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Evaluaton of regional cerebral blood flow alterations by limbic activation in patients with schizophrenia

Ikbal Inanli[1], Ibrahim Eren[1], Harun Suslu[2], Mustafa Yildiz[3]

[1]*Konya Research and Training Hospital, Clinic of Psychiatry, Konya, Turkey*
[2]*Antalya Medical Park Hospital, Department of Nuclear Medicine, Antalya, Turkey*
[3]*Suleyman Demirel University Faculty of Medicine, Department of Nuclear Medicine, Isparta, Turkey*

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Abstract
The aim of study is to evaluate the functions of limbic system in schizophrenia by performing procaine administration and using single photon emission computed tomography (SPECT). 22 patients and 19 controls were included in the study. Patients were evaluated with Positive and Negative Syndrome Scale (PANSS), Brief Psychiatric Rating Scale (BPRS), Hamilton Rating Scale for Depression (HAM-D) and Hamilton Rating Scale for Anxiety (HAM-A), Calgary Depression Rating Scale for Schizophrenia (CDSS) and the controls were evaluated with BPRS, HAM-D and HAM-A. The regional cerebral blood flow (rCBF) values were measured by SPECT. The rCBF following placebo and the alteration ratios of the rCBF following procaine of patients compared to controls. The rCBF following placebo in bilateral frontal, bilateral superior temporal, bilateral parahippocampus, thalamus, insula and alteration ratios of rCBF following procaine in bilateral anterior cingulate cortex (ACC) and left thalamus of patients were lower. Poor limbic activation to procaine was observed in the ACC and left thalamus of patients with schizophrenia. These findings support that the ACC and thalamus are crucial role in pathophysiology of schizophrenia.

Keywords: Schizophrenia, limbic system, procaine, SPECT

Introduction

Schizophrenia is a chronic psychiatric disorder, affecting approximately 1% of the general population [1]. Recent studies suggest functional and structural changes in different brain regions and it has been accepted as a brain disorder since the 1980s [2]. Many neuroimaging studies have suggested that else white-matter deficits and decreased gray-matter volume, particularly limbic structures [3]. The limbic system is a multifunctional structure for cortical and subcortical structures, which provide mood, emotion, reward, memory and impulse to external events [4].

The pathophysiology of schizophrenia likely involves changes within number of brain circuits within involving limbic system and prefrontal cortex [PFC] [5,6]. Many studies showed that changes of metabolism in limbic regions by functional imaging studies using PET and SPECT [2,6,7]. The studies founded significantly results such as decreased rCBF in ACC associated with positive symptoms, increased rCBF in ventral striatum, hippocampus and parahippocampus associated with delusions and hallucinations, increased rCBF in striatum and thalamus associated with positive symptoms [8,9].

***Coresponding Author:** Ikbal Inanli, Konya Research and Training Hospital, Department of Psychiatry, Konya, Turkey
E-mail: kasimturgut@yahoo.com

Hazlett et al. reported lower metabolism in thalamic subdivisions with distinct cortical connections and have specific associations with clinical symptoms with PET study [8].

Due to its deep brain location, extensive neurochemical nature and different anatomical structures, the limbic system isn't a region that can easily be investigated. However, brain imaging and pharmacological activation studies have made it possible to reduce existing difficulties [10]. Procaine is generally associated with blockade of voltage-gated sodium channels and produces local anesthetic effect which synthesized [11]. Procaine shows relatively specific activation of limbic structures and has been used by investigators to assess limbic system [12]. Adinoff et al. [2002] demonstrated specific increases in rCBF in limbic and paralimbic areas following 1.38 mg/kg procaine in healthy individuals by SPECT [10]. Procaine interacts with many neurochemical systems, but is most potent at muscarinic cholinergic receptor. In the animal based study, it was verified that procaine increased activation of limbic region via muscarinic receptors [11]. M1 and M2 receptors are present in core limbic areas, as well as primary sensory regions, which play an important role in emotional processing [13]. Also, studies have displayed relationship between measures of emotional response such as fear, anxiety, and activation of limbic system following procaine [12]. Emotional deficits including expression, experience, and recognition of emotions are among core features in schizophrenia, which altered brain activation in hippocampus,

amygdala, and PFC [13]. Adinoff et al. used procaine and SPECT to investigate limbic responsiveness in cocaine-addicted subjects [14,15].

