;

Table 1 Characteristics of the two subject groups studied

	Controls (n = 19)	Cases (n = 21)	p
Age (years)	25.6 (4.4)	26.6 (6.5)	0.640
Gender: men/ women (%)	73.3/26.7	88.2/11.8	0.383
Hand preference	16/3	19/2	0.601
Study level: basic/middle/ university (%)	0/26.7/73.3	31.3/62.5/6.3	0.043

SD: 6.32; h/m: 13/5) and another of patients with schizophrenia (n = 21; age: 25.59; SD: 6.28; m/w: 19/2) (table 1). The common inclusion criteria for both groups were: being between 18 and 60 years old, not nursing, not having a previous history of substance dependence or abstinence or recent one of intoxication (last 6 months) and not having another disease at the time of the study. Informed consent was required from all the participants.

In the control group, another inclusion criterion was that of not having any personal or family psychiatric and/or neurological background. Exclusion criteria were: intelligence quotient less than 85, according to Raven test, and diagnosis of any mental disorder other than schizophrenia in the patient group.

The patients had to fulfill the schizophrenia diagnostic criteria according to the international classification of disease (ICD-10) and those of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV). The diagnostic evaluation was done by two independent psychiatrists, one being the investigator and the other, the one assigned to the patient for treatment. Only patients who had diagnostic concordance between the two raters were included. Prior to the SPECT tests, a psychopathological evaluation was done with the system of the Association of Methodology and Documentation in Psychiatry (AMDP) and the positive and negative syndrome scale (PANSS) in schizophrenia was filled out[14,15]. Four[4] neuroleptic-naive patients and 17 who were being treated with some of the following atypical neuroleptics were included: risperidone (n = 10; dose range of 3 to 9 mg; mean dose: 6 mg), clozapine (n = 3; dose range of 250 to 450 mg; mean dose: 300 mg) and olanzapine (n = 4; dose range of 10 to 20 mg; mean dose: 15 mg).

Table 2 Comparison of means between correct responses (CR) and perservative errors (PE) of controls and patients

Student's t test	n	t	Sign. (bilateral)
Total CR			
Controls	13	0.128	0.899
Patients	18		
Mann Whitney U test	**n**	**U**	**Sign. (bilateral)**
Total CR			
Controls	13	93,000	0.336
Patients	18		
Total PE			
Controls	13	47,500	0.005**
Patients	18		

The patients were enrolled from among those who came to our Department for a visit in the period between 1997 and 1999. They had a mean disease evolution of 4.5 years (SD: 3.3) and 1.91 previous admissions (SD: 1.90). During this same time period, the control group volunteers, made up by medical students, physicians-in-training and clinical assistants, took part in the study.

Hand preference of the participants was assessed with the Annet questionnaire for hand preference[16] in its Spanish adapted version[17]. According to this, the hand preference was divided into right handed and non-right handed (grouping left-handed and mixed forms). The right handed/non-right handed ratio in the patients was 19/2 and in the controls 15/3. There were no significant differences in age, distribution by gender and hand preference between both groups. A significant difference was only observed in study level (p = 0.043), with a mean years of study greater in the controls (mean: 18.94; SD: 6.32) than in the patients (mean: 14.82; SD: 4.63).

SPECT Procedure

In the SPECT studies, the perfusion tracer ^{99m}technetium hexamethyl propylene amine oxime (^{99m}Tc-HMPAO) was used. The dose administered was 740-1110 Mbq (13.32 Mbq/kg of weight) ^{99m}Tc-HMPAO injected intravenously.

The SPECT studies were acquired 20 minutes after the injection, using a gamma camera (Siemens, Orbiter 75) equipped with an astigmatic collimator (Neurofocal) in a 128 by 128 matrix. Reconstruction was done using a ramp filter (cut-off frequency: 1 Nyquist). After, the images were processed in a Microdelta computer (Siemens), correcting for attenuation (Chang method) and was reoriented according to the line formed by the inferior frontal pole and inferior occipital border. Nine mm thick tomographic slices in the coronal, saggital, and cross-sectional or axial levels were obtained.

