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Ethanol changes the electroencephalographic correlation of the ventral tegmental area and nucleus accumbens, components of the mesoaccumbens system in rats

Marisela Hernández-González [a,*], Mariana Martinez-Pelayo [a], Marcela Arteaga Silva [b], Herlinda Bonilla-Jaime [b], Miguel Angel Guevara [a]

[a] Instituto de Neurociencias, Universidad de Guadalajara, Francisco de Quevedo 180, Colonia Arcos-Vallarta, C.P. 44130, Guadalajara, Jalisco, Mexico
[b] Departamento de Biología de la Reproducción, Universidad Autónoma Metropolitana, Campus Iztapalapa, Av. San Rafael Atlixco 186, Colonia Vicentina, C.P. 09340, México, D.F. Mexico

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ABSTRACT

Alcohol consumption produces various behavioral effects and its administration is associated with increases of dopamine (DA) levels in the nucleus accumbens (Acc). However, it is not yet clear if these behavioral and neurochemical effects are associated with a different functionality of the Acc and ventral tegmental area (VTA), the neural structures that constitute the dopaminergic mesoaccumbens system. The present study was designed to analyze whether the electroencephalographic (EEG) correlation between the Acc and VTA shows characteristic changes after the forced administration of ethanol in male rats. Simultaneous EEG recordings were obtained from the left and right Acc and VTA in adult male rats during a 40 min period immediately after an i.p. injection of ethanol (15% v/v in a dose of 0.75 g/kg). During the 40 min period after i.p. ethanol administration, an increase of the fast frequencies (13–21 Hz) was observed in Acc and VTA. The interaccumbens correlation showed a significant decrease in the theta frequencies (4–7 and 8–12 Hz bands), whereas the intrahemispheric *r* in both hemispheres showed a clear increase in the fast frequencies. The possible participation of the mesoaccumbens system in the arousal state and in the motivo-emotional aspects of ethanol is suggested.

1. Introduction

Many lines of evidence suggest that the dopamine (DA) neurons from the ventral tegmental area (VTA) that innervate the nucleus accumbens (Acc), referred to as the meso-accumbens DA system, play an important role in mediating reward-seeking effects as well as in the activational effects that result from the processing of incentive stimuli associated with rewards (Berridge and Robinson, 1998; Ikemoto and Panksepp, 1999; Salamone, 1994). Results of many behavioral, pharmacological and neurochemical studies support the notion that the reinforcing properties of alcohol are mediated by Acc neuron activity (Di Chiara, 1995; Kiianmaa et al., 1995; Koob, 1992a,b; Koob et al., 1998; Weiss and Porrino, 2002; Wise, 1998). In addition, it has been demonstrated that the direct administration of ethanol to the VTA increases the activity of DA neurons in this structure, resulting in an increase in the levels of DA primarily in the shell of the Acc, and that the action of ethanol in the Acc is mediated by serotonergic 5-HT3 receptors (Yoshimoto and McBride, 1991; Yoshimoto et al., 1996). The acute injection of different doses of ethanol (0.5, 1.0, 2.0 g/kg) produces this effect in a dose-dependent manner (Brodie et al., 1999; Di Chiara, 1997; Gil-Verona et al., 2003; Kaczmarek and Kiefer, 2000; Kiianmaa et al., 2003; Klemm, 1979; Slawecki, 2002; Tizabi et al., 2002; Weiss and Porrino, 2002; Yavich and Tiihonen, 2000). In vivo microdialysis studies have shown that a gradual alcohol-induced increase in the release of Acc DA occurs regardless of the route of alcohol administration (Yoshimoto and McBride, 1991; Yoshimoto et al., 1991, 1996), and that DA levels reach their peak about 20 min after the acute administration in both the Acc and the VTA (Di Chiara et al., 1998; Tizabi et al., 2002), as well as in the central nucleus of the amygdala.

It is relevant to note that the Acc has been divided into two regions: the core and the shell, which differ in both their efferent and afferent neuronal connections. It is now believed that the shell region constitutes a mesolimbic sector that is deeply involved in emotional and motivational processes, whereas the core region comprises a striatal sector predominantly involved in motor functions (Heimer et al., 1991).

The electroencephalogram (EEG) is a useful and widely-used technique that has proven to be highly effective in studying the functional organization of the brain. Thus, certain studies have demonstrated that the electroencephalographic activity of some of the structures of the mesolimbic system is modified by the acute administration or voluntary consumption of ethanol. In the parietal cortex and hippocampus, Slawecki (2002) found that the acute intraperitoneal (i.p.) administration of a 1.5 g/kg dose of ethanol increased the power of slow frequencies (4–6 Hz) by 75% with respect

* Corresponding author. Tel./fax: +52 33 38 18 07 40.
E-mail addresses: mariselh@cencar.udg.mx (M. Hernández-González), oylaepram@yahoo.com (M. Martinez-Pelayo), asm@xanum.uam.mx (M. Arteaga Silva), bjh@xanum.uam.mx (H. Bonilla-Jaime), mguevara@cencar.udg.mx (M.A. Guevara).

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to the baseline, an effect they associated with the sedative action of ethanol. Other studies have shown that especially high doses of ethanol (>2 g/kg) have similar effects on spontaneous EEG (Ehlers and Reed, 1987; Grupp, 1981; Perrin et al., 1975), while still others have shown that at low doses (<1 g/kg) ethanol produced generalized low-voltage and increased high-frequency electroencephalographic activity (Prado de Carvalho and Izquierdo, 1977; Wishaw, 1976).

It has been further suggested that the degree of correlation (r) between two neural regions is indicated by the degree of similarity between EEG activity at the two sites (Lopes da Silva, 1991; Shaw, 1984), such that a low correlation reflects higher functional differences, and vice versa. Correlation analysis provides information on the linear relationships between two signals and we (Guevara et al., 2008; Hernández-González et al., 2005, 2007) as well other researchers, have used this technique previously to elucidate temporal coupling between regions (Guevara and Corsi-Cabrera, 1996; Guevara et al., 2002; Shaw, 1984) in both rats and humans.

