

# Effects of chronic ethanol treatment on monoamine levels in rat hippocampus and striatum

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## Abstract

We studied the effects of ethanol on concentrations of noradrenaline (NE), dopamine (DA) and serotonin (5-HT) and their metabolites in rat hippocampus and striatum. Ethanol (2 or 4 g/kg, *po*, from a 20% aqueous solution) was administered daily to male Wistar rats (4-13 per group) for 30 days and animals were sacrificed 30 min or 48 h after the last administration. Monoamines were measured by HPLC and considered significant at  $P < 0.05$ . A 47% increase in 5-HT levels was observed in the hippocampus with 4 g/kg ethanol in the 30-min protocol. Ethanol (2 and 4 g/kg) decreased DA ( $2114.5 \pm 126.4$  and  $1785.1 \pm 234.2$  ng/g wet tissue, respectively) and 3,4-dihydroxyphenylacetic acid (DOPAC,  $1477.6 \pm 132.1$  and  $1218.8 \pm 271.7$  ng/g wet tissue, respectively) levels, while the higher dose also decreased NE ( $159.8 \pm 13.5$ ), 5-HT ( $228.0 \pm 46.8$ ) and 5-hydroxy-3-indoleacetic acid (5-HIAA,  $304.4 \pm 37.2$  ng/g wet tissue), in the striatum after a 48-h withdrawal as compared to controls (DA:  $3063.9 \pm 321.3$ ; DOPAC:  $2379.6 \pm 256.0$ ; NE:  $292.8 \pm 50.2$ ; 5-HT:  $412.4 \pm 36.2$ ; 5-HIAA:  $703.9 \pm 61.4$  ng/g wet tissue). In the 30-min protocol, ethanol (2 or 4 g/kg) decreased striatal NE (66 and 70%) and DA (50 and 36%) levels. On the other hand, increases were seen in 5-HIAA (146 and 153%) and 5-HT (59 and 86%) levels. Ethanol (2 g/kg, *po*) increased the homovanillic acid (HVA)/DA ratio (129%) in the striatum in the 30-min protocol, while at the higher dose it increased the HVA/DA ratio in the 48-h protocol (61%). These results indicate alterations in monoamines, mainly in the striatum, after chronic ethanol, which are influenced by dose and by the length of time after the last drug administration.

## Key words

- Hippocampus
- Striatum
- Ethanol
- Noradrenaline
- Dopamine
- Serotonin

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## Introduction

Acute or chronic ethanol ingestion has been shown to induce significant changes in neurotransmitter systems (1-3). Among these, dopamine (DA) and 5-hydroxytryptamine (5-HT) have received special attention because

of their putative role in the motivational effects of ethanol (4-6). Administration of ethanol induces DA release (1,7,8) in the caudate nucleus and nucleus accumbens of freely moving rats, but the mechanisms responsible for this action remain to be defined (9).

Several investigators have reported

changes in the levels of 5-HT and 5-hydroxy-3-indoleacetic acid (5-HIAA), its main metabolite, in the nucleus accumbens, hippocampus, and striatum (10,11). Clinical studies indicate that 5-HT re-uptake inhibitors exhibit some efficacy in reducing alcoholism (12). The site of action of the 5-HT re-uptake inhibitors is unknown, but it is possible that these agents act somewhere in central reward pathways to modify the action of ethanol (13). Ethanol is also known to affect the release of noradrenaline, but this effect is probably dependent on the duration of the drug intake (14).

Although some studies have investigated changes in the levels of DA, 5-HT, norepinephrine (NE), and their metabolites in several regions of the rodent brain, many of them involved ethanol treatment for a short period of time and withdrawal (15,16). Thus, in the present study we investigated the effects of repeated and prolonged ethanol administration (30 days) at two doses (2 and 4 g/kg, *po*) and at 30 min and 48 h after drug withdrawal, on the levels of NE, DA, 5-HT and their metabolites in the rat hippocampus and striatum.

## Material and Methods

### Animals

Male Wistar rats (150-200 g) from the animal house of the Federal University of Ceará, Fortaleza, CE, Brazil, were used. Animals had free access to a commercial diet (Purina, Campinas, SP, Brazil) and to water and were housed in groups of 4-5 per cage in a room with a 10- to 14-h on and off lighting schedule (lights on at 7:00 am). Experiments were performed according to the Guide for the Care and Use of laboratory animals of the US Department of Health and Human Services.