The aspect considered most for the patient's preparation was his/her collaboration capacity. The test was cancelled in those cases in which there was clear movement. No participant had to be sedated.

Two brain SPECT studies were carried out in each subject with ^{99m}Tc-HMPAO: one at rest and another during activation with the WCST[18].

At rest condition

The subjects were lying down on an examination bed, in a room without noise. Their eyes were open for 10 minutes before and after the ^{99m}Tc-HMPAO injection. They were asked to not speak or read.

Activation condition

The day after the at rest study, the activation situation was provoked with the WCST. The WCST[19] is a problem resolution test by abstract reasoning that hypothetically requires the frontal lobe function. The test was performed in a well lighted room, with a desk and two facing chairs, one for the examiner and the other for the index case. Before beginning the test, a line to inject radio-tracer was placed in the subject. The response cards (2 × 64) had to be ordered according to the 4 initiation cards. The radio-tracer was injected when the subject was capable of correctly and consecutively ordering 5 cards in order to guarantee that he/she was already involved in the performance of the test and continued until the total 128 cards were completed. If any participant could not make 5 consecutive correct answers, the tracer would be administered in the middle of the test, that is, after ordering 64 cards.

Analysis of the SPECT data

At rest and activation images during WCST were evaluated qualitatively (visual examination) by two specialists in nuclear medicine and semi-quantitatively by the perfusion index calculation. Four cross-sectional or axial slice levels (superior, middle, inferior and cerebellum) were chosen for the semi-quantitative approach. Regions of interest (ROI) were drawn on three levels: *a)* superior level: superior frontal and superior parietal cortex (anterior, middle and posterior); *b)* middle level: middle frontal cortex (anterior and posterior) and parietal (anterior, middle and posterior); *c)* inferior level: inferior frontal cortex (anterior and posterior), temporal (anterior and posterior) and occipital. The ROI were always drawn by the same specialist, first in the left hemisphere (14 ROI) and then duplicated with mirror image on the right (28 ROI in all). In each cerebral region, the ROI were drawn in 3 consecutive slices and mean counts per pixel were obtained. The absolute values of each ROI were normalized with the following formula RDI = 100 × mean counts per pixel of the ROI/mean counts per pixel of all the ROI drawn.

The right and left occipital, frontal, temporal, parietal indexes were obtained by calculating the mean of the respective ROI. The indexes were calculated both at rest and in activation.

To carry out the first objective it was especially interesting, on the one hand, to compare possible intergroup differences in the different indexes. On the other hand, it was interesting to verify possible differences between at rest and activation state, within each group.

Within the first objective, it was also of interest to evaluate if the hypothetical frontal activation with the WCST was done laterally in the controls and if this lateralization effect differentiated them from schizophrenic patients using a single parameter. To do so, we calculated the asymmetry (AI), frontal, temporal, parietal and occipital indexes in both at rest and activation. We compared at rest and activation in each group. Using the right and left frontal, temporal, parietal and occipital indexes, we obtained the 4 asymmetry indexes, respectively applying the following formula: AI = 100 × D–I/D + I. A positive asymmetry index means that the flow was lateralized towards the right and vice versa.

Statistical methods

The Shapiro-Wilk test was used to study the normality of the continuous variables. A comparison of the perfusion and asymmetry indexes, at rest and activation, between controls and cases was performed with the Student's *t* test for independent samples, when the variable followed a normal distribution and by the Mann-Whitney U test on the contrary. Comparison between WCST parameters between patients and controls was done with the same instruments.

At rest and activation perfusion indexes were also compared with the Student's t test for paired samples, when the variable followed a normal distribution and the Wilcoxon signed rank test, when to the contrary.

The Spearman (rS) correlation was used to study the association between psychopathological indexes and variables of the PANSS, due to the ordinal nature of the latter. The χ^2 contingency tests and Fisher's exact test (when necessary) were performed to study the association of qualitative variables. The results are presented as mean (standard deviation). A bilateral *p* value less than 0.05 was defined as significant.

