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S. Guerra López[1, 2]
J. Iglesias Fuster[1]
M. Martín Reyes[1]
T. M. Bravo Collazo[1]
R. Mendoza Quiñones[1]
A. Reyes Berazain[1]
M. A. Pedroso Rodríguez[2]
T. Días de Villarvilla[1]
M. Antonieta Bobés[1]
M. Valdés-Sosa[1]

Attentional network task in schizophrenic patients and theirs unaffected first degree relatives: a potential endofenotype

[1]Department of Biological Psychiatry
Cuban Neuroscience Center
Havana

[2]Department of Clinical Neurophysiology
Moron General Hospital
Ciego de Ávila, Cuba

Introduction. In recent years, reports of attentional deficits in schizophrenic patients and in their biological relatives have rapidly increased, including an important effort to search for the endophenotypes in order to link specific genes to this illness. Posner et al. developed a test, the Attention Network Test (ANT), to study the neural networks. This test provides a separate measure for each one of the three anatomically-defined attention networks (alerting, orienting and executive control).

Methodology. In this paper, we investigate the attentional performance in 32 schizophrenic patients, 29 unaffected first degree relatives and 29 healthy controls using the ANT through a study of family association. We have studied the efficiency of the segregated executive control, alerting and orienting networks by measuring how response latencies (reaction time) were modified by the cue position and the flanking stimuli. We also studied the familial association of these attentional alterations.

Results. The ANOVA revealed main effects of flanker and cue condition and a significant interaction effect between flanker and groups studied. The schizophrenic patients and their relatives had a longer median reaction time than the control group. The probands and their relatives significantly differed from the healthy controls in terms of their conflict resolution; however, the alerting network appeared to be conserved.

Conclusions. Our results support the thesis of a specific attentional deficit in schizophrenia and show the segregation of the three attentional networks. The family association of these reported alterations supports the idea of a potential endophenotype in schizophrenia.

Key words:
Attention. Alerting. Executive control. Schizophrenia. Endophenotype. Orienting.

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Correspondence:
Seidel Guerra López
Departamento de Psiquiatría Biológica
Centro de Neurociencias de Cuba
Avenida 25, 152002. Cubanacan Playa, La Habana, Cuba
Fax: +537 208 67 07.
E-mail: seidel@moron.cav.sld.cu

Redes neurales de la atencion en pacientes con esquizofrenia y sus familiares no afectados de primer grado: un endofenotipo potencial

Introducción. Existe un notable incremento de reportes sobre déficit atencionales en pacientes con esquizofrenia y sus familiares de primer grado, incluyendo un gran esfuerzo por la búsqueda de endofenotipos para llegar a genes específicos responsables de la enfermedad. Posner y col. desarrollaron una prueba para explorar las redes neurales de la atención (ANT). Esta provee mediciones por separado para cada una de las tres redes neurales anatómicamente definidas (alerta, orientación y control ejecutivo).

Metodología: Investigamos a través de un estudio de asociación familiar el desempeño atencional en 32 pacientes con esquizofrenia, 29 familiares sanos y 29 controles utilizando el ANT. Examinamos segregadamente la eficiencia para las redes del control ejecutivo, la alerta y la orientación, evaluando como los tiempos de reacción eran modificados por la posición de la señal orientadora ("cue") y la congruencia contextual del estímulo ("flanker"). También exploramos la asociación familiar de estas alteraciones atencionales.

Resultados: Un ANOVA reveló un efecto principal del "flanker" y la condición del "cue" y una interacción significativa entre el "flanker" y los grupos estudiados. Los pacientes con esquizofrenia y sus familiares tienen un tiempo de reacción medio superior al grupo control. Los probandos y sus familiares difieren significativamente del grupo control en términos de resolución de conflictos, sin embargo, la alerta aparece conservada.

Conclusiones: Nuestros resultados apoyan la tesis de un déficit atencional específico en la esquizofrenia y evidencia la segregación de las tres redes neuro-atencionales. La asociación familiar de estas alteraciones soporta la idea de un endofenotipo potencial en la esquizofrenia.

Palabras claves:
Atención. Alerta. Control ejecutivo. Esquizofrenia. Endofenotipo. Orientación.

INTRODUCTION

The current development of cognitive neurosciences and neuroimaging has made it possible to conceive attention as a complex of neuronal networks that perform very specific operations to control mental activity.[1-4]

Recent research on attention has shown the existence of three segregated neural networks, with specific anatomical localizations associated to the release of specific neurotransmitters, which fulfill the functions of alerting, orienting and executive control. Alerting is defined as a mechanism that makes it possible to achieve and maintain a state of high sensitivity to incoming external stimuli. Specifically, this function involves changes in the internal state that allows the brain to be ready for the entry of any event that has occurred. It is an important source of attention, in the sense that it maintains an adequate vigilance level that is critical to achieve optimum performance. Neuroimaging studies show that the neural alerting networks are fundamentally located in frontal and parietal areas of the right hemisphere.[3, 5, 6]

On the other hand, orienting allows for selective focalization in one or in a few stimuli of several entry candidates. This attentional network rests on cortical areas in the superior and inferior part of the parietal and frontal lobe as well as in the subcortical areas related with ocular movements in the superior colliculus of the midbrain and in the thalamic pulvinar and reticular nuclei.[2, 6]

Executive control makes it possible to resolve conflicts between thoughts, feelings and responses and it is frequently necessary in operations requiring a high mental level, such as planning actions, detecting errors and making decisions, whether new or well-known, and in the latter, it allows them to be overcome. These networks are found in frontal areas, which include the anterior gyrus and the prefrontal lateral cortex.[2]

Pharmacological studies conducted in alert monkeys have made it possible to relate neural attention networks with specific chemical neurotransmitters.[7-9] Thus, it is thought that alerting is related with cerebral areas where norepinephrine is distributed. Lesions of the cholinergic system and use of beta blocker drugs of transmission with acetylcholine have an effect on the orienting of visual attention in monkeys. Finally, the neural networks of the executive control affect dopamine rich regimes of the anterior cingulate and pre-frontal cortex.[3, 7, 8]

Anatomical-functional failures of these complex mechanisms that underline attention are responsible for part of the alterations present in several neuro-psychiatric diseases such as schizophrenia, attention deficit hyperactivity disorder and autism.