### Drugs

Sodium octanesulfonic acid, acetonitrile,

tetrahydrofuran, NE, DA, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-HT, and 5-HIAA were purchased from Sigma, St. Louis, MO, USA. All other drugs were of analytical or HPLC grade.

### Treatment

A 20% ethanol solution was diluted in distilled water and administered in volumes of 1 ml/kg body weight. Rats were divided into groups of 4 to 13 animals each and treated daily by gavage with a 20% aqueous solution of ethanol (2 or 4 g/kg, *po*) at noon for 30 days and sacrificed 30 min or 48 h after the last administration for monoamine determination. Controls received an equivalent volume of distilled water.

### Chemical analyses

Animals were decapitated, and the hippocampus and striatum dissected according to methodology previously described (17) for the preparation of 10% homogenates. Brain tissue samples were sonicated in 0.5-1 ml of 0.1 M HClO<sub>4</sub> for 30 s, and centrifuged for 15 min at 26,000 g, 4°C. Then, a 20- $\mu$ l supernatant aliquot was injected directly into the HPLC column. For monoamine analyses, a CLC-ODS(M) Shimadzu column (Kyoto, Japan) was used. The mobile phase was 0.163 M citric acid, pH 3.0, containing 0.02 mM EDTA with 0.69 mM sodium octanesulfonic acid as an ion-pairing reagent, 4% (v/v) acetonitrile and 1.7% (v/v) tetrahydrofuran. NE, DA, DOPAC, 5-HT, 5-HIAA, and HVA were electrochemically detected using an amperometric detector (Model L-ECD-6A, Shimadzu), by oxidation on a glass carbon electrode at 0.85 V in relation to an Ag-AgCl reference electrode. The amounts of neurotransmitters and metabolites in the supernatants were calculated by comparing their elution times and peak heights with those of standards. Results are reported as ng/g wet tissue.

## Statistical analysis

Data are reported as means  $\pm$  SEM. For data regarding monoamine determinations, one-way ANOVA and the Tukey test (as a *post hoc* test, which is based on the Studentized range distribution) were used for comparing results among treatments. The statistical package GraphPad Software, Inc. (San Diego, CA, USA) was used. The level of significance was set at  $P < 0.05$  in all analyses.

## Results

### Hippocampus

Table 1 shows the effects of ethanol (2 and 4 g/kg, *po*, for 30 days), 30 min and 48 h after its last administration, on monoamine and metabolite concentrations in the rat hippocampus. There were no changes in NE, DA, DOPAC, or HVA levels after ethanol treatment in either experimental protocol (30 min or 48 h). A significant change was observed in 5-HT levels (an increase of about 47%) with the higher dose compared to control and to the lower ethanol dose [ $F(2,9) = 7.041$ ,  $P = 0.0144$ ] in the 30-min protocol. After 48 h of withdrawal, although the ethanol-treated groups were not different from controls, there was a 42% decrease in 5-

HIAA [ $F(2,16) = 5.495$ ,  $P = 0.0153$ ] as well as in 5-HT [ $F(2,15) = 3.417$ ,  $P = 0.0599$ ] levels in the group treated with the 4 g/kg dose compared to the group treated with the 2 g/kg dose.

### Striatum

Table 2 shows the effects of ethanol (2 and 4 g/kg, *po*, for 30 days), 30 min and 48 h after the last administration, on the concentrations of monoamines and their metabolites in the rat striatum. There was a significant decrease in NE (66 and 70%) and DA (50 and 36%) levels with the doses of 2 and 4 g/kg ethanol, respectively, 30 min after the last administration [NE:  $F(2,15) = 10.413$ ,  $P = 0.0015$ ; DA:  $F(2,17) = 7.020$ ,  $P = 0.0060$ ], as well as in DOPAC levels (42% decrease) with the higher dose under the same conditions [ $F(2,19) = 3.790$ ,  $P = 0.0412$ ] compared to controls. In contrast, increases of 146 and 153%, and 59 and 86% were observed in 5-HIAA [ $F(2,21) = 40.352$ ,  $P < 0.0001$ ] and 5-HT [ $F(2,19) = 12.556$ ,  $P = 0.0003$ ] levels, with the doses of 2 and 4 g/kg, respectively, compared to control under the same conditions. After 48 h of withdrawal, ethanol at the lower dose decreased DA and DOPAC levels by 31 and 38%, respectively. The higher dose induced a broad spectrum of changes, decreasing NE (45%), DA

Table 1. Effects of chronic ethanol treatment on concentrations of monoamines and metabolites in the rat hippocampus.