RESULTS

Comparison of the perfusion indexes at rest and activation between both groups (table 3)

The left frontal index is significantly greater in the control group, both at rest (p = 0.017) and in activation (p = 0.025). The right frontal index in activation in this group is also significantly greater (p = 0.004).

The right parietal index at rest is significantly greater in patients (p = 0.007); the difference not being significant (p = 0.08) in activation, although this same tendency to be superior in the patients is observed.

No significant differences are observed in the temporal or occipital indexes.

Comparison of perfusion indexes: at rest versus activation, in each group (table 3)

In the control group, the right frontal index has a significant increase (p = 0.003).

Table 3	Comparison of perfusion indexes		
ROIs	Controls (n = 18)	Patients (n = 19)	p (controls versus patients)
Right frontal			
Rest	99.69 (2.6)	98.80 (3.0)	0.348
Activation	101.46 (2.7)	98.81 (2.5)	0.004
p (rest vs activation)	0.003	0.98	
Left frontal			
Rest	97.39 (2.8)	94.96 (2.9)	0.017
Activation	97.78 (3.1)	95.12 (2.5)	0.025
p (rest vs activation)	0.576	0.79	
Right temporal			
Rest	100.51 (3.3)	100.65 (2.2)	0.88
Activation	99.77(3.4)	100.96 (1.8)	0.927
p (rest vs activation)	0.454	0.626	
Left temporal			
Rest	100.06 (4.1)	98.44 (2.5)	0.171
Activation	99.19 (4.4)	98.34 (2.2)	0.464
p (rest vs activation)	0.461	0.885	
Right parietal			
Rest	100.09 (3.2)	102.68 (2.1)	0.007
Activation	100.46 (2.9)	101.92 (2.2)	0.08
p (rest vs activation)	0.66	0.171	
Left parietal			
Rest	99.9 (1.8)	100.47 (2.3)	0.439
Activation	100.26 (3.1)	100.55 (2.0)	0.48
p (rest vs activation)	0.63	0.856	
Right occipital			
Rest	106.22 (3.9)	106.90 (3.3)	0.578
Activation	108.65 (5.7)	108.89 (3.2)	0.82
p (rest vs activation)	0.057	0.028	
Left occipital			
Rest	106.75 (5.3)	108.02 (3.4)	0.397
Activation	108.15 (6.8)	108.61 (4.2)	0.84
p (rest vs activation)	0.23	0.49	

In the patient group, the right occipital index has a significant increase (p = 0.028) while it is almost significant in the controls (p = 0.057)

No significant variations are observed in any of the groups in the temporal and parietal indexes.

Comparison of asymmetry indexes (AI) (table 4)

At rest, both the frontal AI (p = 0.0027) and the parietal AI (p = 0.036) are significantly greater in patients. No differences in the temporal or occipital AI between both groups are observed.

When the AI are compared between both situations, we see that the frontal AI in the controls has a significant increase, that is, the right lateralization increases. A non-significant decrease is seen in the patients.

No variations are seen in the parietal or temporal AI in any of the groups.

Table 4	Comparison of asymmetry indexes in at rest and activation conditions during WCST in controls and schizophrenic patients		
Regions/subjects	Controls (n = 18)	Schizophrenic patients (n = 19)	p (controls vs schizophrenic patients)
Frontal			
Rest	1.17 (0.9)	1.98 (1.1)	0.027
Activation	1.87 (0.9)	1.90 (0.9)	0.647
p (rest vs activation)	0.032	0.815	
Temporal			
Rest	0.23 (2.3)	1.11 (1.9)	0.223
Activation	0.40 (2.3)	0.80 (2.2)	0.577
p (rest vs activation)	0.927	0.663	
Parietal			
Rest	0.08 (1.5)	1.08 (1.2)	0.036
Activation	0.17 (1.5)	0.59 (1.2)	0.341
p (rest vs activation)	0.959	0.277	
Occipital			
Rest	–0.22 (1.0)	–0.52 (1.2)	0.427
Activation	0.26 (1.7)	0.14 (1.4)	0.535
p (rest vs activation)	0.142	0.047	

In both groups, there are increases in the occipital AI, in both groups, although they are only significant in the patient group (p = 0.047).