Thus, although it has been shown that the dopaminergic mesoaccumbens pathway constitutes the neural substrate of the reinforcement produced by ethanol and that DA levels show a gradual increase in the Acc and VTA after alcohol administration, it is not yet known if the neural structures that constitute the mesoaccumbens system show temporal changes in their functionality after ethanol administration. The aim of the present study was to ascertain whether the electrophysiological correlates of VTA–Acc interactions show different changes after the i.p. administration of ethanol in rats. We hypothesized that the functioning and interactions of these structures would be distinct during the different time periods recorded immediately after forced ethanol administration.

2. Materials and methods

2.1. Subjects

Wistar male rats, 80–90 days old, were obtained from a colony bred at the Institute of Neurosciences, University of Guadalajara. All rats were maintained in a room at 22–23 °C under a 12:12 h reversed light/dark cycle (lights on from 2000 to 0800 h) and housed individually. Food and water were available ad libitum. Temperature, feeding and the light–dark cycle conditions were maintained constant throughout the course of the study. Animal care and all procedures involving animals were approved by our Institutional Animal Care and Use Committee in accordance with NIH specifications.

2.2. Surgery

For surgery, a total of 20 male rats were anaesthetized with sodium pentobarbital (35 mg/kg i.p.). Stainless steel electrodes (0.2 mm in diameter) were implanted bilaterally simultaneously in both hemispheres, specifically into the Acc shell (2.7 mm anterior to bregma, 0.8 mm lateral to midline, 7 mm below the dura mater) and the VTA (6.4 mm posterior to bregma, 0.5 mm lateral to midline, 8.2 mm below the dura mater) with the incisor bar set at −3.5 mm following Paxinos and Watson's stereotaxic atlas (Paxinos and Watson, 1997). Two stainless steel screws were placed in the anterior and posterior parts of the skull to serve as “reference” and “ground” electrodes, respectively. All electrodes were attached to a miniature connector that was fixed on the skull by means of stainless-steel hooks and acrylic cement. Adequate care was taken to minimize pain or discomfort in the animals throughout the experiment. After surgery, all subjects were housed in individual cages with food and water ad libitum.

2.3. EEG tests

Seven days after electrode implantation, and three or four days before the EEG tests, all rats were given 30 min to adapt to the recording room and connection cable. On the day of each EEG test, the animals were allowed to adapt to the recording room for another 30 min before being submitted to EEG recordings.

2.4. Acute alcohol administration

After the adaptation period, the animals were connected to the cable to record 30 2-second segments while they were immobile and had their eyes open; conditions that were established as the basal state (awake–quiet). At that point, the animals were taken and a dose of 0.75 g/kg of ethanol (99.8%, MERK, Germany) was administered intraperitoneally (i.p.) as a 15% (v/v) solution in physiologic saline to minimize concentration-induced differences in the alcohol absorption rate and tissue irritation at the injection site. Subjects were immediately returned to their cages and connected. The EEGs from the Acc and VTA were recorded simultaneously in 2 second epochs at three time points: (1) during the first 15 min; (2) from the 15 to 25 min interval; and (3) from the 25 to 40 min interval. All recordings were obtained while subjects were in the awake–quiet behavior state.

2.5. Acute saline administration

In order to obtain a control test for this acute administration, one week later the rats were re-weighed and then received an i.p. injection with the same volume of saline solution as in the ethanol injection. This was administered in order to assess the possible stress caused by the injection and the volume administered. EEG recording before (basal state) and up to the 40 min after the saline injection was performed.

Both the saline test and the acute administration of ethanol were conducted in a counter-balanced way, such that half of the rats began with the acute ethanol administration test and received the saline test one week later, while the other half had the tests in reverse order.

2.6. Recording and sampling of EEG activity

To record the EEGs, the connection cable from the electrodes implanted in the rats was hung freely from the polygraph to allow them free movement within their cages while the EEGs from the Acc and VTA of both hemispheres were recorded. The cable was connected to the headstage AC preamplifiers (model 7P5C) of a Grass 7B polygraph (band pass 3–30 Hz), and their outputs were plugged into a PCL-812 analog-to-digital converter (Advantech), which functioned as an interface to a microcomputer. The EEG signals were recorded at a sampling rate of 512 Hz during 2-s EEG epochs and were calibrated at a pulse of 50 μV, produced by the preamplifiers and delivered to a PC as a reference to convert the output of the analog-to-digital converter to microvolts. The capture of the EEG signals corresponding to each one of the different conditions was performed by means of a board unit with eight on/off buttons connected to the digital input lines of the analog-to-digital converter. Thus, the capture of the 2-s segments of EEG corresponding to the different periods (0–15, 15–25, 25–40 min) of each recording session began when a specific button of the board unit was pressed; another button was pushed to end signal input.

By means of specific computer programs (Hernandez-González et al., 1997), the capture of the simultaneous bilateral recording of the EEG signals from the Acc and VTA was made in a precise temporal relation to each time period after the forced administration of the two solutions. Several 2-s EEG segments were captured in each condition and were independently stored in files for off-line analysis.

2.7. EEG analysis

The EEG signals were carefully inspected before analysis and all segments containing artifacts were discarded. At least 30 2-s EEG