	NE	DA	DOPAC	HVA	5-HT	5-HIAA
30 min						
Control	233.6 $\pm$ 39.7 (4)	51.6 $\pm$ 6.2 (4)	37.4 $\pm$ 3.3 (4)	105.2 $\pm$ 7.9 (4)	244.9 $\pm$ 19.3 (4)	573.5 $\pm$ 68.1 (4)
Ethanol 2	177.9 $\pm$ 16.7 (4)	47.5 $\pm$ 5.1 (4)	36.9 $\pm$ 3.4 (4)	103.8 $\pm$ 7.2 (4)	244.5 $\pm$ 26.1 (4)	653.6 $\pm$ 54.2 (4)
Ethanol 4	276.7 $\pm$ 25.1 (4)	66.3 $\pm$ 13.9 (4)	61.6 $\pm$ 11.2 (5)	106.1 $\pm$ 7.4 (5)	361.2 $\pm$ 29.4 (4) <sup>a,b</sup>	566.5 $\pm$ 41.3 (5)
48 h						
Control	243.0 $\pm$ 36.3 (6)	63.7 $\pm$ 10.1 (6)	39.9 $\pm$ 8.7 (6)	124.5 $\pm$ 23.9 (6)	213.5 $\pm$ 29.4 (7)	533.5 $\pm$ 58.0 (7)
Ethanol 2	194.3 $\pm$ 24.0 (6)	71.7 $\pm$ 17.9 (6)	40.4 $\pm$ 8.2 (7)	136.0 $\pm$ 23.3 (6)	340.1 $\pm$ 61.6 (6)	691.7 $\pm$ 70.5 (6)
Ethanol 4	235.0 $\pm$ 20.1 (7)	77.5 $\pm$ 16.3 (6)	30.3 $\pm$ 4.3 (5)	151.6 $\pm$ 25.8 (6)	197.6 $\pm$ 18.7 (5)	400.0 $\pm$ 52.2 (6) <sup>b</sup>

NE = norepinephrine; DA = dopamine; DOPAC = 3,4-dihydroxyphenylacetic acid; HVA = homovanillic acid; 5-HT = serotonin; 5-HIAA = 5-hydroxy-3-indoleacetic acid. Data are reported as mean  $\pm$  SEM ng/g wet tissue in the hippocampus from rats treated daily with ethanol (2 or 4 g/kg, *po*) for 30 days and sacrificed 30 min or 48 h after the last administration. The numbers in parentheses indicate the number of rats in each group.

<sup>a</sup> $P < 0.05$  compared to controls (30 min or 48 h) and <sup>b</sup> $P < 0.05$  compared to ethanol-2 (ANOVA followed by the *post hoc* Tukey test).

(42%), DOPAC (49%), 5-HIAA (57%), and 5-HT (45%) [NE:  $F(2,16) = 3.868$ ,  $P = 0.0426$ ; DA:  $F(2,20) = 7.800$ ,  $P = 0.0031$ ; DOPAC:  $F(2,21) = 6.469$ ,  $P = 0.0065$ ; 5-HT:  $F(2,21) = 6.299$ ,  $P = 0.0072$ ; 5-HIAA:  $F(2,22) = 8.368$ ,  $P = 0.0020$ ] compared to control. However, ethanol administration did not alter HVA levels [ $F(2,20) = 0.1386$ ,  $P = 0.8715$ ].

Figure 1 presents the effects of ethanol (2 and 4 g/kg, *po*, for 30 days) 30 min and 48 h after the last administration on the DOPAC/DA, HVA/DA and 5-HIAA/5-HT ratios in the rat striatum. There was a significant in-

crease (129%) in the HVA/DA ratio ( $0.94 \pm 0.16$  ng/g wet tissue) in the striatum in the 30-min protocol [ $F(2,17) = 6.914$ ;  $P = 0.0063$ ] with the dose of 2 g/kg ethanol, while the higher dose increased by 61% the HVA/DA ratio ( $0.61 \pm 0.06$  ng/g wet tissue) after 48 h of withdrawal [ $F(2,16) = 5.141$ ;  $P = 0.0189$ ] compared to control (30 min =  $0.41 \pm 0.07$ ; 48 h =  $0.38 \pm 0.03$  ng/g wet tissue). A tendency to a decrease in the 5-HIAA/5-HT ratio was also observed in the hippocampus at the higher dose of alcohol at 30 min and 48 h (data not shown).

