Average spectral power changes at the hippocampal electroencephalogram in schizophrenia model induced by ketamine

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\textbf{ABSTRACT}

The use of ketamine (Ket) as a pharmacological model of schizophrenia is an important tool for understanding the main mechanisms of glutamatergic regulated neural oscillations. Thus, the aim of the current study was to evaluate Ket-induced changes in the average spectral power using the hippocampal quantitative electroencephalography (QEEG). To this end, male Wistar rats were submitted to a stereotactic surgery for the implantation of an electrode in the right hippocampus. After three days, the animals were divided into four groups that were treated for 10 consecutive days with Ket (10, 50, or 100 mg/kg). Brainwaves were captured on the 1st or 10th day, respectively, to acute or repeated treatments. The administration of Ket (10, 50, or 100 mg/kg), compared with controls, induced changes in the hippocampal average spectral power of delta, theta, alpha, gamma low or high waves, after acute or repeated treatments. Therefore, based on the alterations in the average spectral power of hippocampal waves induced by Ket, our findings might provide a basis for the use of hippocampal QEEG in animal models of schizophrenia.

\textbf{INTRODUCTION}

Schizophrenia is a serious and disabling chronic mental illness that affects around 1% of the world population [1]. Schizophrenia include positive (delusions and hallucinations) and negative (blunted affect and social withdrawal) symptoms as well as cognitive impairments [2]. The pathophysiology of this mental disorder remains unknown. These symptoms are related to alterations in distinct brain areas, mainly hippocampus (HC) [3,4]. Patients with schizophrenia, besides from presenting behavioral symptoms, also show electroencephalographic changes mostly during crises [5]. This abnormal electrical activity is related to the malfunction of brain structures like the hippocampus, amygdala, thalamus, temporal, frontal, and cingulate cortices [6].

The electroencephalogram (EEG) is the graphic registry of the electrical activity of the brain [7]. It is based on a mathematical process called the fast Fourier transform (FFT), which separates a complex sinusoidal wave into a sum of simple wave forms of specific frequency and voltage [8]. Thus, brain rhythms can be used as biomarkers of physiological and pathological...
states of rest and activity. Neural oscillations and their synchronization may represent a versatile signal to understand the flexible communication within and between cortical areas. There is broad evidence that cognitive functions are associated with the synchronized oscillatory activity, suggesting a functional mechanism of neural oscillations in cortical networks. In addition to its role in normal brain function, there is growing evidence that the modified oscillatory activity may be associated with certain neuropsychiatric disorders such as schizophrenia, which involve dysfunctional behavior and cognition [9]. That way, schizophrenia is often considered a disconnection syndrome, as abnormal interactions between a wide range of functional brain networks result in cognitive and perception deficits [10].

Ketamine, a noncompetitive antagonist of NMDA glutamate receptor, presents a broad range of pharmacological effects depending on the dose. In this regard, at high doses (160 mg/kg) this drug presents anesthetics properties [11]. However, not anesthetics doses of ketamine (10 mg/kg [12]: 20 mg/kg [13,14]; 25, 50, or 75 mg/kg [15]; and 100 mg/kg [16]) induce cognitive impairment, psychosis, and exacerbates schizophrenic symptoms. Thus, ketamine is widely used for schizophrenia-like induction in rodents [12–14,16,17]. In line with this, several studies have demonstrated that a single dose of ketamine induces a state in the brain characterized by an increase in the power and intrinsic frequency of brain oscillations [18–20]. Spectral analysis showed an increase in absolute power after ketamine doses of 9 or 30 mg/kg, with the highest increase being achieved in the delta, beta, and gamma bands. Changes were most prominent at 10–15 min after administration, which temporally correlates with the highest ketamine and norketamine levels in the brain [21].