Attention and schizophrenia

Several studies have stressed attentional deficit as the central axis of the cognitive alterations observed in schizophrenia.[10, 11] There are contradictions on which one or ones of the attentional components are most affected in this condition (alerting, orienting or executive control). Seemingly, a failure in the attention inhibitory mechanisms constitutes the basis of some of the signs and symptoms present in the disease.[9-11] The main body of the reports indicate that this deficit is restricted to the inhibitory mechanisms of the executive neural networks.[12-15] These results are complemented with studies that examine other functions of the frontal lobe, as the executive component of the working memory, by the Wisconsin Card Sorting Test (WCST) that is also altered in patients with schizophrenia.[12,13] These patients have an abnormal activation of associated remote semantics in indirect semantic facilitation tasks.[12-14] These alterations could be attributed to an alteration in the lexical network functioning and possibly in the working memory. Many investigations have been carried out to demonstrate this possible semantic alteration and to be able to understand its specific nature.[12-15]

A line of research indicates a disorder in the activation within the semantic neural networks, devoid of inhibition in schizophrenia. The other line defends evidence in favor of poor contextual use in these patients. This indicates that the semantic activation created by the context is not efficiently used in the subsequent processing of the stimulus.[16]

In tasks that investigate the contextual usage (flanker task), patients with schizophrenia reveal an important effect of the contextual usage. The reaction time (RT) of the patients with schizophrenia is slower when there is incongruency between the target stimulus and the context and the RTs are more rapid when there are congruences between both. These findings suggest that patients with schizophrenia have difficulties in conflict resolution that requires multiple and simultaneous responses.[17]

There are several reports in the literature consistent with a deficit of the alerting mechanisms in schizophrenia. Cornblatt and Malhotra, in 2001, using a version of the Continuous Performance Test or CPT, demonstrated that patients with schizophrenia had a sustained attention deficit. This suggests a dysfunction in the alerting mechanisms, even though this test involves several processes such as working memory, which is a function inherent to the executive control network.[17-19]

Studies in patients with schizophrenia using tasks that use orienting signals to measure reaction time, that is, cued reaction time tasks, showed significant effects with the use of the signal, the RTs being more rapid in the validated trials (with signals) and slower in the non-validated ones (without signals).[17]

Neurocognitive endophenotypes in schizophrenia

In the search for specific biological markers for schizophrenia, a high risk of morbidity for schizophrenia in their first degree relatives compared with the general population has been demonstrated, suggesting an elevated familial association in the disease.[18]

Endophenotypes have emerged with special emphasis in the familial studies of patients with schizophrenia. An endophenotype is an observable or measurable expression of vulnerability for the disease, which is hereditarily transmitted.[19] Gottesman and Gould defined the criteria for the evaluation of an endophenotype: 1. the endophenotype is associated with illness in the population, 2. the endophenotype is heritable. 3. the endophenotype is primarily state-independent (it may be detected in patients in remission phase of the disease who do not activity suffer it), 4. the endophenotype has greater prevalence in the affected relatives of the patients compared with the healthy relatives. Therefore, the endophenotype co-segregates with the clinical phenotypes and 5. the endotype found in members affected by the disease should be found in the healthy members of the family in a higher proportion than in the general population.[20]

Longitudinal studies in descendents of parents with schizophrenia reveal that the attentional alterations precede the appearance of the disease. Studying a sample of siblings pairs discordant for schizophrenia and normal subjects, Cannon et al. found that siblings discordant for the disease were affected during the performance of tasks that explore working memory, executive functions, attention, learning and memory (Trail making tests, Wisconsin Card Sorting Test (WCST), Stroop Test, verbal fluency test and other tests).[21-23]

Elemental visual processing, episodic verbal memory and spatial working memory are considered endophentypic candidates of schizophrenia.[21, 23] Patients with schizophrenia and their unaffected first degree relatives have difficulties in following moving objects, with alterations in smooth persuit eye movements of the visual stimulus and the occurrence of corrective saccadic movements. Similar alterations have been observed regarding the suppression of the middle latency P50 auditory evoked potential component in a stimulus conditioning and test task, where the suppression of the response to the second auditory stimulus is less in patients with schizophrenia and their first degree relatives regarding control subjects.[21, 23, 24]

Neural networks of attention in schizophrenia

Fan et al. (2002) developed a test for the examination of the neural attention networks (Attention Network Task, ANT) that makes it possible to evaluate the efficacy of the neural networks separately and to study the interrelationship between them. The advantages of these measurements over others obtained with the neuropsychological techniques is because they make it possible to rapidly examine the efficacy of each one of the neural attention networks, associating them to anatomical sites and specific chemical neuromodulators.[17,25-29] Studies with the ANT in healthy subjects have revealed high indexes in the average efficiency values for each one of the attentional networks (47 ms., 51 ms., and 84 ms. for alerting, orienting and executive control, respectively).[13,30] That is why it has been used in studies of different populations, including attention deficit hyperactivity disorders,[25] borderline personality disorders[31] and in patients with schizophrenia.[17,25,32] Studies have also been performed to evaluate genetic variations of the attention networks using the ANT.[33]