Our aim was to investigate rCBF of limbic system and related regions measured by SPECT imaging method via comparing the response to procaine activation of patients with schizophrenia and healthy controls in order to evaluate role of the limbic structures in schizophrenia. Additionally, limbic system rCBF response to procaine activation would be correlated with clinical features.

Material and Methods

Subjects

A total of 22 patients were diagnosed with schizophrenia according to DSM–IV criteria by interviewer were included in study along with 19 healthy volunteers. All subjects were right handed, between 18 and 65 years and not pregnant. Patients who had no mental retardation, history of head trauma, another psychiatric, neurological, medical disease, or substance abuse except nicotine and not using antipsychotic drugs in the last three weeks or in the last four weeks long-acting antipsychotic. Additionally, they didn't receive any electroconvulsive therapy in the last 6 months. During study, patients were not allowed to take antipsychotics. Control group was composed of right handed who were not using any psychotropic drugs and didn't have any psychiatric, neurological or medical disease. The study was approved by the ethical board first, then the aims and the methods of investigation were explained to all participants and written information forms were obtained.

Scales

All participants were assessed by BPRS, HAM-D, HAM-A and Edinburgh Handedness Inventory. Additionally, patients were assessed by semi-structured interview schedule for sociodemographic data, PANSS and CDSS.

Study Sessions

All subjects experienced two separate study sessions. Sessions were minimum two days apart and at same time of day. Subjects were administered either saline or 1.38 mg/kg procaine. Study sessions took place at Department of Nuclear Medicine. All subjects were asked not to take caffeine for 24 hours and nicotine for four hours before the imaging. On session day, intravenous line was placed into the peripheral veins of the subjects for administration of placebo, procaine, Tecnetium-99m Hexamethyl propylene amine oxime (Tc-99m HMPAO), and blood pressure and pulse were measured. Subjects remained in a semi-seated position with closed eyes in a dim lighted room with a low-noise level, where visual, auditory and cognitive stimuli were kept at minimum at least 20 minute at rest position. 20 or 30 minutes later, either saline or procaine (1.38 mg/kg) was administered over 60 s by slow push, followed by three ml saline flush over 45 s. After these processes, 555-740 MBq (15-20 mCi) Tc-99m HMPAO was injected over 30 s, and 10 ml saline flush over 30 s. Five minutes following radiotracer administration, blood pressure and pulse were measured.

SPECT Imaging

After 45 minutes from radiopharmaceutical injection a total of 128 images with a 128x128 matrix, 30 second duration and 2.813 degree angles of each were recorded by using a dual detector gamma camera system with high resolution collimators. Images were processed by iterative method using a Gaussian filter. Transaxial sections with 3.9 mm/pixel were obtained. On the sections where they were best demonstrated, regions of interest (ROI) were drawn for right and left superior, medial, inferior frontal; right and left superior, medial, inferior temporal; right and left parahippocampus; right and left caudate nucleus; right and left lentiform nucleus; right and left thalamus; right and left insula; right and left superior, inferior parietal; right and left occipital; right and left ACC; and cerebellum. Mean counts were obtained in these regions. Since cerebellar perfusion is independent from age, cerebellum values were used as reference for normalization of rCBF values. But the rCBF values of cerebellum are not used in study. All ROI count values were normalized by proportioning to the count rate of cerebellar ROI. These values were defined as rCBF ratio of related region.

Statistics

Statistical analyses were performed using Statistical Package for the Social Sciences, "SPSS 15.0 for Windows". In comparing sociodemographic and clinical variables two groups according to their distributions, student's T Test and Mann-Whitney U test was used. The Chi-square test was used to compare categorical variables. The Wilcoxon signed-rank test was used to compare rCBF following placebo and procaine in groups. Alteration ratio of rCBF was calculated with the following formula: [(rCBF following procaine– rCBF following placebo) ÷ rCBF following placebo]. Alteration ratio of rCBF in both groups were compared by using Mann-Whitney U test for two independent group averages. Spearman correlation analysis used to determine correlation between the rCBF following placebo and clinical features, between the alteration ratios of rCBF following procaine and clinical features. P values <0.05 were considered as statistically significant.