Pharmacological models of schizophrenia induced by ketamine are an important tool for understanding the main mechanisms of glutamatergic regulated neural oscillations. These neural oscillations are evaluated through quantitative EEG (QEEG) of rats after the administration of ketamine providing a valuable animal-clinical interface for the study of glutamatergic dysfunction in schizophrenia. Thus, our study aimed to evaluate changes in the average spectral power of hippocampal QEEG in animals submitted to the model of schizophrenia induced by ketamine.

MATERIAL AND METHODS

Animals
The experiments were performed in male Wistar rats (200–300 grams). The animals were kept at a room with controlled temperature (23 ± 1°C) with a cycle of 12-h light/dark and free access to food and water. Experiments were conducted during the light phase between 09:00 and 13:00.

All experimental procedures were performed in accordance with Guide for the Care and Use of Laboratory Animals from the National Research Council. The protocols were approved by the Ethics Committee of Federal University of Ceará (No. 92/2009).

Drugs
Ketamine (Ket) hydrochloride (Vetanarcol®, 50 mg/mL, König, Avellaneda, Argentina) was dissolved in 0.9% saline solution and administered intraperitoneally (ip). The control animals received 0.9% saline. All solutions were administered in a volume of 0.1 mL for each 100 g of body weight.

Outline of the study

Electroencephalographic study

Stereotactic surgery and electrodes implantation. The rats were first anesthetized with ketamine (100 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.). During the surgical procedure, bipolar and twisted electrodes of NiCr wire (diameter 150 μm) were implanted in the hippocampus through stereotactic device (Stoelting®, EUA) at the following coordinates (mm): AP = −4.0, ML = ±2.6, and DV = −3.5 from bregma, according to the Atlas of Paxinos and Watson [22]. An additional screw was placed in the frontal bone cavity as the reference electrode. The electrodes were fixed to the skull with dental acrylic cement. The correct location of the implanted electrodes in the hippocampus was verified by histological analysis using violet cresyl staining according to [23] and [24].

Treatment protocol. Three days after implantation of the electrodes [23], the animals were randomly divided into four groups (n = 6 animals/group). The treatment groups received 10 days administration of the drugs (each one administered once a day) being divided as follows: group 1 – control – 0.9% saline intraperitoneal (IP) injection; group 2 – Ket 10 mg/kg, IP; group 3 – Ket 50 mg/kg, IP; group 4 – Ket 100 mg/kg, IP (Figure 1). Ketamine was used in three different doses.
which are indicated in the literature for induction of schizophrenia-like alterations (10 mg/kg [12]; 50 [15,25] or 100 mg/kg [16]). The EEG recordings were taken from the hippocampus of animals in vivo on the 1st and 10th days of treatment, immediately after the last drug administration during the light phase between the hours of 09:00 and 13:00 (Figure 1).

**Electroencephalographic records.** The quantitative EEG was recorded continuously for 20 min through a polygraph digital system (PowerLab 4/30 device) [26]. The signals were recorded with a 1,000-Hz sampling rate and recorded using version 7.3.8 of the LabChart Acquisition Software. EEG was recorded individually in a glass box (0.3 × 0.5 × 0.4 m), with the floor covered with sawdust. The acquisition cable was connected to a microconnector on the animal’s head, with the electrical activity signals being captured by a PowerLab® system. The EEG recordings were analyzed by the LabChart 7.3.8. The data were segmented into 1,024 points, and the signals were converted to spectral activity signals being captured by a PowerLab system.

**Spectral power changes at the hippocampal EEG by ketamine.**

Statistical analysis

D’Agostino and Pearson omnibus normality test was used to confirm the normally distribution of data. The comparisons were performed by analysis of variance (two-way ANOVA) using GraphPad Software 5.0 version for Windows, GraphPad Software (San Diego, CA, USA) with Bonferroni as post hoc test. Results were considered significant at \( P < 0.05 \) and were presented as mean ± SEM.

**RESULTS**

Typical examples of the EEG, mean power spectral in the hippocampus of rats in acute treatment (Figure 2a), or repeated treatment for 10 days (Figure 2b) with ketamine were 10, 50, or 100 mg/kg.