Fan J. et al. (2001) studied 26 pairs of monozygotic and dizygotic twins demonstrating that the network of executive control has high indexes of heritability ($h_F^2 = 0.89$, $h_H^2 = 0.62$), while alerting and the average of RT had a lower heritability ($h_F^2 = 0.18$, $h_H^2 = 0.14$ and $h_F^2 = 0.16$, $h_H^2 = .24$, respectively). However, orienting did not show heritability.[33] These results explain how the genetic variations contribute to differences observed in the executive functions of healthy individuals.[33, 34]

Fossella J. (2002) evaluated the efficiency of the neural networks by applying the ANT to 200 healthy subjects. He examined the genetic polymorphism in four candidate genes (DRD4, DAT, COMT and MAOA), finding an association of several polymorphisms with the efficacy of the executive control, but not with other measurements performed. These results support how the genetic variations are responsible for the inter-individual differences observed in the executive performance of healthy subjects. It was also demonstrated that the genetic influences on decision-making are specific for certain anatomically well-defined neural networks and that they do not affect the executive attentional performance globally or nonspecifically.[30]

A recent study evaluated the attentional performance of 77 patients with schizophrenia, comparing them with normal subjects using the application of the ANT. The controls in patients with schizophrenia did not differ in terms of verbal fluency and performance of memory. However, ANT data revealed significant differences in the conflict resolution between the patients with schizophrenia and the controls, showing a selective attentional deficit in the disease. Furthermore, the patients also showed significant differences in the functioning of the neural orientation network, although smaller than those reported in conflict resolution. However, the alerting network appeared to be functionally intact.[32]

Table 1 Demographic variables and clinical data for the three groups studied (patients with schizophrenia, controls and relatives)

	Probands	Relatives	Controls
n	32	29	29
Gender (M/F)	19M/13F	17M/12F	19M/10F
Age	35.87 ± 5.77*	37.14 ± 12.45*	36.65 ± 12.15*
Years of schooling	11.02 ± 3.01*	14.15 ± 3.38*	13.54 ± 2.79*
Skin color (N)			
White	18	16	18
Black	5	4	4
Mixed race	9	9	7
WAIS I.Q.	104.06 ± 12.01*	111.53 ± 11.59*	129.0 ± 11.06*
Onset age of disease	19.88 ± 3.33*	-	-
Duration of the disease (years)	12.30 ± 6.18*	-	-
Antipsychotic medication (n)			
Typical Neuroleptics	25		
Atypical Neuroleptics	3		
Both types	4		

* Means and standard deviation.

Gooding DC. et al. (2006) investigated attentional performance in 26 patients with schizophrenia using the ANT. They only reported alterations in the executive control of patients with schizophrenia, supporting the thesis of a selective attentional deficit.[17]

Paul G. et al. (2007) performed a study applying the ANT and demonstrated that patients with schizophrenia had a decrease in efficacy of the neural networks that cover alerting.[35]

The present study

In the present study, we examined the attentional behavior in patients with schizophrenia and their first degree relatives, using the ANT. We have tried to establish if the attentional alterations constitute a global phenomena or specific one of any of their components. Furthermore, we evaluated the family association of these attentional alterations, trying to propose them as a neurocognitive endophenotype in schizophrenia. Parallelly, the executive component was examined with the Wisconsin card sorting test.

METHODOLOGY

We studied 90 subjects from mental health centers and from the Cuban Neuroscience Center (CNC), divided into 3 groups. The first two, belonging to 60 nuclear families, were affected by Multiplex Schizophrenia" (MS). Of these, 32 patients had schizophrenia (GROUP 1) and there were 29 unaffected first degree relatives of these patients (parents and siblings) (GROUP II). The remaining 29 were healthy subjects (Group III) and were matched by age, gender and years of schooling with the probands.

Table 1 shows the demographic and clinical data of the sample. No significant differences were found between the 3 groups studied regarding age (H (2, 78)= 2.69 p=0.39), gender (χ^2(2)=4.09, n.s.), race (χ^2(4)= 3.16, n.s.), and education level (H (2.83)= 0.96, p= 0.92). At the time of the study, all the patients were receiving antipsychotic medication and were exempt of concomitant medications (benzodiazepines and anticholinergics) (Table 1).

All the participants except for the controls had a family background of multiplex schizophrenia. In the family study, the FIGS (NIMH-Molecular Genetics Initiative, 1992) interview for genetic studies was used. This interview makes it possible to obtain diagnostic information in families of patients with schizophrenia. The genealogical tree was constituted after applying the FIGS and was verified with the informers. When other relatives in addition to the proband with schizophrenia were found, the family was classified as "Multiplex Schizophrenia" and was included in the study. The relatives included were not positive in any of the list of symptoms of the FIGS.

All the participants were right handed (Edinburgh Handedness Inventory, Odfield, 1971) and had normal vision or lens-corrected vision. Exclusion criteria for relatives and controls were presence of any psychiatric symptom or disease, neurological diseases, histories of traumas with brain damage, use of drugs or medications that affect cognitive functions, history of substance abuse or addictions, use of neuroleptics or body motor injuries. The study was approved by the ethics committee of the CNC and the participants signed the informed consent.