Results

A total of 22 patients, and 19 controls were included in the study. No statistically difference was observed between groups in sociodemographic features and clinical features (Table 1). The mean HAM-D, HAM-A and BPRS scores of the patients were higher than controls (Table 2). In the patients, the total PANSS, positive and negative symptoms and general psychopathology sub-scale scores are presented in Table 2.

Table 1. Sociodemographic features of groups and clinical features of patient group

	Patient Group	Control Group	Comparisons
Age	33.81±11.84	34.47±10.89	z=-0.262, p=0.794
Gender			
Male	13 (59.1%)	11 (57.9%)	
Female	9 (40.9%)	8 (42.1%)	x2=0.06, p=1.00
Education (years)	10±3.39	10.57±4.65	z=-0.868, p=0.385
Marital Status			
Married	14 (63.6%)	13 (68.6%)	
Single	6 (27.3)	6 (31.6%)	
Divorced	1 (2.4%)		
Living separately	1 (2.4%)		x2=7.6, p=0.055
Age of onset (Mean±SD)	24.50±7.76		
Disease duration (months) (Mean±SD)	116.81±81.56		

Table 2. Psychiatric assessment scale scores of patient and control groups.

	Patient Group (Mean±SD)	Control Group (Mean±SD)	
HAM-D	9.86±5.21	2.26±2.05	z=-4.90 p<0.0001
HAM-A	11.50±6.23	4.89±3.21	z=-3.63 p<0.0001
BPRS	62.22±14.22	26.10±1.69	z=-5.48 p<0.0001
PANSS			
Positive	20.91±5.75	-	
Negative	24.68±7.65	-	
General	44.54±9.75	-	
Total	90.13±20.91	-	
CDSS	6.09±4.74	-	

The comparison of rCBF

The comparison of rCBF following placebo between groups revealed that rCBF in the right superior (p=0.003), medial (p<0.001), inferior frontal (p<0.001), left superior (p=0.01), medial (p<0.001) and inferior frontal (p<0.001), left superior temporal (p=0.02), bilateral parahippocampus (p=0.008, p=0.003, respectively), bilateral thalamus (p=0.01, p=0.02, respectively) and bilateral insula (p=0.04, p=0.03, respectively) of patients were lower than controls (Table 3). The comparison of rCBF following placebo and procaine in patients revealed that rCBF following procaine in right superior frontal (p<0.001), left inferior frontal (p=0.01), bilateral caudate (p=0.001, p<0.001, respectively) and bilateral lentiform nucleus (p=0.007, p=0.04, respectively) were higher than rCBF obtained by placebo administration (Table 3).

The comparison of rCBF following placebo and procaine in the controls revealed that rCBF following procaine at right medial and inferior frontal (p=0.005, p=0.007, respectively), left superior and inferior frontal (p=0.007, p=0.04, respectively), bilateral parahippocampus (p=0.006, p=0.001, respectively), bilateral thalamus (p<0.001, p<0.001, respectively), bilateral ACC (p<0.0001, p<0.0001, respectively), bilateral caudate nucleus (p<0.001, p<0.0001, respectively) and bilateral lentiform nucleus (p<0.001, p<0.001 respectively) were higher than rCBF following placebo (Table 3).

The comparison of rCBF following procaine between groups revealed that rCBF in the right superior frontal (p=0.02), bilateral medial frontal (p=0.001, p<0.0001, respectively) and bilateral inferior frontal (p<0.0001, p<0.001, respectively), bilateral parahippocampus (p=0.001, p<0.0001, respectively), bilateral thalamus (p<0.0001, p<0.0001, respectively), bilateral ACC (p=0.008, p=0.01, respectively), left lentiform nucleus (p=0.03) and right superior parietal (p=0.025) of controls were higher than patients (Table 3).