Correlation between indexes and score on positive and negative syndrome scale (PANSS) (table 4).

As is observed in table 4, the score of the positive syndrome scale (PANSS-P) positively and significantly correlates only with the left frontal index at rest (r = 0.47; p = 0.048).

The negative scale (PANSS-N) does not significantly correlate with any of the indexes, either at rest or in activation. When the symptoms making up the PANSS-P and PANSS-N scales are examined individually, significant correlations are observed between the right frontal index at rest and the excitation symptoms (P4) (r = 0.51; p = 0.028), and inverse correlation with difficulty in abstract thinking (N5) (r = –0.49; p = 0.034). The negative symptom lack of spontaneity and flow of conversation (N6) negatively correlates with the left frontal index at rest (r = –0.47; p = 0.047). On the other hand, the negative symptom affective blunting (N1) correlates negatively with the left frontal index in activation (r = –0.50; p = 0.029).

DISCUSSION

The results suggest that the controls base the performance of WCST on a greater stimulation of the frontal cortex, especially the right side. This result is congruent with the previous study findings done in controls by PET[20] and fMRI[21]. The frontal cortex is involved in planning and monitoring processes[22]. What is expected in a complex task, such as WCST performance, is that the frontal area increases its activity under normal conditions[21]. The group of patients has less left frontal activity at rest and in activation than the control group and is also incapable of increasing this activity in response to the activation. It also cannot increase its right frontal activity in response to the activation, which significantly differentiates them from the control group. These data agree with the previous findings in regards to the incapacity of the schizophrenic patients to activate the frontal cortex during WCST performance[8] and could be related with planning, monitoring and selection processes of attention towards a specific stimulus, inhibiting others to avoid distraction. This last process seems to be related with the anterior cingulate cortex that is within the frontal area. This cerebral region is also classically associated to affective and emotional aspects of the setting. Failure of the patients to increase their activity during the WCST performance could be a reflection of affective blunting.

Other data of secondary interest are that the WCST stimulates the occipital cortex, above all the right side, in both groups. This result agrees with that of a study by SPECT conducted in controls that has detected increases in visual cortex perfusion during WCST performance[9]. Thus, going from an at rest situation with the eyes open to one of visualization and performance of the WCST produces increases in occipital areas corresponding to a greater perfusion of the visual cortex. Although only in the schizophrenia patient group, the increases in the right occipital index reach statistical significance. This could be related with a reorganization of their cerebral blood flow, where the occipital activity may be relatively greater in the patients while the frontal activity may be relatively less than in the controls. However, given that no significant differences have been observed between groups in the occipital indexes in our study, we cannot conclude that they differ in activation of the occipital cortex.

The correlation of the PANSS scale with the frontal indexes has supplied results concordant with previous studies that would suggest the association of hyperfrontality with positive symptom intensity in schizophrenia[8] and supports the congruency of the classification of this group of symptoms into a subgroup defined within the total of the schizophrenia symptoms.

We have not found significant data that relate frontal hypoperfusion at rest with intensity of the negative symptoms, even though previous studies suggest this[5-7]. However, when each symptom is analyzed independently, we observe inverse correlations of rCBF with difficulty of abstract thinking (N5) and lack of spontaneity and flow of conversation (N6). This means that the greater intensity of these two negative symptoms is associated to a lower frontal perfusion at rest. This favors the role played by the frontal cortex in language modulation and integration of the setting in executive functions.

The intensity of affective blunting is associated to left frontal hypoperfusion in activation. This result could mean that affective blunting is manifested as an incapacity to increase frontal CBF in response to an executive task or that the increase of the frontal activity is necessary for the regulation of affectivity in the executive task context.

In this study, the subgroup of negative symptoms could not be significantly associated to frontal hypoperfusion in at rest or activation and certain symptoms that also have presented a congruent association pattern (inverse correlation) with that expected could be associated. This could mean a lack of homogeneity of this subgroup of symptoms, on the contrary to that which occurs with the negative symptoms.