Clinical and neuropsychological evaluation

The patients were interviewed using part two of the Current Status Examination of the structured surveys of the Scan (Schedules for Clinical Assessment in Neuropsychiatry) System.[36] For the diagnoses of schizophrenia, the criteria used were those of the Diagnostic and Statistical Manual of Mental Disorders (DSM IV),[37] and those of the International Classification of Diseases (ICD-10).[38] The subjects of the control group were evaluated by a psychiatrist, applying a scale to them, that included questions above the cutoff of the PSE-10 on the family disease backgrounds. For the neuropsychological evaluation, the Wisconsin card sorting test was applied to all of the participants.

Design of the task for the examination of the neural networks

A stimulation system was programmed in order to examine the neural attentional networks.

Stimulus: our ANT is a combination of the spatial orientation task and flanker task, which contains 3 experimental blocks with 60 tests each and 12 conditions in all (4 levels of signal by 3 levels of target stimulus). The stimulus is formed by a row of 5 arrows in horizontal direction (→→→→→) and the participant in the task should indicate in which direction the center arrow - the target- is pointing (→→→→→). When the central arrow points in the same direction as the rest, it is considered a congruent condition (→→→→→), and when this central arrow points in the opposite direction from the rest of the arrows, we are in the presence of the incongruent condition (→→←→→). This other condition is called neutral (→ or ←) where only one arrow is shown, which may point to the right or to the left.

Manipulations of the stimulus in the task

The stimulus was not presented in a fixed location, it being able to appear above or below a fixation point. Consequently, to identify the direction of the target stimulus, the participant in the task probably should change their attention upward or downward in search of the arrow.

Variations in the condition of the signal: the stimulus may be preceded or not by an orienting signal (asterisk *). When it is preceded by it, it is called signal condition and, on the contrary, in its absence, it receives the name of no signal condition.

When the signal appears, it may be presented in the center of the fixation point (central signal) or above or below the fixation point, in the location where the stimulus arrow will appear (spatial signal) and in a third condition, the signal will appear simultaneously above or below the fixation point (double signal). Consequently, when the spatial signal appears, the participant can accurately predict where the stimulus will occur, while in the conditions of the central signal or double signal, the subject does not have information on the precise site where the stimulus will appear.

Application of the test

The experiment was applied in an isolated room. An IBM computer controlled the presentation of the stimuli and collected the responses. The participants observed the monitor screen at a distance of 65 cm. The tests were presented in random order. In each ANT test, the subject had to look at the center of the screen attentively and wait for the stimulus to appear. Previously, the subject had undergone training to respond as rapidly and precisely as possible the direction in which the central arrow of the display presented was pointing (right or left), using the right and left keys of the mouse for it. The participants initiated the task after learning the instructions well. The work session lasted ≈ 23 minutes. In the course of the task, the RTs of all the conditions presented were collected.

Procedure

Each trial began with the presentation of a fixation point for a randomized time interval between 400 and 1600 ms. After, in some trials, an alerting signal appeared for 100 ms. After the alerting signal disappeared, the fixation point appeared for the second time for 400 ms. After, the target stimulus and the contextual information or only the target stimulus appeared together. These stimuli were shown until the subject responded, with a maximum presentation time of 1700 ms, after this time, the trial being declared as non-valid.

Collection of the data and statistical analysis

The RT of the responses occurring between 200 and 1700 ms were considered valid. The responses performed before and after this time were counted as errors and were evaluated as 0. The RT analysis was only based on the correct responses. The meaning of the RTs was calculated for each one of the 12 conditions of the test. The efficacy of each one of the neural attention networks was calculated from the RT using the following methodology.

- Alerting efficiency = (Mean of the RT of the no signal condition) – (Mean of the RT of the double signal condition).
- Orienting efficiency = (Mean of the RT of the condition with central signal) – (Mean of the RT of the spatial signal condition). In both conditions, the subject is alerted, but the necessary information provided for orientation in the search of the stimulus was only provided in the spatial signal condition.
- Efficiency of the executive control = (Mean of the RT of the incongruent condition) – (Mean of the RT of the congruent condition).

The means of proportion of alerting, of orienting, and executive control were calculated by dividing the value of each one by the average of the meaning of the RT of the ANT (in accordance with Wang et al., 2005).

The statistical analysis of the data was made using version 7 of the STATISTICA program. For the examination of the RT data, a Repeated Measures Analysis of Variance (rmANOVA) was performed, including a between subjects factor (probands, relatives and controls) and two within-subjects factors: Condition of the signal (no signal, central signal, double signal and spatial signal) and type of context (neutral, congruent and incongruent). To analyze the effect of the signal and that of the context, two analyses of variance (ANOVA) were performed between controls, probands and relatives, following the plan of the primary analysis. In order to avoid violations of the sphericity of the data, when the degrees of freedom exceeded two, the Greenhouse-Geisser correction was applied, reporting the corrected probability. To make more precise comparisons (effect of context on group), the Fisher-LSD test, with Bonferroni correction was used.

The analysis of the correlations between the different neural attention networks was evaluated using the analysis of the Pearson's product correlation. For the ANOVA, a significance level of $p< 0.05$ was used and for the analysis of correlation, more restrictive significance levels were used ($p<0.01$).

The neuropsychological evaluation of the WCST focused on the variables of percent of errors and percent of perseverative errors. An ANOVA of these measurements between probands, relatives and controls was made.

RESULTS

Efficiency of the attentional networks

Table 2 shows the means of the RT in milliseconds (A) and the percentage of errors (B) for each one of the conditions of the signal and type of context.

In the analyses of the RT, statistical differences were obtained between the three groups [$F_{(1,69)}=7.87$, $p<0.00$] (Figure 1). The patients with schizophrenia had a very delayed RT (972.36 ms) compared to the control subjects (749.3 ms). The relatives had intermediate values (897.9) between both groups. A previous analyses on all of the conditions did not show differences between the localizations of the target stimulus (above or below) or between the different directions of the arrow (pointing to the right of the left). Thus, all combinations were used in the subsequent analyses (Table 2) (Figure 1).