Table 3. The rCBF values following placebo and procaine administrations in patient and control groups

	Placebo (Mean±SD)				Procaine (Mean±SD)							
Brain Regions	**Patients**	**Controls**	**Comparisons 1**		**Patients**	**Controls**	**Comparisons 2**		**Comparisons 3**		**Comparisons 4**	
Right superior frontal	1.00±0.08	1.09±0.09	-2.98	0.003	1.05±0.12	1.15±0.09	-2.41	0.02	-4.11	<0.0001	-1.61	0.11
Left superior frontal	1.01±0.11	1.09±0.08	-2.46	0.01	1.05±0.16	1.15±0.09	-1.96	0.05	-1.93	0.05	-2.69	0.007
Right medial frontal	1.02±0.12	1.16±0.09	-3.43	0.001	1.09±0.12	1.23±0.10	-3.29	0.001	-2.09	0.03	-2.80	0.005
Left medial frontal	1.02±0.12	1.17±0.07	-3.58	<0.0001	1.06±0.11	1.22±0.10	-3.69	<0.0001	-1.48	0.14	-1.69	0.09
Right inferior frontal	1.04±0.09	1.20±0.09	-4.31	<0.0001	1.08±0.08	1.24±0.08	-4.42	<0.0001	-1.28	0.20	-2.70	0.007
Left inferior frontal	1.05±0.10	1.20±0.08	-3.87	<0.0001	1.11±0.09	1.23±0.08	-3.47	0.001	-2.52	0.01	-2.01	0.04
Right superior temporal	1.02±0.12	1.09±0.11	-1.94	0.05	1.05±0.08	1.09±0.09	-1.88	0.06	-1.35	0.18	-0.28	0.78
Left superior temporal	1.01±0.16	1.08±0.10	-2.30	0.002	1.05±0.13	1.09±0.11	-1.12	0.26	-1.93	0.05	-0.24	0.81
Right medial temporal	1.09±0.12	1.12±0.10	-1.05	0.30	1.10±0.11	1.11±0.08	-0.62	0.53	-0.44	0.66	-0.16	0.87
Left medial temporal	1.08±0.13	1.13±0.11	-1.57	0.12	1.11±0.10	1.13±0.09	-0.37	0.71	-1.02	0.31	-0.20	0.84
Right inferior temporal	1.09±0.12	1.09±0.09	-0.15	0.71	1.09±0.09	1.09±0.05	0.03	0.98	-0.67	0.51	-0.12	0.90
Left inferior temporal	1.10±0.12	1.07±0.08	-0.60	0.55	1.11±0.10	1.11±0.10	-0.60	0.55	-0.70	0.49	-0.97	0.33
Right parahippocampus	0.77±0.09	0.87±0.11	-2.67	0.008	0.81±0.10	0.94±0.13	-3.40	0.001	-1.41	0.16	-2.74	0.006
Left parahippocampus	0.75±0.09	0.87±0.12	-2.95	0.003	0.79±0.09	0.94±0.13	-3.66	<0.0001	-1.87	0.06	-3.34	0.001
Right caudate nucleus	0.86±0.09	0.88±0.05	-0.99	0.32	0.94±0.08	0.97±0.08	-0.97	0.33	-3.36	0.001	-3.66	< 0.0001
Left caudate nucleus	0.84±0.10	0.88±0.07	-1.62	0.11	0.92±0.10	0.95±0.07	-1.12	0.26	-3.75	< 0.0001	-3.58	< 0.0001
Right lentiform nucleus	0.95±0.12	0.96±0.07	-0.71	0.48	1.02±0.08	1.05±0.09	-0.63	0.53	-2.68	0.007	-3.06	0.002
Left lentiform nucleus	0.94±0.12	0.94±0.09	-0.08	0.94	0.98±0.08	1.04±0.10	-2.14	0.03	-1.99	0.04	-3.02	0.003
Right thalamus	0.83±0.08	0.90±0.08	-2.54	0.01	0.84±0.08	0.97±0.11	-3.77	<0.0001	-0.50	0.62	-3.50	< 0.0001
Left thalamus	0.82±0.08	0.88±0.09	-2.28	0.02	0.83±0.07	0.97±0.11	-3.82	<0.0001	-0.67	0.51	-3.74	< 0.0001
Right insula	0.96±0.11	1.03±0.10	-1.99	0.04	1.02±0.10	1.06±0.09	-0.99	0.32	-1.90	0.05	-1.01	0.31
Left insula	0.93±0.11	1.00±0.09	-2.17	0.03	0.98±0.10	1.04±0.11	-1.37	0.166	-1.70	0.09	-1.45	0.15
Right superior parietal	1.00±0.11	1.03±0.07	-1.89	0.06	1.02±0.09	1.08±0.07	-2.25	0.025	-1.15	0.25	-1.77	0.08
Left superior parietal	1.04±0.08	1.04±0.06	-0.76	0.45	1.04±0.08	1.08±0.06	-1.70	0.08	-0.31	0.75	-1.93	0.05
Right inferior parietal	1.03±0.13	1.11±0.11	-1.87	0.06	1.05±0.12	1.11±0.12	-1.73	0.08	-0.99	0.32	-0.32	0.75
Left inferior parietal	1.04±0.13	1.11±0.10	-1.88	0.06	1.04±0.15	1.10±0.08	-1.20	0.230	-0.28	0.78	-0.60	0.55
Right occipital	1.08±1.12	1.10±0.07	-0.76	0.45	1.08±0.10	1.11±0.10	-0.58	0.57	-0.86	0.39	-0.44	0.66
Left occipital	1.07±0.14	1.08±0.08	-0.99	0.32	1.06±0.10	1.09±0.09	-1.20	0.23	-0.02	0.99	-0.32	0.76
Right anterior cingulate	0.94±0.09	0.94±0.06	-0.18	0.86	0.96±0.11	1.04±0.07	-2.61	0.008	-1.19	0.23	-3.82	< 0.0001
Left anterior cingulate	0.94±0.08	0.94±0.07	0.05	0.96	0.97±0.09	1.05±0.10	-2.45	0.01	-1.34	0.18	-3.78	< 0.0001