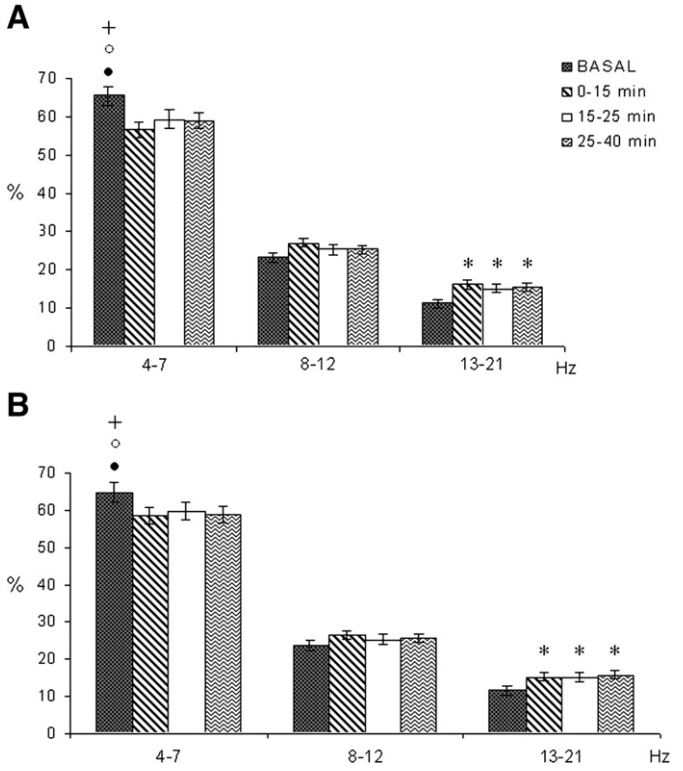

Fig. 1. Relative power (%, mean ± S.E.M.) of the three frequency bands in the left (A) and right Acc (B) during the different time periods after forced ethanol administration. * increased over basal; ● increased over 0–15 min; ° increased over 15–25 min; + increased over 25–40 min.

segments from each subject and each condition on each test were analyzed. Relative power (RP) (the proportional contribution of each band expressed as a percentage of total power between 4 and 21 Hz) was calculated by means of a Fast Fourier Transformation (FFT) for the 4–7 (low theta), 8–12 (high theta) and 13–21 Hz bands, following the typical EEG division made by Vanderwolf in rats (Vanderwolf, 1969, 1990). Correlation (r) analyses provide information on the linear relationships between two signals and have therefore been used to elucidate temporal coupling between regions (Guevara and Corsi-Cabrera, 1996; Shaw, 1984). Thus, with the aim of obtaining the degree of functional synchronization between bilateral regions (inter-hemispheric r) and regions within one hemisphere (intra-hemispheric r), the r was calculated in the time domain for the same bands by means of Pearson product-moment correlation coefficients between successive amplitude values of EEG segments from the left and right accumbens and tegmental regions. Analyses of the EEGs corresponding to each time period after the forced administration of each solution were performed, as well as comparisons of the specific 15–25 min periods after administration of the two solutions.

2.8. Histology

At the end of the experiment, the animals were deeply anaesthetized with sodium pentobarbital. An intracardial infusion of isotonic saline (0.9%) followed by a 5.0% buffered paraformaldehyde solution fixed the brain, which was then removed and stored in formalin for at least 2 weeks. 50 μm-thick sections were made with a microtome and stained with cresyl violet. Inspection under a stereoscopic microscope following the stereotaxic coordinates made it possible to reconstruct the path followed by the recording electrode. Only those recordings obtained from the shell region of the Acc and the VTA were included in data analysis.

2.9. Statistics

Statistical analysis was limited to the 4–21 Hz band. A one-factor analysis of variance (ANOVA) to compare the RP and correlation parameters of the different frequency bands by each structure (Acc and VTA) was performed, followed by Tukey's tests for post-hoc comparisons. Each ANOVA compared the basal condition and the three time periods (0–15, 15–25, 25–40 min) of post-ethanol administration. Differences were considered significant when $p<0.05$ was reached. A Student t test was applied to compare the specific 15 to 25 min periods following the i.p. applications of the saline and ethanol solutions.

3. Results

3.1. Histology

Sixteen of the twenty male rats had the correct location of the electrode tips. Electrode tips in the shell region of the Acc were bilaterally located between 1.2 and 2.2 mm anterior to bregma, while those of the VTA were located between 5.2 and 6.4 mm posterior to bregma.

3.2. Acute alcohol administration

3.2.1. Relative power (RP)

In the left Acc, the RP of the 4–7 Hz band showed a significant decrease after acute ethanol administration (0–15, 15–25 and 25–40 min) [$F_{(3,45)}=19.3$, $p<0.001$] compared to the basal condition (Fig. 1A). A similar pattern was observed during all periods in the right Acc [$F_{(3,45)}=11.6$, $p<0.001$] (Fig. 1B). In the 13–21 Hz band, all three periods after acute ethanol administration showed a significant increase in the left Acc [$F_{(3,45)}=39.1$, $p<0.001$] (Fig. 1A), and in the right Acc [$F_{(3,45)}=17.1$, $p<0.001$] (Fig. 1B) compared to the basal period.

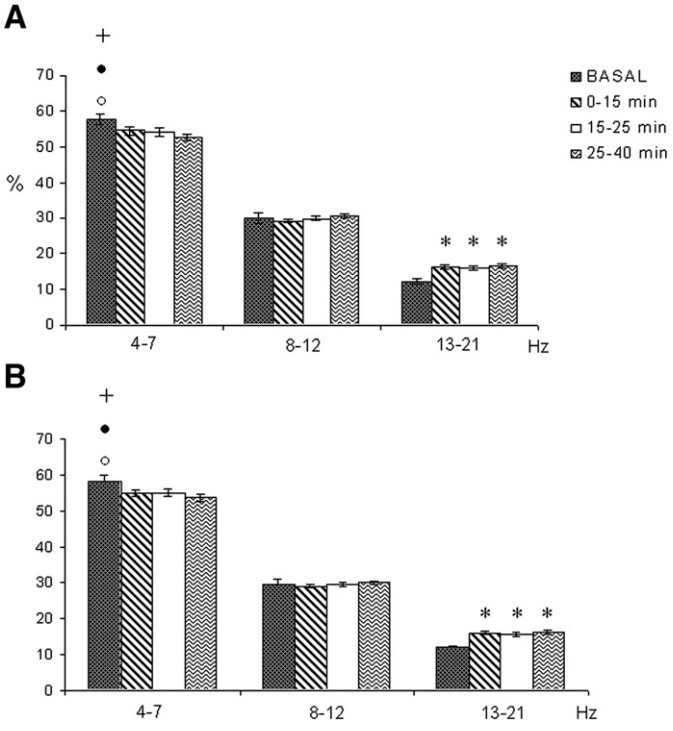

Fig. 2. Relative power (%, mean ± S.E.M.) of the three frequency bands in the left (A) and right VTA (B) during the different time periods after forced ethanol administration. * increased over basal; ● increased over 0–15 min; ° increased over 15–25 min; + increased over 25–40 min.