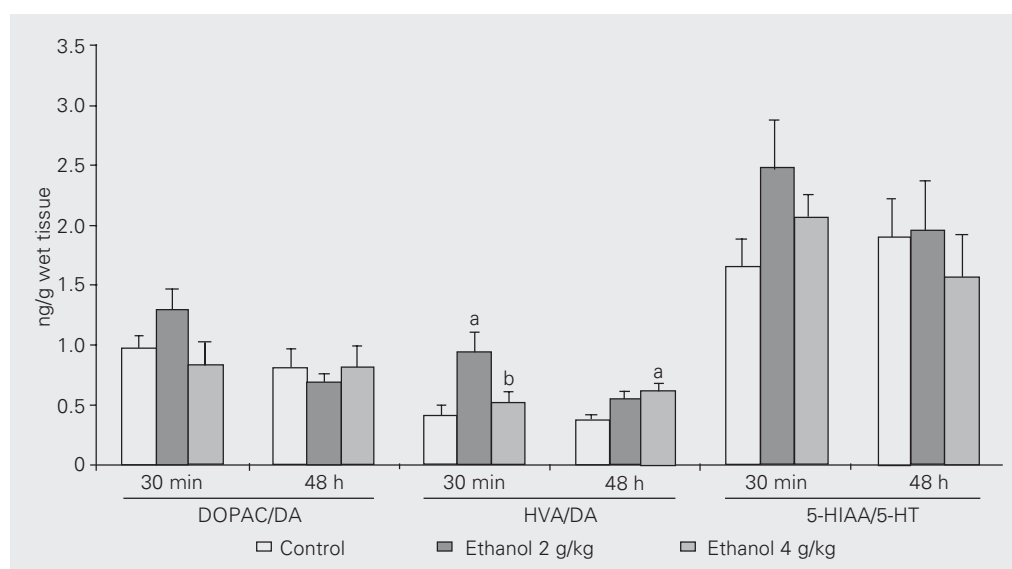
Table 2. Effects of chronic ethanol treatment on the concentrations of monoamines and metabolites in the rat striatum.

	NE	DA	DOPAC	HVA	5-HT	5-HIAA
30 min						
Control	291.8 ± 44.5 (8)	3133.5 ± 375.2 (9)	2645.6 ± 278.9 (12)	1106.6 ± 85.6 (11)	402.1 ± 33.6 (11)	604.9 ± 44.4 (13)
Ethanol 2	98.9 ± 19.4 (5) <sup>a</sup>	1560.9 ± 144.8 (5) <sup>a</sup>	1998.8 ± 234.2 (5)	1391.4 ± 119.7 (5)	637.9 ± 95.1 (5) <sup>a</sup>	1488.1 ± 149.2 (5) <sup>a</sup>
Ethanol 4	87.5 ± 19.6 (5) <sup>a</sup>	2001.9 ± 154.5 (6) <sup>a</sup>	1527.8 ± 131.2 (5) <sup>a</sup>	994.2 ± 124.9 (6)	746.8 ± 51.9 (6) <sup>a</sup>	1527.8 ± 131.2 (6) <sup>a</sup>
48 h						
Control	292.8 ± 50.2 (7)	3063.9 ± 321.3 (7)	2379.6 ± 256.0 (11)	1088.3 ± 88.1 (10)	412.4 ± 36.2 (10)	703.9 ± 61.4 (12)
Ethanol 2	204.9 ± 20.5 (6)	2114.5 ± 126.4 (8) <sup>a</sup>	1477.6 ± 132.1 (6) <sup>a</sup>	1182.4 ± 125.2 (6)	310.6 ± 25.8 (8)	540.0 ± 85.2 (7)
Ethanol 4	159.8 ± 13.5 (6) <sup>a</sup>	1785.1 ± 234.2 (8) <sup>a</sup>	1218.8 ± 271.7 (7) <sup>a</sup>	1119.7 ± 192.7 (6)	228.0 ± 46.8 (6) <sup>a</sup>	304.4 ± 37.2 (6) <sup>a</sup>

NE = norepinephrine; DA = dopamine; DOPAC = 3,4-dihydroxyphenylacetic acid; HVA = homovanillic acid; 5-HT = serotonin; 5-HIAA = 5-hydroxy-3-indoleacetic acid. Data are reported as mean ± SEM ng/g wet tissue in the striatum from rats treated daily with ethanol (2 or 4 g/kg, *po*) for 30 days and sacrificed 30 min or 48 h after the last administration. The numbers in parentheses indicate the number of rats in each group.