The comparison of groups in terms of the alteration ratio of rCBF following procaine revealed that alteration ratio of rCBF in the left thalamus (p=0.036), right ACC (p=0.004), left ACC (p=0.02) and left occipital (p=0.02) of controls were higher than patients (Table 4).

The relationship between the rCBF following placebo, alteration ratio of rCBF following procaine and clinical features

In the patients, the correlation analysis of the rCBF following placebo revealed negative correlation between the rCBF of bilateral superior frontal and duration of education (p=0.049, p=0.037, respectively), between right medial frontal rCBF and disease duration (p=0.044) and positive correlation between rCBF of right lentiform nucleus, PANSS negative, PANNS total and BPRS scores (p=0.006, p=0.036, p=0.018, respectively). A negative correlation was observed between right lentiform nucleus alteration ratio of rCBF and HAM-D, CDSS, PANSS negative, PANSS general, PANSS total and BPRS scores (p=0.02, p=0.04, p=0.0001, p=0.02, p=0.01, p=0.004, respectively) in patients. Left lentiform nucleus alteration ratio of rCBF were negatively correlated with PANSS negative (p=0.036), and left superior frontal rCBF alteration ratio were negatively correlated with CDSS (p=0.016) (Table 4).

Table 4. The rCBF alteration ratios following procaine administrations in patient and control groups

Brain Regions	Patients	Controls		
Right superior frontal	4.98±9.26	5.92±13.77	z=-0.24	p=0.81
Left superior frontal	4.48±13.91	5.88±10.45	z=-0.39	p=0.69
Right medial frontal	6.96±12.69	6.14±8.37	z=0.60	p=0.55
Left medial frontal	5.23±12.93	5.03±9.03	z=-0.08	p=0.94
Right inferior frontal	4.35±10.89	3.57±5.43	z=-0.50	p=0.62
Left inferior frontal	6.01±9.29	2.89±5.41	z=-0.92	p=0.36
Right superior temporal	3.70±10.81	1.60±11.17	z=-0.81	p=0.42
Left superior temporal	5.12±11.87	2.15±11.74	z=-1.36	p=0.17
Right medial temporal	2.15±12.57	0.50±12.63	z=-0.44	p=0.66
Left medial temporal	3.47±13.31	0.30±10.19	z=-0.60	p=0.55
Right inferior temporal	1.14±12.25	1.5±12.49	z=-0.55	p=0.58
Left inferior temporal	1.87±13.38	4±11.98	z=-0.08	p=0.94
Right parahippocampus	5.13±13.88	8.26±10.49	z=-0.89	p=0.37
Left parahippocampus	6.66±13.88	8.89±9.76	z=-0.50	p=0.62
Right caudate nucleus	9.78±10.89	10.22±9.79	z=-0.03	p=0.98
Left caudate nucleus	10.17±10	8.25±8.11	z=-0.58	p=0.57
Right lentiform nucleus	8.30±13.49	9.24±11.18	z=-0.08	p=0.94
Left lentiform nucleus	5.14±13	10.53±13.11	z=-0.97	p=0.33
Right thalamus	2.93±14.32	7.57±7.749	z=-1.26	p=0.21
Left thalamus	2.52±11.51	9.18±7.32	z=-2.09	p=0.036
Right insula	8.35±16.39	3.02±10.61	z=-1.02	p=0.308
Left insula	6.63±15.69	4.83±10.42	z=-0.84	p=0.40
Right superior parietal	2.80±10.14	4.83±10.42	z=-0.18	p=0.86
Left superior parietal	0.55±7.54	3.82±7.20	z=-1.46	p=0.14
Right inferior parietal	1.92±10.39	1.73±18.41	z=-0.84	p=0.40
Left inferior parietal	0.69±13.81	-0.19±14.26	z=-0.55	p=0.58
Right occipital	0.80±9.15	1.47±10.92	z=-0.24	p=0.81
Left occipital	3.98±10.52	1.24±10.47	z=-2.29	p=0.02
Right anterior cingulate	1.87±9.38	11.60±10.47	z=-2.83	p=0.004
Left anterior cingulate	3.98±12.43	12.43±12.38	z=-2.29	p=0.02