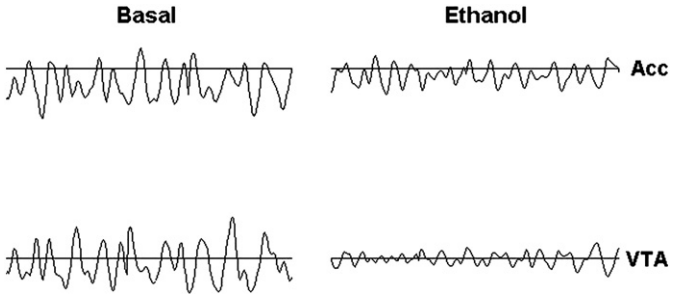

Fig. 3. Electroencephalographic (EEG) 1 second segments from the nucleus Accumbens (Acc) and ventral tegmental area (VTA) of a male rat before (Basal) and after forced ethanol administration (Ethanol). All EEG recordings were obtained while subjects were in the awake–quiet state. Note the higher proportion of low-voltage fast frequencies obtained after ethanol administration.

In the VTA, the three periods after acute ethanol administration showed a significant decrease of the RP in the 4–7 Hz band compared to the basal period. This was statistically significant in both, the left [$F_{(3,45)}$=7.2, p<0.001] (Fig. 2A) and right VTA [$F_{(3,45)}$=6.7, p<0.001] (Fig. 2B). The RP of the 13–21 Hz band showed an increase in the three periods after the acute injection of ethanol. This was significant in both the left [$F_{(3,45)}$=67.1, p<0.001] (Fig. 2A) and right VTA [$F_{(3,45)}$= 68.1, p<0.001] compared to the basal period (Fig. 2B). The increased proportion of the fast frequencies that was obtained in both structures during the entire period of 40 min post-ethanol administration is shown in Fig. 3.

3.2.2. Inter-hemispheric correlation

The interaccumbens r (between the left and right Acc) of the 4–7 Hz band showed a decreased r in 0–15, 15–25 and 25–40-min periods [$F_{(3,45)}$=29.8, p<0.001] compared to the basal period (Fig. 4A). Though this decrease was also observed in the 8–12 and 13–21 Hz bands, it was only statistically significant in the former [$F_{(3,45)}$=13.3, p<0.001] compared to the basal period (Fig. 4A).

The inter-tegmental correlation after acute ethanol administration showed no changes in any of the frequency bands with respect to the basal condition (data not shown).

3.2.3. Intrahemispheric correlation

The intrahemispheric correlation between the Acc and the VTA showed a similar pattern in both hemispheres during the 40 min after acute ethanol administration. This pattern was characterized by an increased r of the fast frequencies (13–21 Hz) in both, the left [$F_{(3,45)}$= 8.0, p<0.01] (Fig. 4B) and right hemispheres [$F_{(3,45)}$=13.3, p<0.01] compared to the basal period (data not shown).

3.3. Acute saline administration

The RP and r values of the three different frequency bands analyzed showed no changes as compared to the RP and r values recorded during the basal condition (before i.p. administration of the saline solution).

3.4. Comparison of saline vs alcohol administration

It has been reported that the higher release of DA in the Acc and the VTA (Tizabi et al., 2002; Di Chiara et al., 1998) as well as in the central nucleus of the amygdala (Yoshimoto et al., 2000) occurs about 20 min after ethanol administration. Thus, in order to elucidate the differences between the saline solution injection (saline) and the forced administration of ethanol, an analysis comparing the 15–25 min period after the saline injection and the 15–25 min period after the acute ethanol administration was performed.

3.4.1. Relative power

The RP values of the 8–12 (t=2.236; p<0.05) and 13–21 Hz bands (t=2.253; p<0.05) showed an increase during the 15–25 min period

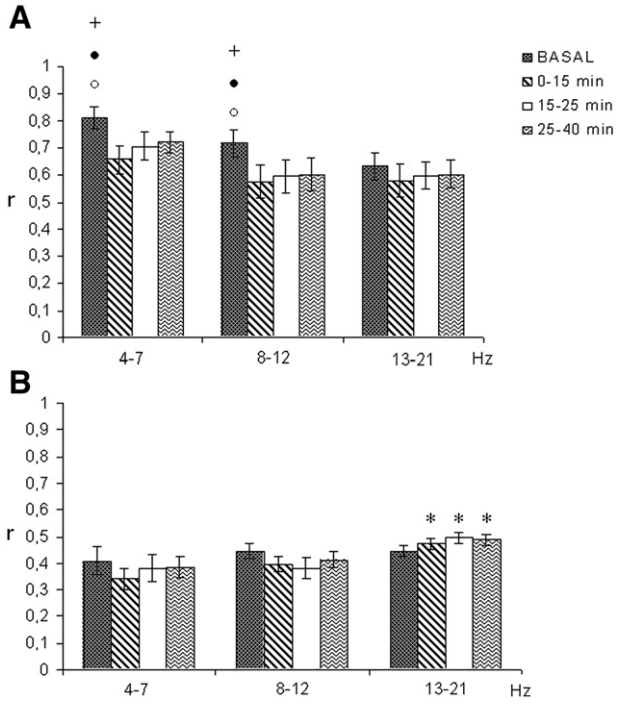

Fig. 4. Mean±S.E.M of the interaccumbens (A) and left intra-hemispheric correlation (r) (B) of the three frequency bands during the different time periods after forced ethanol administration. * increased over basal; ● increased over 0–15 min; ° increased over 15–25 min; + increased over 25–40 min.