The acute administration of ketamine did not change the average spectral power in the delta band (0–4 Hz) at any time when compared to the control group (Figure 3a). However, ketamine repeated administration for 10 days at the lowest dose (10 mg/kg) induced increases in the average spectral power in the delta band (Figure 3b). This increase was observed in the fifth (3.44 ± 0.02; \( P < 0.05 \)), tenth (3.44 ± 0.01; \( P < 0.01 \)), fifteenth (3.44 ± 0.01; \( P < 0.01 \)), and twentieth minute (3.44 ± 0.00; \( P < 0.01 \)) compared to group control (5 min: 3.37 ± 0.01; 10 min: 3.36 ± 0.01; 15 min: 3.36 ± 0.00; 20 min: 3.37 ± 0.01) [\( F_{3,50}=30.23; P < 0.001 \)].

For the theta band (4–8 Hz), ketamine (Figure 4a) only at the dose of 50 mg/kg decreased its average spectral power in the 1st day of treatment (5 min: \( P < 0.05 \); 10 min: \( P < 0.05 \); 15 min: \( P < 0.01 \); 20 min: \( P < 0.01 \)) when compared to the control group (5 min: 3.96 ± 0.06; 10 min: 3.99 ± 0.06; 15 min: 4.02 ± 0.09; 20 min: 3.98 ± 0.08) [\( F_{3,49}=24.85; P < 0.0001 \)]. A similar effect (Figure 4b) was observed after repeated ketamine administration for 10 days at 50 mg/kg (5 min: \( P < 0.01 \); 10 min: \( P < 0.05 \); 15 min: \( P < 0.05 \); 20 min: \( P < 0.01 \)) or 100 mg/kg (5 min: \( P < 0.001 \); 10 min: \( P < 0.01 \); 15 min: \( P < 0.05 \); 20 min: \( P < 0.01 \)) when compared to the control group (5 min: 4.23 ± 0.10; 10 min: 4.14 ± 0.07; 15 min: 4.00 ± 0.08; 20 min: 4.18 ± 0.11) [\( F_{3,62}=42.36; P < 0.0001 \)]. On the other hand, Ket 10 mg/kg induced an increase, although not statistically significant, in theta band (Figure 4a and b).

An increase in the average spectral power of alpha band (>8–13 Hz) was evidenced by ketamine administration (Figure 5) only at the lowest dose, both on the 1st day (5 min: \( P < 0.01 \); 10 min: \( P < 0.001 \); 15 min: \( P < 0.05 \)) as on the 10th day of treatment (5 min: \( P < 0.01 \); 10 min: \( P < 0.01 \); 15 min: \( P < 0.001 \); 20 min: \( P < 0.01 \)) when compared to the control group [1st day (5 min: 4.87 ± 0.12; 10 min: 4.84 ± 0.12; 15 min: 4.88 ± 0.16) [\( F_{3,58} = 23.63; P < 0.0001 \)].

**Figure 1** Treatment protocol with saline (Control), ketamine 10 mg/kg (Ket 10), ketamine 50 mg/kg (Ket 50), or ketamine 100 mg/kg (Ket 100).
Bands Treatment for 10 days

<table>
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(b)

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Figure 2: Specimen recording in the hippocampal EEG of rats filtered between 0–4 Hz (delta); 4–8 Hz (theta); >8–13 Hz (alpha); 30–50 Hz (gamma low); and 50–100 Hz (gamma high), after acute treatment (Figure 2a) or repeated treatment for 10 days (Figure 2b) with ketamine (10, 50 or 100 mg/kg). All EEG traces had calibration scale in mV, and polygraphic records were cut in 10-s epochs.
P < 0.0001]; 10th day (5 min: 5.26 ± 0.19; 10 min: 5.07 ± 0.09; 15 min: 4.88 ± 0.08; 20 min: 4.99 ± 0.16) [F_{3,64} = 41.70; P < 0.0001].