Discussion

In this research; we found that administration of procaine to controls led to the activation of right medial and inferior frontal, left superior and inferior frontal, bilateral parahippocampus, thalamus, ACC, caudate nucleus and lentiform nucleus regions. Similarly in previous studies, this finding indicates in healthy individuals [10,12,15,16]. In the schizophrenia group, procaine activation was observed in the regions of right superior frontal, left inferior frontal, right and left caudate nucleus as well as bilateral lentiform nucleus in our study. This finding indicates that procaine can provide activation of some areas of the limbic system in patients with schizophrenia. There are no previous studies conducted with limbic system activation with procaine in schizophrenia. Limbic system activations with procaine have been investigated in patients with substance abuse and, founded results of these investigations revealed the limbic response to procaine [14,15].

In our study, rCBF in the bilateral superior, medial, inferior frontal, superior temporal, parahippocampus, thalamus and insula were found to be lower in patients. Several neuroimaging studies showed a decrease in frontal rCBF, which was named as “hypofrontality” [17]. Our results support the hypoftrontality in schizophrenia.

In the functional imaging studies, there have been findings indicating different activity of limbic system. In our study the rCBF following placebo in parahippocampus and insula were observed to be lower in patients. While the parahippocampus has extensive connections with amygdala, hippocampus, and PFC, it plays role in important processes, such as episodic and spatial memory, learning and emotion [4]. Hu et al. founded that most differences in regional gray matter observed in the bilateral parahippocampus

and hippocampus, and reported that decreased volume of these regions may be potential endophenotype for schizophrenia, and there may be a relationship between gray matter structural changes and heritable [18]. It has been demonstrated in schizophrenia that; structural and functional abnormalities take part in emotion processing; especially in assigning emotional feeling to body senses [19]. Chen et al. reported that progression of schizophrenia is associated with lower right insula volume [20]. This findings support that abnormalities of parahippocampus and insula may be important ethiopathogenesis of schizophrenia.

When alteration ratios of rCBF following procaine were compared, rCBF increase in bilateral ACC, left thalamus and left occipital regions was found to be significantly lower in patients. The ACC, having connections with PFC, temporolimbic structures, thalamus and basal ganglion, is an important structure, performing integrative role in executive functions and emotional regulations [5]. An abnormalities of ACC have been reported in many studies in schizophrenia. According to our study, the lower activation of ACC in patients, indicates the impairment in ACC activity, and is consistent to previous studies. In the neuroimaging studies, reduction in ACC metabolism was also reported, and the poorly performing showed only a modest increase in rCBF in the left ACC during cognitive activation [21]. Recent evidence suggests may be expressed at level of a spatially distributed network, which contains multiple densely interconnected regions of the cortical and subcortical regions [22]. A recent study, ACC activation to emotional stimuli was reduced and associated with negative symptoms [23]. We could not find relationship between psychotic symptoms. This result may be related to the relatively clinically stable of patients according to PANSS. Our findings support that ACC may be the key region in patophysiology of schizophrenia.