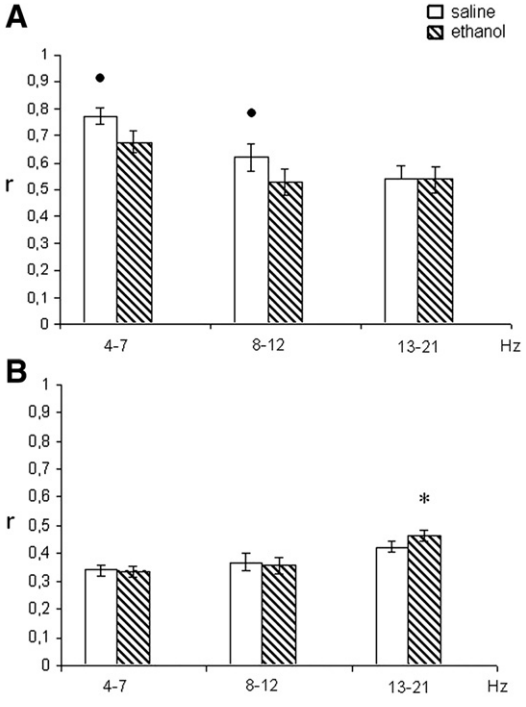

Fig. 5. Mean±S.E.M of the interaccumbens r (A) and left intra-hemispheric r (B) of the three frequency bands during the 15–25 min period after saline and 15–25 min period after forced ethanol administration. ● increased over ethanol administration; * increased over saline administration.

after ethanol administration as compared to the RP values obtained after saline administration in both the Acc and VTA (data not shown).

3.4.2. Interhemispheric correlation

The interaccumbens *r* of the 4–7 Hz band decreased during the 15–25 min period after acute ethanol administration as compared to the 15–25 min period after saline ($t=3.017$; $p<0.01$). Similarly, in the 8–12 Hz band, the 15–25 min period after acute ethanol administration showed a decreased *r* compared to the same period after saline administration ($t=2.948$; $p<0.01$). In the 13–21 Hz band, no changes in the interaccumbens *r* were observed (Fig. 5A).

The intertegmental *r* (between left and right VTA) showed no significant differences between the 15–25 min periods after saline and ethanol administration.

3.4.3. Intrahemispheric correlation

The only significant difference observed in both hemispheres was obtained in the 13–21 Hz band, where the 15–25 min period after acute ethanol administration ($t=-2.901$; $p<0.01$) showed an increased *r*, compared to the same period after saline administration (Fig. 5B).

4. Discussion

In male rats, ethanol produced clear differences in the proportion and correlation of the different frequency bands between the nucleus accumbens and ventral tegmental area during the entire 40 min period after its acute administration, thus demonstrating that the functionality of the dopaminergic mesoaccumbens system is sensitive to moderately intoxicating doses of ethanol.

Biochemical and pharmacological evidence suggests that the DA mesolimbic system plays a key role in mediating the reinforcing properties of alcohol and other drugs of abuse (Koob, 1992b). The reinforcing and arousal effects of alcohol have been postulated to be partially mediated by a neurobiological mechanism that involves the alcohol-induced elevation of DA in the Acc (Brodie et al., 1999; Di Chiara, 1997; Gil-Verona et al., 2003; Kaczmarek and Kiefer, 2000; Kiianmaa et al., 2003; Klemm, 1979; Slawecki, 2002; Tizabi et al., 2002; Weiss and Porrino, 2002; Yavich and Tiihonen, 2000). On the other hand, several studies have demonstrated the anxiolytic and sedative properties of alcohol in both humans (Grove and Cadoret, 1983; Sullivan et al., 2005) and animals (Annemoon et al., 2001; Varlinskaya et al., 2001; Wilson et al., 2004), which are mediated mainly through their interaction with the $GABA_A$ receptor (for a review, see Davies, 2003).

In this experiment, rats were exposed to forced ethanol administration. After the male rats received the i.p. ethanol injection (0.75 g/kg), the RP of the low theta frequencies (4–7 Hz) decreased while that of the fast frequencies (13–21 Hz) increased in both structures during the entire 40 min period after acute administration. This finding supports the results of previous studies that have shown that similar doses of alcohol induce an increase of low-voltage fast frequencies (Ehlers and Reed, 1987; Grupp, 1981; Perrin et al., 1975; Prado de Carvalho and Izquierdo, 1977; Wishaw, 1976). An EEG pattern of increased low-voltage fast frequencies has been traditionally associated with an activated state (Coult, 1998), so this elevated EEG activity has been considered as an index of a state of arousal in animals and humans. Similarly, numerous studies have described irregular, low-voltage fast activity (from 12 to over 40 Hz) in the cortex under various behavioral situations of increased alertness (Murthy and Fetz, 1992), or as a response to optimal sensory stimuli in animals and humans. Thus, the prevalence of fast frequencies in the Acc and the VTA indicates that after ethanol administration both structures are activated and this activation may be associated with the aroused state induced in subjects immediately after the administration of ethanol, as has been reported in other studies (Prado de Carvalho and Izquierdo, 1977; Wishaw, 1976). The overall effects after forced ethanol administration on relative power – decreasing low theta frequencies (4–7 Hz) and increasing fast frequencies (13–21 Hz) throughout the 40 min post-administration period – are consistent with other studies in which low doses of diazepam, a well-known benzodiazepine with anxiolytic effects, produced a similar increase of the fast frequencies in the parietal cortex of male rats (Ugalde et al., 1998). Diazepam, like alcohol, exerts its pharmacological effects through an allosteric modulatory site within the $GABA_A$ receptor and modulates GABA-ergic activity by affecting ligand binding and increasing the frequency of the opening of the GABA-dependent chloride channels (Greenblatt et al., 2000; Squires and Braestrup, 1977). Thus, these data could suggest two possible aspects: first, that the low theta frequencies are most sensitive to the effects of ethanol and, second, that it may be exerted by means of a similar neurochemical mechanism; i.e., through interaction with the $GABA_A$ receptors.

We hypothesized that the functioning and interactions of these structures would be distinct during the different time periods recorded immediately after forced ethanol administration; however, the EEG changes were similar during the entire 40 min post-ethanol period, indicating that the effects induced by this dose are accompanied by an immediate increased activation of the brain structures that constitute the dopaminergic mesoaccumbens system.

It is well documented that alcohol and benzodiazepines affect several processes by interacting with the membrane receptors coupled primarily to the $GABA_A$ receptor subtype (Davies, 2003; Squires and Braestrup, 1977). It is worth mentioning that both ethanol and diazepam produce similar actions on the EEG, such as an increased percentage of fast frequencies. It has been proposed that all agonistic modulators of the $GABA_A$ receptor (most of them with anxiolytic-like activity), including progesterone (Fernández-Guasti et al., 2003; Ugalde et al., 1998), induce a general enhancement of fast frequency EEG signals. Present data agree with this observation.