Analyzing the percentage of errors committed, no significant differences were observed between the groups and the type of context used [$F_{(4,70)}=1.73$, $p<0.19$, $\varepsilon=0.80$, Adj.$p<0.21$]. However, the probands were less exact in the performance of the tasks than their relatives and then the control subjects (figure 2).

The principal effect of the signal condition (Figure 3) was highly significant [$F_{(3,333)}=15.03$, $p<0.00$, $\varepsilon=0.86$, Adj. $p<0.00$]. The participants had more efficient performance when they were alerted on the site where the stimulus would appear and when their attention was oriented towards the correct spatial position of its occurrence. On the other part, the participants were slower when they were not warned about the presentation of the stimulus (figure 3).

The effect exerted by the condition context was also highly significant [$F_{(2,116)}=164.09$, $p<0.00$, $\varepsilon=0.80$, Adj. $p<0.00$]. The participants were slower when they responded to stimuli located in an incongruent contexts compared to responses obtained for contexts that were neutral or congruent with the target stimulus. In this analysis of variance, a significant interaction was observed between the condition signal and type of the context used [$F_{(6,761)}=2.39$, $p<0.01$, $\varepsilon=0.83$, Adj. $p<0.01$] (Figure 3). The presence of an incongruent context slowed down the RT in all the conditions of signals use, although this effect of the context was reinforced when the participants were not alerted by signals with spatial information of the occurrence of the stimulus.

More precise comparisons of the effect of the context on the group (least significant difference [LSD] test, with Bonferroni correction) indicated significant differences in the mean of the RT between the trials that used incongruent contexts and those that use neutral contexts (MS = 32865,

Table 2 **Means of reaction times in ms. (A) and proportions of errors (B) for each one of the conditions of the signal, type of context and groups studied (standard deviations shown in parenthesis)**

Type of signal	Type of context used								
	Probands			Relatives			Controls		
	Neutral	Congruent	Incongruent	Neutral	Congruent	Incongruent	Neutral	Congruent	Incongruent
A. Response time									
No signal	917.1 (223.7)	1005 (235.2)	1020.7 (221.8)	804.9 (181.4)	897.1 (221.6)	1024.9 (227.1)	688.9 (152.6)	741.5 (168.9)	863.1 (197.5)
Central signal	933.2 (242.6)	966.8 (229.1)	1013.3 (250.1)	815.3 (200.5)	899.8 (220.4)	986.0 (218.5)	699.6 (130.9)	743.6 (161.9)	834.2 (195.4)
Double signal	899.4 (221.9)	977.0 (281.2)	1063.3 (239.4)	819.0 (185.5)	886.2 (237.5)	1011.8 (205.1)	711.0 (133.3)	718.3 (172.2)	848.2(184.4)
Spatial signal	879.8 (218.7)	940.3 (255.2)	1056.2 (278.4)	791.9 (174.3)	871.9 (212.7)	966.2 (230.1)	672.1 (123.2)	682.6 (157.9)	788.5 (169.8)
B. Percentage of errors.									
No signal	0.76 (1.83)	0.50 (0.81)	1.93 (3.20)	0.58 (1.28)	0.72 (0.88)	0.84 (1.55)	0.31 (0.73)	0.41 (0.15)	1.13 (0.16)
Central signal	1.03 (2.09)	1.22 (2.60)	1.92 (3.24)	0.17 (0.47)	0.28 (0.68)	0.61 (1.84)	0.52 (0.59)	0.20 (0.56)	0.52 (1.28)
Double signal	0.78 (2.02)	0.17 (0.50)	2.22 (3.37)	0.57 (0.90)	0.11 (0.30)	1.57 (1.03)	0.10 (0.28)	0.20 (0.48)	0.67 (0.14)
Spatial signal	1.45 (2.81)	0.58 (0.99)	1.29 (2.89)	0.40 (0.65)	0.16 (0.64)	0.53 (1.64)	0.05 (0.20)	0.15 (0.33)	0.44 (1.18)

df = 77, 04), it being greater in patients with schizophrenia compared to the control group (p<0.01) and also in their relatives in relationship to the healthy subjects (p<0.01). However, no significant differences were found between the probands and their unaffected relatives. This result, in which patients with schizophrenia and their unaffected first degree relatives require more time to solve conflicts in relationships to the healthy subjects is very suggestive of a defect in the neural networks that govern the executive control in them. In accordance with the hypothesis of our study, the first degree relatives of patients with schizophrenia have a similar deficit to that reported in them for the neural -network of

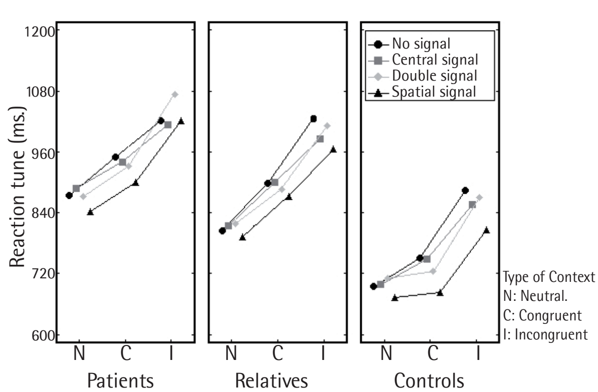

Figure 1 General results of a repeated measure ANOVA performed on the reaction times in the three groups studied