<sup>a</sup> $P < 0.05$  compared to controls (30 min or 48 h; ANOVA followed by the *post hoc* Tukey test).

Figure 1. Effects of ethanol treatment on the DOPAC/DA, HVA/DA and 5-HIAA/5-HT ratios in the rat striatum determined 30 min or 48 h after the last drug administration. Measurements were performed in 5-11 areas of each group. N = 5-9 (30-min protocol) and 6-7 (48-h protocol) for DOPAC/DA, N = 5-9 (30-min protocol) and 6-7 (48-h protocol) for HVA/DA, and N = 5-11 (30-min protocol) and 6-10 (48-h protocol) for 5-HIAA/5-HT. DOPAC = 3,4-dihydroxyphenylacetic acid; DA = dopamine; HVA = homovanillic acid; 5-HIAA = 5-hydroxy-3-indoleacetic acid; 5-HT = serotonin. <sup>a</sup> $P < 0.05$  compared to controls (30 min or 48 h) and <sup>b</sup> $P < 0.05$  compared to 2 g/kg ethanol (Ethanol 2), (ANOVA followed by the *post hoc* Tukey test).



## Discussion

In the present study, we showed that rats chronically exposed to ethanol presented significant alterations in the levels of monoamines and metabolites in both the hippocampus and striatum. Although, NE, DA, DOPAC, and HVA levels were unchanged in the hippocampus, an increase was observed in 5-HT levels after ethanol treatment 30 min after the last administration and a small but significant decrease in 5-HIAA levels was detected with the high dose compared with the lower one after 48 h of withdrawal. These results suggest that ethanol altered 5-HT levels in the hippocampus after chronic treatment in the 30-min protocol, levels returning to normal after 48 h.

Our results agree with those of Bare et al. (18) who showed that acute intraperitoneal injection of a high ethanol dose (2.5 g/kg) significantly increased the extracellular concentration of 5-HT in the rat hippocampus to a maximum of approximately 180% of baseline values, within 50 min. Thereafter, the levels of 5-HT began to return to baseline. However, this dose had no effect on the extracellular levels of 5-HT if the rat received a single dose of ethanol (2.5 g/kg) 24 h earlier, suggesting in this case the development of tolerance. A lower ethanol dose (1 g/kg) had no effect. Others (19) have shown that the 2.5 g/kg ethanol dose significantly increased the extracellular levels of 5-HT in the hippocampus from ethanol preferring rats pre-treated with the same dose of ethanol 18 to 24 h earlier. It is widely accepted that the ventral hippocampus receives a major 5-HT input from the median raphe nucleus, and the activation of this pathway by acute ethanol administration may be followed by the development of a rapid tolerance process. Recently (20), it was shown that rats receiving 50% ethanol administered subcutaneously for 14 days presented a significant increase in the rate of 5-HT synthesis in several brain areas in nigrostriatal struc-

tures and in the hippocampus.

Uzbay et al. (11) observed that, after chronic ethanol (7.2% v/v) ingestion, 5-HIAA levels in the hippocampus of Wistar rats were increased after 10 days of exposure, and these levels returned to control values after 21 days of ethanol consumption. Earlier work using an experimental protocol similar to ours (21) has shown that 5-HIAA levels were significantly reduced in the hippocampus after 2 h of ethanol withdrawal. These results suggest that serotonergic functioning is altered in the hippocampus and that the effects of ethanol depend on the duration of treatment, dose, and time of withdrawal.

The present study indicated that major alterations occurred in the striatum, where ethanol significantly decreased DA and DOPAC levels at both doses after chronic treatment, 30 min or 48 h after drug discontinuation. These effects could be related to the higher dopaminergic innervation in the striatum compared to the hippocampus. Our results agree with those of Gil et al. (22) who showed noticeable decreases in striatal levels of DA and DOPAC in rats submitted to a 40-day ethanol treatment, 24 h after withdrawal. Similarly, Bailey et al. (23) observed decreases in the levels of dopamine and its metabolites in the striatum during the withdrawal phase, after 24 h of chronic ethanol administration. However, they did not observe any alteration in the striatum 6 days or 2 months after withdrawal.