In this work, only the electrical activity of the shell region of the Acc was recorded and it was found that after the i.p. administration of ethanol, in addition to power spectral changes, forced ethanol administration decreased the interaccumbens *r* of the low theta frequencies without affecting the intertegmental *r*, such that a higher functional difference was observed between the left and right Acc, which was maintained during the 40 min period after the administration of ethanol. Considering that the shell region of the Acc plays an important role in motivo-emotional processes, it could be suggested that this temporal uncoupling in the low theta frequencies between the left and right Acc could be associated with the motivo-emotional experience associated with ethanol, which was clearly different from that obtained after the i.p. administration of saline.

This suggestion may be supported by other studies which have demonstrated that the theta rhythm in rats, with frequencies between 5 and 12 Hz, has been associated with motivo-emotional conditions in alert immobility (Kramis et al., 1975) as well as with pleasant behavioral conditions or "relaxed behaviors", including milk ingestion, grooming and nursing (Cervantes et al., 1992; Lincoln, 1980). A high proportion and interprefrontal *r* of the low theta frequencies has been found in lactating rats during the olfaction of nest-bedding (Hernández-González et al., 2005) as well as in sexually-motivated male rats while remained near to a receptive female (Hernández-González et al., 2007). The fact that in this study the interaccumbens *r* decreased after the ethanol administration supports the suggestion that ethanol changes the functionality of the Acc and VTA by increasing the proportion of fast frequencies and decreasing the coupling between the left and right Acc. These data taken together may represent a characteristic functionality of the mesoaccumbens system that could be associated with the motivo-emotional effects (probably anxiolytic) generated after ethanol administration, or with the activational effects that result from the processing of the significant and novel stimuli

(ethanol administration). This suggestion could be supported by studies that have suggested that Acc DA is involved in the behavioral activation produced by motivational stimuli of an appetitive as well as of an aversive character (Salamone, 1994).

The inter-tegmental correlation of the different frequency bands showed no changes, a finding that may be associated with the motor inactivity that subjects showed after ethanol administration. This suggestion is supported by the fact that the VTA has been associated with the adequate multisensory processing needed to perform the motor activity of voluntary acts (Mogenson et al., 1980).

Another interesting result was that the intrahemispheric correlation (between Acc and VTA) of the fast frequencies was increased in both the left and right hemispheres. This high coupling in the functioning of the Acc and VTA could be associated with the increase of DA levels that, as numerous studies have shown, occurs in those structures after ethanol administration. Although in this study the DA levels in these structures were not measured, there are numerous works in which one can find support for this suggestion (Di Chiara et al., 1998; Tizabi et al., 2002; Yoshimoto and McBride, 1991; Yoshimoto et al., 1991, 1996).

It has also been reported that the increase in the Acc DA levels occurs regardless of the route of alcohol administration (Yoshimoto and McBride, 1991; Yoshimoto et al., 1991, 1996), and that DA levels reach their peak about 20 min after acute administration in both the Acc and the VTA (Di Chiara et al., 1998; Tizabi et al., 2002), as well as in the central nucleus of the amygdala (Yoshimoto et al., 2000). It is believed that the mesolimbic DA system is intimately involved in mediating the self-administration of ethanol in animal models and that $GABA_A$ receptors play a role in mediating DA levels in this system. For example, the infusion of muscimol directly into the VTA results in a dose-dependent increase in DA levels (Kalivas et al., 1990).

Thus, for the purpose of determining if this increase in the coupling of the Acc and VTA could effectively be associated with ethanol administration (and probably with the increased DA levels) and not only with the anxiety provoked by the i.p. injection, we compared the effects of the saline administration with those of the ethanol administration. In general terms, the saline administration did not affect the relative power of the three frequency bands as compared to ethanol administration, however, it did decrease the interaccumbens r of the low frequencies and increased the intrahemispheric r of the fast frequencies in both hemispheres, specifically in the 15–25 min period after ethanol administration, the period during which other studies have shown that the highest levels of DA were reached.

In conclusion, the present results indicate that a moderate dose of ethanol induced a higher activation in the Acc and VTA, together with lower interaccumbens coupling and a high coupling of the intrahemispheric r between Acc and VTA. These data could be associated with the aroused state and the possible motivo-emotional effects (probably anxiolytic) generated by moderate doses of alcohol, a suggestion that could be supported by other studies that have shown correlation changes among mesolimbic structure that depend on the emotional/motivational state of the rats (Guevara et al., 2008; Hernández-González et al., 2005, 2007; Korzeniewska et al., 1997). Similarly, these data may support the hypothesis that the effects of ethanol are at least partially mediated by the different functionality of the dopaminergic mesoaccumbens system, which is mediated by different neurotransmitters.

There are, however, various points that should be considered: first, the activity in the Acc and the VTA does not reflect exclusively that of the DA neurons; moreover, the activity in the Acc and the VTA may not be related only to the release of DA, since it has also been reported that after alcohol administration, serotonin levels are increased in both Acc and the amygdala (Lovinger, 1997, 1999; Yoshimoto et al., 1991, 2000). Furthermore, other neurotransmitters, such as the cholinergic (Ericson et al., 1998; Soderpalm et al., 2000), GABAergic (Kalivas et al., 1990) and opioid systems, (Méndez et al., 2001; Ulm et al., 1995) may also influence the mesoaccumbens system or mediate their behavioral effects. Additional research, particularly the measurement of DA levels with simultaneous recording of EEG during and after ethanol administration, the pharmacological manipulation of different neurotransmitter systems involved in the functioning of the Acc and VTA, and simultaneous EEG recordings and correlation measures among other structures that receive projections from the VTA, will be required to further sustain the specific role of these areas in the motivo-emotional and activational states related to the effects of ethanol.

References

Annemoon MM, Erp V, Miczek KA. Persistent suppression of ethanol self-administration by brief social stress in rats and decreased startle responses as index of withdrawal. Physiol Behav 2001;73:301–11.