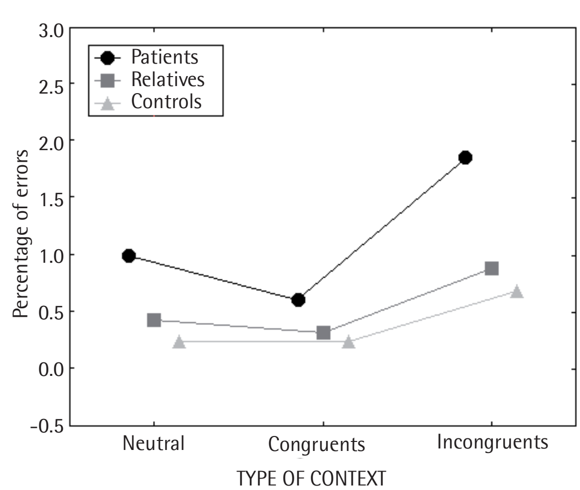

Figure 2 Percentage of errors based on the group and type of context

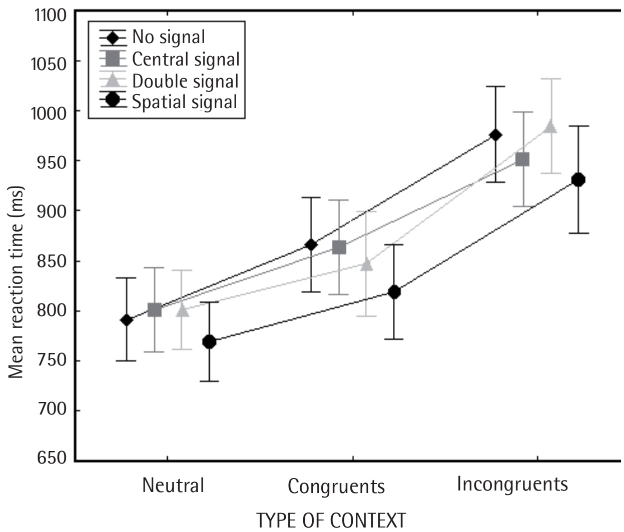

Figure 3 Mean reaction time based on the signal condition and type of context

the executive control (see figure 4-left). The three groups study showed a similar pattern of responses (RT) for the congruent and neutral tests.

The results obtained with the ANT coincide with the evaluation of the executive functions obtained with the WCST, in which it was seen that the groups of patients and relatives had the same behavior and both groups differentiated significantly from the controls in the variables studied: percentage of errors, (p=0.28), and (p<0.003), (p<0.01), and percentage of perseverative errors (p=0.29) (p<0.01), (p<0.04), respectively (see figure 4- Right). (Figure 4).

Analysis of the efficiency and independence of the neural attentional networks

The mean efficiency for each one of the three attentional networks was calculated starting from the raw RT obtained in each one of the stimulation conditions. In table 3, the values (in milliseconds) of the means, standard deviations and proportions calculated in each one of the three groups studied are shown. The efficiency for alerting had similar values in the probands (42.40 ± 58.9) and relatives (40.42 ± 29.9). However, both differed from the controls (24.51 ± 22.1). Similar behavior was observed in orienting. The probands (66.38 ± 44.5) and their relatives (60.01 ± 40.8) had similar values, both were very distant from the efficiency for the orientation obtained in the healthy subjects (47.62 ± 30.0). However, executive control had inferior values (114.88 ± 73.3 and 120.18 ± 47.5) in the probands and their first degree relatives, respectively, compared to the better executive performance shown by the healthy subjects (132.75 ± 63.7). In spite of the similar behavior in the efficiency of the three attentional networks between probands and their relatives, and the differences