The low gamma band (30–50 Hz) was increased after acute administration of ketamine (Figure 6a) in the doses of 10 (5 min: P < 0.001; 10 min: P < 0.001; 15 min: P < 0.001; 20 min: P < 0.001) or 50 mg/kg (5 min: P < 0.001; 10 min: P < 0.001; 15 min: P < 0.001; 20 min: P < 0.001) when compared to control group (5 min: 35.48 ± 0.15; 10 min: 35.60 ± 0.11; 15 min: 35.58 ± 0.09; 20 min: 35.68 ± 0.10) [F_{3,51} = 109.29; P < 0.0001]. A similar effect was observed after ketamine repeated treatment (Figure 6b) at the doses of 10 (5 min: P < 0.001; 10 min: P < 0.001; 15 min: P < 0.001; 20 min: P < 0.001) or 50 mg/kg (5 min: P < 0.01; 10 min: P < 0.01; 15 min: P < 0.01; 20 min: P < 0.01) when compared to control group (5 min: 35.58 ± 0.07; 10 min: 35.63 ± 0.12; 15 min: 35.56 ± 0.07; 20 min: 35.67 ± 0.12) [F_{3,33} = 48.45; P < 0.0001].

In the high gamma band (50–100 Hz), the results of the average spectral power were increased by acute [F_{3,44} = 17.14; P < 0.0001] or repeated [F_{3,46} = 26.43; P < 0.0001] treatment with ketamine (Figure 7) at the dose of 100 mg/kg [1st day (5 min: P < 0.05; 10 min: P < 0.05; 15 min: P < 0.05; 20 min: P < 0.01); 10th day (5 min: P < 0.001; 10 min: P < 0.001; 15 min: P < 0.01; 20 min: P < 0.001)]. However, a similar effect was observed with the acute administration of ketamine 50 mg/kg (5 min: P < 0.05; 20 min:
and with the repeated administration of ketamine 10 mg/kg (5 min: \( P < 0.05 \); 10 min: \( P < 0.05 \); 15 min: \( P < 0.05 \); 20 min: \( P < 0.05 \)) when compared to control group [1st day (5 min: 69.40 ± 0.19; 10 min: 69.45 ± 0.29; 15 min: 69.44 ± 0.14; 20 min: 69.43 ± 0.39); 10th day (5 min: 69.42 ± 0.36; 10 min: 69.44 ± 0.35; 15 min: 69.44 ± 0.14; 20 min: 69.43 ± 0.39)].

**DISCUSSION**

From the quantitative analysis of rats hippocampal EEG under the pharmacological model of schizophrenia induced by ketamine, our results showed that only ketamine in low doses (10 mg/kg) caused an increase in the average spectral power in the delta band after repeated treatment. This result suggests that the action of ketamine in rats’ hippocampal delta wave will depend on the dose of administration and the treatment time. In other words, we observed that the frequency of the delta wave was increased to near the maximum peak frequency stipulated in this study (4 Hz) only after the repeated treatment with ketamine 10 mg/kg. A similar result was obtained in an in vivo study showing the effects of the administration of NMDA receptor (NMDAR) antagonists on delta oscillations in the CA1 area of the hippocampus [27]. These authors observed an increase in the average spectral power with ketamine at a dose of 50 mg/kg, which was not seen with the dose of 20 mg/kg [27].
It is believed that the slow delta activity is originated in oscillatory neurons in the deep cortical layers of the thalamus, normally inhibited by the ascending reticular input. The delta activity might reflect the hyperpolarization of cortical neurons resulting in de-differentiation of neural activity [28] [29]. This supports the concept that schizophrenia may involve the filtering of sensory signals input to the cortex by means of the thalamus [30]. Many researchers have supported the concept that the negative symptoms of schizophrenia might be related to a decrease in delta wave during sleep [31–34].