Berridge K, Robinson T. What is the role of dopamine in reward: hedonic impact, reward learning or incentive salience? Brain Res Rev 1998;28:309–69.

Brodie MS, Pesold C, Appel SB. Ethanol directly excites dopaminergic ventral tegmental area reward neurons. Alcoholism Clin Exp Res 1999;23:1848–52.

Cervantes M, Ruelas R, Alcalá V. EEG signs of "relaxation behavior" during breast-feeding in a nursing woman. Arch Med Res 1992;23:123–7.

Coult JT. Neural correlates of attention and arousal from electrophysiology, functional neuroimaging and psychopharmacology. Prog Neurobiol 1998;55:343–61.

Davies M. The role of GABAA receptors in mediating the effects of alcohol in the central nervous system. J Psychiatry Neurosci 2003;28:263–74.

Di Chiara G. The role of dopamine in drug abuse viewed from the perspective of its role in motivation. Drug Alcohol Depend 1995;38:95-137.

Di Chiara G. Alcohol and dopamine. Alcohol Health Res World 1997;21:108–14.

Di Chiara G, Acquas E, Carboni E. Drug motivation and abuse: a neurobiological perspective. In: Kalivas PW, Samson HH, editors. The neurobiology of drug and alcohol addiction. New York: New York Academy of Sciences; 1998. p. 207–19.

Ehlers CL, Reed TK. Ethanol effects on EEG spectra in monkeys: comparison to morphine and diazepam. Electroencephalogr Clin Neurophys 1987;66:317–21.

Ericson M, Blomqvist O, Engel JA, Soderpalm B. Voluntary ethanol intake in the rat and the associated accumbal dopamine overflow are blocked by ventral tegmental mecamylamine. Eur J Phharmacol 1998;358:189–96.

Fernández-Guasti A, del Río portilla IY, Ugalde E, Corsi-Cabrera M. Diazepam and progesterone produce sexually dimorphic actions on the rat EEG: role of the neonatal sexual differentiation process. Psychoneuroendocrinology 2003;28:85-100.

Gil-Verona JA, Pastor JF, De Paz F, Barbosa M, Macías-Fernández JA, Maniega MA, et al. Neurobiología de la adicción a las drogas de abuso. Rev Neurol 2003;36:361–5.

Greenblatt DJ, Harmatz JS, Von Moltke LL, Wright E, Durol ALB, Harrel-Joseph LM, et al. Comparative kinetics and response to the benzodiazepine agonists triazolam and zolpidem: evaluation of sex-dependent differences. J Pharmacol Exp Ther 2000;93:435–43.

Grove WM, Cadoret RJ. Genetic factors in alcoholism. In: Kissin B, Begleiter H, editors. The pathogenesis of alcoholism. Biological factors. New York: Plenum Press; 1983.

Grupp LA. The influence of some neurotransmitter agonists and antagonists on the response of hippocampal units and the cortical EEG to ethanol in the awake rat. Psychopharmacology 1981;75:327–34.

Guevara MA, Corsi-Cabrera M. EEG coherence or EEG correlation? Int J Psycophysiol 1996;23:145–53.

Guevara MA, Hernández-González M, Zarabozo D, Corsi-Cabrera M. POTENCOR: a program to calculate power and correlation spectra of EEG signals. Comp Meth Prog Biomed 2002;72:241–50.

Guevara MA, Martinez-Pelayo M, Arteaga Silva M, Bonilla-Jaime H, Hernández-González M. Electrophysiological correlates of the mesoaccumbens system during male rat sexual behaviour. Physiol Behav 2008, doi:10.1016/j.physbeh.2008.07.025.

Heimer L, Zahm DS, Churchill L, Salivas PW, Wohltmann C. Specificity in the projection patterns of accumbal core and shell in the rat. Neuroscience 1991;41:89-125.

Hernandez-González M, Guevara MA, Moralí M, Cervantes G. Computer programs to analyze brain electrical activity during copulatory pelvic thrusting in male rats. Physiol Behav 1997;62:701–8.

Hernández-González M, Prieto-Beracoechea CA, Navarro-Meza M, Guevara MA. Prefrontal and tegmental electrical activity during olfactory stimulation in virgin and lactating rats. Physiol Behav 2005;83:749–58.

Hernández-González M, Prieto-Beracoechea CA, Arteaga-Silva M, Guevara MA. Different functionality of the medial and orbital prefrontal cortex during a sexually motivated task in rats. Physiol Behav 2007;90:450–8.

Ikemoto S, Panksepp J. The role of nucleus accumbens dopamine in motivated behavior: a unifying interpretation with special reference to reward-seeking. Brain Res Rev 1999;31:6-41.

Kaczmarek HJ, Kiefer SW. Microinjections of dopaminergic agents in de nucleus accumbens affect ethanol consumption but not palatability. Pharmacol Biochem Behav 2000;66:307–12.

Kalivas PW, Duffy P, Eberhardt H. Modulation of A10 dopamine neurons by gamma-aminobutyric acid agonists. J Pharmacol Exp Ther 1990;253:858–66.

Kiianmaa K, Nurmi M, Nyanen I, Sinclair JD. Effect of ethanol on extracellular dopamine in the nucleus accumbens of alcohol-preferring AA and alcohol-avoiding ANA rats. Pharmacol Biochem Behav 1995;52:29–34.

Kiianmaa K, Hyytiä P, Samson HH, Angel JA, Svensson L, Söderpalm B, et al. New neuronal networks involved in ethanol reinforcement. Alcoholism Clin Exp Res 2003;27:209–19.