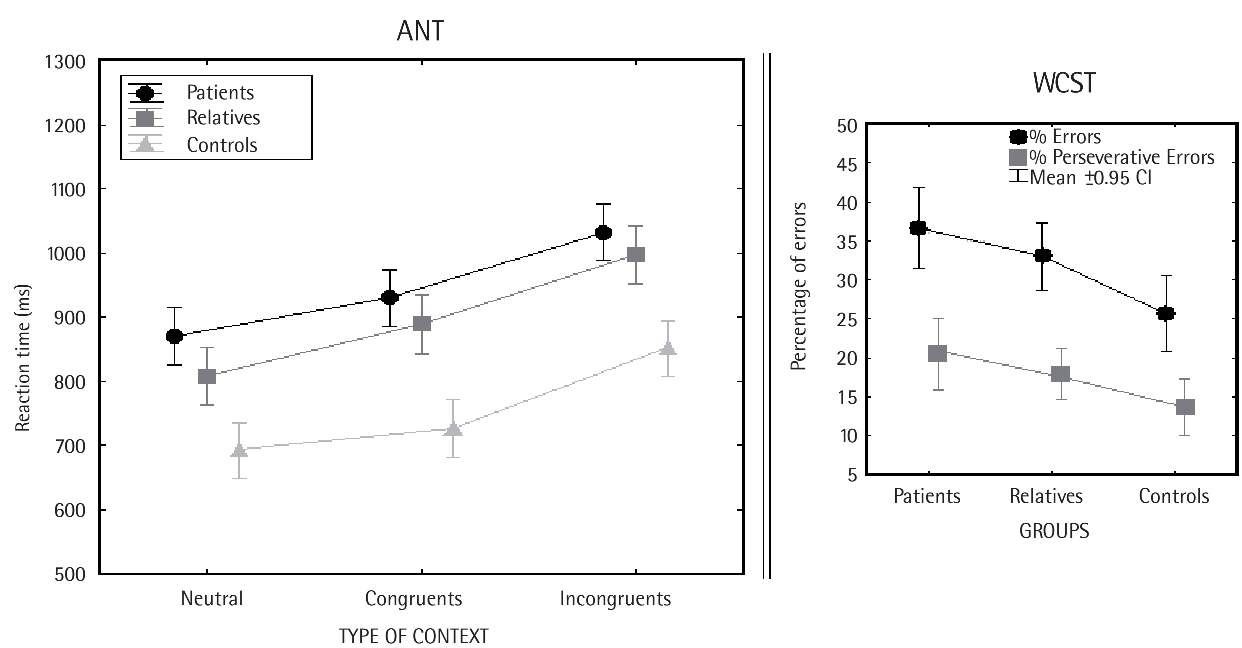

Figure 4 Mean reaction time based on the group and type of context with the ANT (Left). Comparison of percentage of errors and percentage of perseverative errors in the Wisconsin Card Sorting Test between the groups studied (Right)

Table 3 Efficiency of the neural attentional networks (ms.) and proportions calculated for the three groups studied (mean and standard deviation)

Ejective Groups	ATTENTIONAL NETWORKS					
	Alerting		Orienting		Control	
	Media±SD	P	Media±SD	P	Media±SD	P
Probands	42.40±58.93	.044	66.38±44.55	.067	114.88±73.33	.120
Relatives	40.42±29.91	.045	60.01±40.88	.066	120.18±47.54	.133
Controls	24.51±22.12	.032	47.62±30.05	.063	132.75±63.76	.177

P: Proportion

of these regarding the controls, these differences were not statistically significant [$F_{(4,138)}$=0.71, p<0.58] (Table 3).

To demonstrate the functional independence between the three neural attention networks, we performed an analysis of correlation. Table 4 shows the results of the matrix of correlation for alerting, orienting and executive control in the three groups studied. One of the correlations was statistically significant, showing a functional segregation between the three neural attention networks (table 4).

Table 4 Correlation values (r) between the three neural attentional networks for each group studied

Results	Alerting	Orienting	Executive control
Probands			
Alerting		0.33	0.41
Orienting	---	0.13	
Executive control		---	
Relatives			
Alerting		- 0.11	- 0.10
Orienting	---	- 0.05	
Executive control		---	
Controls			
Alerting		0.06	0.11
Orienting	---	0.17	
Executive control		---	

The significance level of the correlations is 0.01.
The correlations were calculated based on the measurements of all the participants.

CONCLUSIONS

In the last decade, there has been a considerable increase in reports of cognitive deficits in patients with schizophrenia and their relatives, including a great effort for the search of endophenotypes for specific genes.

The purpose of this work has been to evaluate the attentional behavior of a group of patients with schizophrenia and their unaffected first degree relatives, using a task to explore neural networks (ANT) and to determine if the attentional alterations found meet the criteria of familial association.

On the other hand, we aim to determine if the attentional disorder observed in the patients with schizophrenia and their relatives constitute a global phenomenon or if only an attentional subcomponent (alerting, orienting, or executive control) was of interest.

The analysis of the behavioral results suggests that the participants correctly understood and carried out the experimental paradigm. There were no significant differences in the percentage of errors committed by the probands and their relatives regarding the controls. This allowed us to propose that these attentional alterations suggest a true deficit in executive functioning of the probands and their relatives.

Although significant differences exist between the group regarding the intelligence quotient estimated with the WAIS-R (Table 1), these differences are usually present in many studies on schizophrenia due to the methodological problems inherent to the matching of subjects by their intelligence.[19, 23] Furthermore, other investigations test the possible hypothesis of a deficit in executive control of patients with schizophrenia and their relatives, present in this paradigm. These may be partially explained through a global cognitive disorder that involves functions such as working memory, required in the performance of this task and reported with alterations in schizophrenia.[30]

Gold JM et al. have interpreted the results of the WCST in the patients with schizophrenia (perseveration errors) as being a direct consequence of their incapacity to make rapid comparative changes between the different concepts and to adopt different perspectives on a concept. This corresponds with the findings observed in the blood flow of the prefrontal cortex of these patients.[13] The ANT data, together with those of the WCST in our sample, strengthened the idea of a disorder in the executive control networks in the patients and their first degree relatives (see figure 4).

In the present study, the patients and their relatives showed a significant effect of the context (congruent versus incongruent) regarding the healthy controls, suggesting a deficit in the neural networks to solve conflicts. The participants were slower to respond when the context was incongruent than when there was congruency or neutrality between the context and the central target stimulus. When there is an incongruent context, the RT suffers a slow down in all the conditions of the orienting signal used, although this effect of the context is reinforced when the participants do not have orientative information on the site of the possible occurrence of the stimulus.

Precise comparisons performed to evaluate the effect of the context on the groups indicate significant differences in the mean of the RT between the congruent trials and the neutral ones, the probands and their relatives having slower RT then the control subjects. However, these differences are not seen when comparing patients versus unaffected relatives. These alterations found in the patients with schizophrenia replicate previous results of other work groups.[17, 32] The difficulty found in the group of relatives to resolve conflicts regarding the control group is also suggestive of the deficit of their executive control networks, results that are coherent with our working hypothesis. Based on our knowledge, this is the first report in the literature on the deficit in the neural attention networks examined with the ANT in first degree unaffected relatives of patients with schizophrenia belonging to families affected by multiplex schizophrenia. This result allows us to propose this executive attentional deficit as a possible dophenotypic marker of schizophrenia.

Several studies of familial association in schizophrenia document a cognitive deterioration that is clearly demonstrable when comparing the attentional performance of relatives of patients with schizophrenia with that of normal subjects without a family background of the disease. However, this is always less severe than that found in the patients with clinical manifestations of schizophrenia. These studies use different neuropsychological tests to evaluate the executive functions,[11,22] nonetheless, the ANT has never been used for this purpose.