For theta band, our findings showed that ketamine in the higher dose decreased the average spectral power. Previous findings showed a similar decrease in the average spectral power in hippocampal theta band oscillations with the administration of NMDAR antagonists, amino-phosphono-valeric acid (10–20 μg APV) [35]; MK801 (0.01–0.2 mg/kg) [36,37], and ketamine (20 mg/kg) [38] [39]. Theta rhythm is a rather slow frequency between 4 and 8 Hz, with an amplitude of 50–100 μV [23,40]. This rhythm is mostly related to REM sleep [40] and may represent the inhibitory action of GABAergic interneurons affecting the corticothalamic network. It could be associated with limbic activity (memory and emotions) [8] in brain areas such as hippocampus and amygdala. The proper organization and function of GABAergic interneuron networks in these systems are essential for many cognitive processes and abnormalities documented in schizophrenic patients. The memory function of the hippocampus depends on two major patterns of oscillations in the theta and gamma ranges [37].

We also observed an increase in the average spectral power of the alpha band with ketamine administration at a dose of 10 mg/kg in the 1st and 10th day of treatment. The effects caused by the administration of NMDAR antagonists on hippocampal alpha oscillations are poorly documented in the literature. The alpha rhythm can be produced by pacemaker neurons distributed through the thalamus that oscillate synchronously in the alpha frequency range (7.5–12.5 Hz), which dominates the EEG of an alert normal person at rest [28]. The reticular nucleus can hyperpolarize cell membranes of thalamic neurons through the release of GABA, decreasing the dominant alpha rhythm, thus passing to the theta spectrum, which is slower (3.5–7.5 Hz) and reducing the sensory activity to the cortex [28]. Therefore, we can infer that the alpha band activity could be increased with low and intermediate doses of ketamine. Based on this premise, we can suggest that the changes induced by ketamine in the alpha band oscillation are related primarily to the thalamus. Thus, the alterations in this frequency band may indicate dysfunction of the inhibitory thalamic neurons by ketamine.

Acute administration of ketamine (10 or 50 mg/kg) increased the spectral power of the gamma low band. This increase in the spectral activity of gamma low band remained after the treatment with ketamine for 10 days in doses of 10 or 50 mg/kg. A similar effect was observed for gamma high band with acute ketamine administration only at the highest dose (50 or

Figure 7 Mean power spectral of gamma high rhythm in the hippocampus of rats in acute treatment (a) or repeated treatment for 10 days (b) with ketamine (10, 50, or 100 mg/kg). Each bar represents mean ± SEM. For all analyzes, *P < 0.05, **P < 0.01, or ***P < 0.001 were considered significant. Two-way ANOVA followed by Bonferroni as post hoc test.
100 mg/kg). In the case of treatment with ketamine for 10 days, this increase in the spectral activity of gamma high band remained, but at the doses of 10 or 100 mg/kg. The activity in the gamma bands (25–50 Hz) may reflect cortico-thalamo-cortical reverberatory circuits as well as back propagation of axonal discharges to the dendrites of cortical pyramidal cells, which may play an important role in perception [28]. Furthermore, there is also evidence that the oscillatory gamma activity may be related to symptoms of schizophrenia such as hallucinations, thought disorders and negative symptoms. Thus, the positive symptoms of schizophrenia can be correlated with an amplitude increase in the gamma-band in brain regions, while the negative symptoms have been linked to low frequency oscillations [8,41] [42]. Similar results were observed in earlier studies demonstrating that the administration of ketamine induced a state in the brain characterized by an increase in the power and intrinsic frequency of gamma oscillations [15,27,37,43].

The present study has some limitations: (i) the beta band (14–30 Hz) is missing in the defined frequency ranges of our study, and (ii) ketamine is a pharmacological model of schizophrenia presenting thus limitations regarding the modeling of the ethiopathogenic mechanisms involved in this mental disorder, such as its developmental course. This may justify some inconsistent results observed in the present study.

In summary, our study provides an analysis of the changes induced by ketamine in the hippocampal electrical activity of rats. We found that acute and repeated administration of ketamine increased the average spectral power of delta, alpha, gamma low, and gamma high bands, indicating that the glutamatergic system dysfunction observed in schizophrenia affects the spectrum of hippocampal bands oscillations.

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