Klemm WR. Effects of ethanol on nerve impulse activity. In: Majchrowic E, Noble EP, editors. Biochemistry and pharmacology of ethanol, vol. 2. New York: Plenum Press; 1979. p. 243–67.
Koob GF. Drug of abuse: anatomy, pharmacology and function of reward pathway. Trends Pharmacol Sci 1992a;13:177–84.
Koob GF. Neural mechanisms of drug reinforcement. Ann NY Acad Sci 1992b;654:171–91.
Koob GF, Roberts AJ, Schulteis G, Parson LH, Heyser CJ, Hyytia P, et al. Neurocircuitry targets in ethanol reward and dependence. Alcohol Clin Exp Res 1998;22:3–9.
Korzeniewska A, Kasicki S, Zagrodzka J. Electrophysiological correlates of the limbic-motor interactions in various behavioral states in rats. Behav Brain Res 1997;87:69–83.
Kramis R, Vanderwolf CH, Bland BH. Two types of hippocampal rhythmical slow activity in both the rabbit and the rat: relations to behavior and effects of atropine, diethyl ether, urethane, and pentobarbital. Exp Neurol 1975;49:58–85.
Lincoln DW. Sleep: a prerequisite for reflex milk ejection in the rat. Exp Brain Res 1980;38:151–62.
Lopes da Silva F. Neural mechanisms underlying brain waves: from neural membranes to networks. Electrenceph Clin Neurophysiol 1991;79:81–93.
Lovinger DM. Serotonin's role in alcohol's effects on the brain. Alcohol Health and research World 1997;21:114–9.
Lovinger DM. 5-HT_3 receptors and the neural actions of alcohol: an increasingly exciting topic. Neurochem Internat 1999;35:125–30.
Méndez M, Leriche M, Calva JC. Acute ethanol administration differentially modulates μ opioid receptors in the rat meso-accumbens and mesocortical pathways. Mol Brain Res 2001;94:148–56.
Mogenson GJ, Jones DL, Yim CY. From motivation to action: functional interface between the limbic system and the motor system. Prog Neurobiol 1980;14:69–97.
Murthy VN, Fetz EE. Coherent 25 to 35 Hz oscillations in the sensorimotor cortex of awake behaving monkeys. Proc Natl Acad Sci 1992;89:5670–4.
Paxinos G, Watson C. The rat brain in stereotaxic coordinates. 2nd ed. California: Academic Press; 1997.
Perrin RG, Kalant H, Livingston KE. Acute effects of ethanol on spontaneous and auditory evoked electrical activity in cat brain. Electroencephalogr Clin Neurophysiol 1975;39:157–62.
Prado de Carvalho L, Izquierdo I. Changes in the frequency of electroencephalographic rhythms of the rat caused by single intraperitoneal injections of ethanol. Arch Int Pharmacodyn 1977;229:157–62.
Salamone JD. The involvement of nucleus accumbens dopamine in appetitive and aversive motivation. Behav Brain Res 1994;61:117–33.
Slawecki CJ. Altered EEG responses to ethanol in adult rats exposed to ethanol during adolescence. Alcoholism Clin Exp Res 2002;26:246–54.
Shaw JC. Correlation and coherence analysis of the EEG. A tutorial review. Int J Psychophysiol 1984;1:255–66.
Soderpalm B, Ericson M, Olausson P, Blomqvist O, Engel JA. Nicotinic mechanisms involved in the dopamine activating and reinforcing properties of ethanol. Behav Brain Res 2000;113:85–96.
Squires RF, Braestrup C. Benzodiazepine receptors in rat brain. Nature 1977;166:732–4.
Sullivan LE, Fiellin DA, O'Connor PG. The prevalence and impact of alcohol problems in major depression: a systematic review. Am J Med 2005;118:330–41.
Tizabi Y, Copeland Jr RL, Louis VA, Taylor RE. Effects of combined systemic alcohol and central nicotine administration into ventral tegmental area on dopamine release in the nucleus accumbens. Alcoholism Clin Exp Res 2002;26:304–99.
Ugalde E, del Río Portilla IY, Juárez J, Roldán A, Guevara MA, Corsi-Cabrera M. Effect of diazepam and sex hormones on EEG of gonadectomized male and female rats. Psychoneuroendocrinology 1998;23:701–12.
Ulm RR, Volpicelli JR, Volpicelli LA. Opiates and alcohol self-administration in animals. J Clin Psychiatry 1995;56(Suppl. 7):5-14.
Vanderwolf C. Hippocampal electrical activity and voluntary movement in the rat. Electroenceph Clin Neurophysiol 1969;26:407–18.
Vanderwolf C. An introduction to the electrical activity of the cerebral cortex: relations to behavior and control by subcortical inputs. In: Kolb B, Tees RC, editors. The cerebral cortex of the rat. Cambridge: The MIT Press; 1990. p. 151–89.
Varlinskaya EI, Spear LP, Spear NE. Acute effects of ethanol on behavior of adolescent rats: role of social context. Alcoholism Clin Exp Res 2001;25:377–85.
Wilson MA, Burghardt PR, Ford KA, Wilkinson MB, Primeaux SD. Anxiolytic effects of diazepam and ethanol in two behavioral models: comparison of males and females. Pharm Biochem Behav 2004;78:445–58.
Weiss F, Porrino LJ. Behavioral neurobiology of alcohol addiction: recent advances and challenges. J Neurosci 2002;22:3332–7.
Wishaw IQ. The effects of alcohol and atropine on EEG and behavior in the rabbit. Psychopharmacologia 1976;48:83–90.
Wise RA. Drug-activation of brain reward pathways. Drug Alcohol Depend 1998;51:13–22.
Yavich L, Tiihonen J. Ethanol modulates evoked dopamine release in mouse nucleus accumbens: dependence on social stress and dose. Eur J Pharmacol 2000;401:365–73.
Yoshimoto K, McBride WJ. Regulation of nucleus accumbens dopamine release by the dorsal raphe nucleus in the rat. Neurochem Res 1991;17:401–7.
Yoshimoto K, McBride WJ, Lumeng L, Li TK. Alcohol stimulates the release of dopamine and serotonin in the nucleus accumbens. Alcohol 1991;9:17–22.
Yoshimoto K, Yayama K, Ogata M, Nishimura A, Yoshida T, Ueda S, et al. Possibility of 5-HT3 receptor involvement in alcohol dependence: a microdialysis study of nucleus accumbens dopamine and serotonin release in rats with chronic alcohol consumption. Alcoholism Clin Exp Res 1996;20:311A–9A.
Yoshimoto K, Ueda S, Kato B, Takeuchi Y, Hawai Y, Noritake K, et al. Alcohol enhances characteristic releases of dopamine and serotonin in the central nucleus of the amygdala. Neurochem Int 2000;37:369–76.