In 2004, Henik and Salo proposed a methodology to test the hypothesis that patients with schizophrenia had more difficulties to resolve conflicts when the attention is diffuse in relationship to when it is focalized.[39.] In our study, we did not make these comparisons (no signal versus spatial signal) because our objective was to observe if the global behavior of the neural networks of patients with schizophrenia from multiplex families corresponded with the functioning of these neural networks in their first degree healthy relatives.

Several reports in the literature coincide in that patients with schizophrenia have an attentional deficit, but there are discrepancies regarding whether this deficit is global or specific to some of the neural networks that govern the attentional process.[31-34] Most of the authors coincide in considering that the fundamental problem is found in a deterioration of their executive control,[30, 33, 40-44] even though some authors have stated that the deficit is exclusive to alerting,[35, 45] or the attention orienting networks.[46] Others suggest a combination of mixed disorders that include orienting and executive control.[17,32,47] Our results coincide with most of the reports in the literature and are based on the results of an evaluation study of heritability in the neural networks conducted by Jin Fan et al. (2001). This suggests that the efficiency of the neural executive attentional networks that mediate between the stimulus and the responses to the conflicts have sufficient heritability in healthy subjects (h^2=0.72) to assure subsequent studies in patients. However, the alerting networks (h^2=0.18) and the average reaction time (h^2=0.16) show little heritability and the neural orienting networks do not show any heritability.[33] They also suggest that this executive portion of the ANT could constitute a potential endophenotype that would permit analysis of molecular genetics in populations of normal subjects. These results justify our study of familial association with ANT in families with multiplex schizophrenia. It is well to stress that heritability of the executive networks has been observed with other tasks to examine conflicts (for example: Stroop task), and that in them, the cingulate and other frontal areas are also activated. However, our context task has advantages over the Stroop because it does not involve language in its performance.[30]

The report of this possible endophenotype constitutes a valuable tool for the integration of genetic information into the identification of candidate genes in schizophrenia. Knowledge of attentional performance in probands and relatives using the ANT allows us to have neuroanatomical and functional information on the areas involved in the process. In the specific case of the neural networks of executive control, the functional neuroimaging studies show activation of the lateral frontal and midline structures, dopamine modulated areas, which would suggest that the genes codifying the synthesis of this neurotransmitter should be examined.[7, 8]

In a previous report of our working group, familial association of the N400 evoked potential was also found in a graphic semantic task (using pairs of congruent and incongruent figures), observing a deficit of the semantic processing in the first degree relatives of patients with schizophrenia, possibly due to poor contextual usage of the stimulus.[48] In keeping with these previous results, we consider that patients with schizophrenia and their first degree relatives certainly have a significant deficit of their executive resources to solve conflicts. This disorder in executive control mechanisms of patients with schizophrenia can be seen by the deterioration observed in other neuro-cognitive tasks and in the manifestations of some of the symptoms of the disease.[48] This deficit in the executive control networks is not exclusive to the schizophrenics spectrum disorders. However, its presence in relatives unaffected by the disease suggests that some of the executive functions may be true markers of the disease.[49] The patients with schizophrenia in our sample generally took typical neuroleptics. Thus, we consider that the neurocognitive changes demonstrated in them and in their first degree relatives free of antipsychotic treatment are not dependent on the medication received.[50]

Do the patients with schizophrenia and their relatives have a global cognitive deficit or a specific deficit of some of the attentional networks?

The efficiency values obtained for each one of the attentional networks as well as the means of the proportions of the efficiency were calculated in accordance with the operational definitions described in the method.

The results obtained for the three attentional networks in the control group are similar to other reports in the revised literature.[17, 26, 32, 33] However, patients with schizophrenia and their first degree relatives have very similar results. However, these results differ greatly from those observed in the control group. The average efficiency for alerting and orienting of the probands and their relatives was superior to the efficiency found in the controls. Nonetheless, the efficiency for the solution of conflicts of the controls is greater than that found in the probands and their relatives. These results support the idea that the probands and their unaffected first degree relatives have a deterioration of their executive functions, conserving integrity of alerting and orienting. The high heritability of the executive control reported in healthy subjects,[33] may justify why these executive alterations segregate in the unaffected relatives of patients with schizophrenia belonging to multiplex families.

The analyses of correlation performed between the results obtained with ANT for the neural networks revealed the existence of total independence between these networks for the three groups studied. This result is similar to that of other reports[17, 32] and suggests that the efficiency of each one of these attentional networks can be measured segregatively with the ANT and that they have functional independence between them.[5]

Finally, our results show that the patients with schizophrenia and their unaffected first degree relatives have an attentional deficit that exclusively involves executive functioning. This reaffirms the hypothesis of a specific attentional deficit in patients with schizophrenia (Gooding et al., 2006) and also in their unaffected relatives, suggesting the existence of a possible endophenotypic marker for the disease.

Future projections and potential limitations of the study

Our results lead us to proposed future investigations linking the ANT to studies of event related potentials (ERP) and functional magnetic resonance (fMRI) in first degree relatives of patients with multiplex schizophrenia, which would make it possible to examine in detail the functional and anatomical bases underlying such a complex phenomena. Nonetheless, we consider that it would be difficult to obtain the ERP with the ANT as this would require a large number of pre-measurements for each condition and these long sessions would interfere with good attentional performance of the subjects studied. However, there are reports by Fan et al., 2005, that show the feasibility of the linking ANT with fMRI studies.[27]

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