Duration of treatment seems to be important regarding changes in monoamine levels after exposure to ethanol. Microdialysis experiments in rodents indicate that ethanol promotes DA release predominantly in the nucleus accumbens, a phenomenon implicated in the reinforcing effect of the drug. In humans, alcohol also promotes DA release, with a preferential effect on the ventral striatum (24). Recently (25), it was reported that the application of ethanol to the nucleus accumbens temporarily increased DA levels

in a dose-dependent manner. We also showed that ethanol (4 g/kg, *po*) after acute or repeated administration for 7 days increased DA levels in the striatum 30 min or 48 h after drug administration (17,26). Other data obtained with short-term ethanol treatment (16) showed that DA and 5-HT levels were increased in the central amygdaloid nucleus dialysate within 20 min of ethanol administration (2 g/kg, *ip*).

Budygin et al. (27) showed that ethanol exerts a profound effect on DA neurons, resulting in a suppression of DA neurotransmission in the striatum at high doses (around 5 g/kg). Others (28) demonstrated that DA and DOPAC levels are significantly decreased in the striatum of rats chronically receiving alcohol. Although these effects suggest that DA and DOPAC levels were depleted after a long ethanol treatment, the literature is not conclusive about changes occurring in HVA levels, with some investigators reporting an increase (21) or a decrease (22) in HVA levels.

These discrepancies may be related to different experimental procedures, such as the period of exposure to ethanol, dose, animal strain, and observation intervals after drug administration. Boone et al. (29) and Devaud et al. (30) observed strain and sex differences in the monoamine response to acute doses of ethanol in several mouse brain areas, including the nucleus accumbens and caudate putamen. These reports showed increased levels of DA and DOPAC in some strains, while no changes were detected in others. Similar effects were observed for serotonergic activity.

We also showed that ethanol treatment increased (30-min protocol) and decreased (48-h protocol) 5-HT and 5-HIAA levels in the striatum. These results show that chronic ethanol administration altered the serotonergic system and that the effects were time dependent. Uzbay et al. (21) observed a decrease in 5-HT levels during the first 6 h of withdrawal, suggesting that the decrease in

serotonergic activity might be involved in the early phase of ethanol withdrawal. Several lines of evidence (10,31-33) also showed reduced 5-HT and 5-HIAA levels in rodent whole brain, as well as in limbic and striatal tissue preparations after ethanol withdrawal. Recently, Berggren et al. (34) observed a negative correlation between prolonged and excessive alcohol consumption and central serotonergic neurotransmission due to a toxic effect of alcohol on 5-HT neurons.

The present investigation demonstrated that the HVA/DA ratio was increased by ethanol while the DOPAC/DA ratio was unchanged in the rat striatum. Furthermore, several studies have indicated that dopamine turnover is increased among abstinent alcoholics (35) and alcohol-induced stimulation of dopaminergic neurotransmission may encode the reinforcing properties of alcohol consumption. We also observed a tendency to a decrease in 5-HIAA/5-HT ratio in the hippocampus at the higher ethanol dose in both protocols. Serotonergic dysfunction has been associated with behavior disinhibition and negative mood states that may predispose to excessive alcohol intake (35). In addition, chronic ethanol consumption has been shown to decrease the content of DA and its metabolites and of 5-HIAA in striatal tissue 24 h after cessation of ethanol intake (23), indicating the occurrence of changes in monoamine turnover. Other investigators (36) observed an increase in the 5-HIAA/5-HT ratio in brain areas, including the striatum, of aged rats.

We also demonstrated significant changes in NE levels in the striatum after repeated administration of the higher ethanol dose and a 48-h withdrawal. Rossetti et al. (37) found a biphasic effect on NE release in the frontal cortex and, while a low ethanol dose (0.2 g/kg) increased NE outflow, a higher one (2 g/kg) inhibited it. The authors suggested that the decrease in cortical NE output might reflect the sedative-hypnotic properties of ethanol at high doses, whereas the

increased NE release may represent a biochemical correlate of the arousal and increased alertness elicited by low doses of ethanol.

Our data showed that after chronic ethanol treatment and 30 min after the last administration, the changes in NE, DA and

DOPAC levels observed in the striatum were similar and in the same direction as those observed after 48 h of drug withdrawal. However, in this condition, where significant decreases were detected in 5-HIAA and 5-HT levels, the reverse was seen 30 min after ethanol administration.

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