Limitations

This study has several methodological limitations:

— The anatomic definition of SPECT is limited. Thus, it may have been better to use other brain neuroima-

Table 5 Correlations between frontal indexes, PANSS-P and PANSS-N scales and isolated symptoms of the negative scale in activation and at rest

Frontal indexes	PANSS-P	PANSS-N	Excitation (P4)	Affective blunting (N1)	Difficulty of abstract thinking (N5)	Lack of spontaneity and flow of conversation (N6)
Right						
Rest	0.30	−0.24	0.51	−0.41	−0.49	−0.06
	(0.219)	(0.320)	(0.028)	(0.087)	(0.034)	(0.81)
Activation	0.12	−0.02	0.43	−0.36	−0.04	0.09
	(0.606)	(0.932)	(0.062)	(0.128)	(0.851)	(0.69)
Left						
Rest	0.47	−0.31	0.17	0.02	−0.28	−0.47
	(0.048)	(0.210)	(0.483)	(0.922)	(0.244)	(0.047)
Activation	0.19	−0.10	0.25	−0.50	−0.19	0.11
	(0.425)	(0.683)	(0.287)	(0.029)	(0.432)	(0.627)
	P	N	P4	N1	N5	N6

ging methods such as the positron emission tomography (PET) or functional magnetic resonance imaging (fMRI). In spite of this, and considering these limitations, anatomical regions that showed clear functional significance and that could be easily defined by SPECT were chosen.

— Those who were receiving, or had received treatment at some time with neuroleptics versus those who had never been treated predominated in the patient group. Although the influence of drugs in the CBF patterns is not well known, it has been documented that the therapeutic effect of risperidone and clozapine in schizophrenia patients is accompanied by variations of cortical perfusion of the temporal and frontal areas in at rest situation[23,24]. It seems certain that patients with schizophrenia may improve initial performance in the WCST after being treated with atypical neuroleptics[25] and that performance on the WCST is related with increases in frontal perfusion. Small improvements of the perseveration symptom after long term treatment have been described[26]. It does not seem that the treatment has negatively influenced performance of our patients, considering that we found no significant differences between both groups in WCST parameters, including the correct responses, except in frequency of perserverative errors. Due to the characteristics of the disease, this was to be expected and constitutes a qualitative difference (observe that a reference of our research team has been eliminated here) more than a difference in global performance. On the other hand, although this question requires more studies that investigate it, it seems clear that the new neuroleptics have a minimum effect on the negative symptoms of the disease[27]. It also implies that some, such as clozapine, have demonstrated capacity to compare the responses of the patients in neurocognitive tests to those of the controls. This is seen by the presence in them of a greater effect of negative priming than in the naive patients or those under treatment with classic neuroleptics[28]. The fact that relative values of CBF were used would reduce the possible effect of the medication on the CBF patterns.

— As mentioned in the Material and Methods sections, we find significant differences between the education level of patients and controls, the years of study being significantly greater in the control group (table 1). It is clear that this difference could influence the performance of a neurocognitive test such as the WCST. However, as has been mentioned in the previous point, no significant differences were found in the global performance of the test, but only in the frequency of perseverative type errors. This implies an expected qualitative difference in schizophrenia patients.

— Regarding the statistical analysis, we should mention that some epidemiologists and specialized journal editors require a stricter criterion for the statistical significance than the conventional $p < 0.05$ when a statistical test is used to analyze the data of a study on some clinical aspect. A central problem in functional neuroimaging studies, due to its nature, is the large number of statistical tests required, which could generate false positives. An attempt could be made to resolve this through Bonferroni's adjustment. However, other authors admit that this method may become unnecessary and even detrimental when obtain-

ing statistical inferences because it is too strict and could eliminate true activations[29, 30]. Adjusting the significance according to the number of tests conducted may thus create more problems than are solved. The simple fact of describing the test and the statistical significance used and why is generally the best way to shortcut the problem of multiple comparisons[31]. We have based our analysis on clinical experience, clearly defining the study objectives and hypotheses.

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