Many studies provide evidence of thalamus abnormality in schizophrenia. In our study, the alteration ratio of rCBF following procaine in left thalamus and rCBF following placebo in bilateral thalamus were lower in patients, and all these findings support talamus dysfunction hypothesis in schizophrenia [24]. Through extensive connections with cerebral cortex and limbic system, the thalamus plays essentials roles in the information input to brain, information processing and attention, also acting as a filter to protect the brain from redundant information input. The faulty connections may be related to wide variety of behavioral and cognitive characteristics seemed in schizophrenia [25]. Many author asserted that decreased thalamic activity might lead to impairment of the sensory filter system. In recent review, it was reported that thalamic nuclei which anatomically and functionally connected with the PFC may play important role, and several genes are associated with thalamic phenotypes which are altered in [24]. The study contributes to role of thalamus in the pathophysiology of schizophrenia.

In this research, lower activation to procaine in bilateral ACC and left thalamus of patients is shown. Low activation might be due to structural changes and related to neurophysiologic disorders in these regions. Impairments at cellular level may inhibit response to procaine. Procaine mainly binds to cholinergic muscarinic receptors as well as 5- HT3 receptors In monkeys, it was observed that there was an increase in procaine activation which was performed by using a cholinergic marker, limbic region activation and cholinergic receptor binding [11]. The central cholinergic system affects working memory, attention, verbal and episodic memory. It is also recognized that working memory and attention deficits are seen in schizophrenia [26]. In a SPECT study, M1 receptor levels were decreased in frontal, temporal, occipital cortex, basal ganglia and thalamus in schizophrenia, In postmortem studies; decrease in cortical expression of M1 receptors and polimorphizm or mutation of M1 receptor gene in cortical expression of M1 receptor gene; linked to more severe cognitive impairment. [27]. In postmortem studies; decrease in cortical expression of M1 receptors and polimorphizm or mutation of M1 receptor gene in cortical expression of M1 receptor gene; linked to more severe cognitive impairment. [28]. Procaine, which is considered to induce limbic system activation by central cholinergic system activation particularly through muscarinic receptors, might lead to lesser limbic system activation in patients than controls due to cholinergic receptor deficiency. Moreover, Zavitsanou et al. [2004] reported that ACC, has abundant muscarinic receptors of M1/M4, found presence of a deficit in M1/M4 receptors in schizophrenia [29].

In many studies, relationship between negative symptoms and hypofrontality has been supported. A negative correlation between PANNS negative and frontal, temporal, cingulate and thalamus rCBF were widely reported [7]. Positive symptoms have been mainly related to limbic system activity. However, in our study negative correlation was observed between the rCBF of right lentiform nucleus and PANSS negative and total scores. Also verified was a negative correlation between lentiform nucleus alteration ratios of rCBF and PANSS negative. The nucleus lentiformis play role of pacemaker for mesolimbic and cortical structures and has close relationship with limbic system [30]. In the study, less number of patients and including different schizophrenia subtypes could have negative influenced to the correlation results.

To our knowledge, it is the first study that investigates effect of procaine administration to patients. However, there are still some limitations. Some regions related to limbic system could not be evaluated due to technical insufficiency. We have observed low response to procaine activation in ACC; nevertheless, this region might not be specific for schizophrenia since ACC abnormalities have also been reported in other disorders, such as obsessive-compulsive disorder. Although it has been shown that the results are not influenced by disease duration and drug doses in structural and functional imaging studies, the patients in our study had different in terms of clinical features, disease duration and treatment. Schizophrenia is accepted as a disease in heterogeneous nature and more homogeneous distinct subtypes have been described. The different clinical attributes of schizophrenia included in our study might have decreased the sensitivity of the imaging results. In order to control this situation, a relatively homogeneous group of right-handed patients who were not under medical treatment were included.

Conclusion

The lesser limbic activation was observed after procaine administration in patients compared to controls. Insufficient limbic activation to procaine was evidenced particularly in ACC, which has both anatomic and functional connections with frontal cortex and temporal-imbic regions, and also in the left thalamus,

which has extensive connections with limbic system. These findings support that both ACC, involved in several cognitive and emotional processes, and thalamus, which functions sensory filter, have crucial roles in pathophysiology of schizophrenia.

Competing interests
The authors declare that they have no competing interest.

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Ethical approval
The study was approved by the ethical board first, then the aims and the methods of investigation were explained to all participants and written information forms were obtained.

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