

Effect of chronic ethanol exposure on rat ventilatory responses to hypoxia and hypercapnia

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OBJECTIVE: The effect of chronic ethanol exposure on chemoreflexes has not been extensively studied in experimental animals. Therefore, this study tested the hypothesis that known ethanol-induced autonomic, neuroendocrine and cardiovascular changes coincide with increased chemoreflex sensitivity, as indicated by increased ventilatory responses to hypoxia and hypercapnia.

METHODS: Male Wistar rats were subjected to increasing ethanol concentrations in their drinking water (first week: 5% v/v, second week: 10% v/v, third and fourth weeks: 20% v/v). At the end of each week of ethanol exposure, ventilatory parameters were measured under basal conditions and in response to hypoxia (evaluation of peripheral chemoreflex sensitivity) and hypercapnia (evaluation of central chemoreflex sensitivity).

RESULTS: Decreased respiratory frequency was observed in rats exposed to ethanol from the first until the fourth week, whereas minute ventilation remained unchanged. Moreover, we observed an increased tidal volume in the second through the fourth week of exposure. The minute ventilation responses to hypoxia were attenuated in the first through the third week but remained unchanged during the last week. The respiratory frequency responses to hypoxia in ethanol-exposed rats were attenuated in the second through the third week but remained unchanged in the first and fourth weeks. There was no significant change in tidal volume responses to hypoxia. With regard to hypercapnic responses, no significant changes in ventilatory parameters were observed.

CONCLUSIONS: Our data are consistent with the notion that chronic ethanol exposure does not increase peripheral or central chemoreflex sensitivity.

KEYWORDS: Ethanol; Chemoreflex; Hypercapnia; Hypoxia.

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INTRODUCTION

Evidence in the literature has shown that ethanol (ETOH) exposure stimulates changes in the neuroendocrine and autonomic nervous systems (1-3). The characterized autonomic nervous system changes include an enhanced cardiac sympathetic baroreflex and a reduced cardiac parasympathetic baroreflex (2,4). In addition, diminished baroreflex control, which is a sympathoinhibitory reflex, may be involved in increased arterial pressure (2), as observed in experimental animals and humans upon ETOH exposure

(2,3,5-10). Moreover, relationships between ETOH consumption and increased mortality have been reported (11,12).

Respiratory reflexes are related to cardiac autonomic control (13). Therefore, the peripheral-chemoreflex (triggered by hypoxia) and central-chemoreflex (triggered by hypercapnia), which are sympathoexcitatory reflexes, may be increased following ETOH consumption. The effect of acute, but not chronic, ETOH exposure on chemoreflexes has been evaluated by several studies in humans (14,15), which demonstrated that acute ETOH intake did not alter the ventilatory response to hypoxia (15) or hypercapnia (14,15). Although there is evidence that ETOH consumption has acute effects on chemoreflexes in humans (14,15), the effect of chronic ETOH exposure on the peripheral and central chemoreflexes of experimental animals has never been assessed.

We hypothesized that chronic ETOH exposure increases peripheral and central chemoreflex sensitivity. To test this

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hypothesis, we established a time course of changes in chemoreflex sensitivity during four weeks of ETOH exposure.

MATERIALS AND METHODS

ETOH exposure

Experiments were performed using male Wistar rats (250 to 300 g, $n = 24$). The rats were collectively housed (4 rats per cage) in plastic cages, had free access to food and water and were maintained with a 12:12 h light-dark cycle. All experimental procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals [Dept. of Health, Education and Welfare, Publication No. (NIH) 85-23, Revised 1985; Office of Science and Health Reports, DRR/NIH, Bethesda, MD]. The experimental protocols used in this study were approved by the Local Committee of Ethics in Animal Research of the School of Medicine of Ribeirão Preto, University of São Paulo (protocol 065/2009). After habituation for 7 days, the rats were randomly divided into two groups: the first group received tap water (control group), and the second group received an increasing concentration (v/v) of ETOH (Labsynth, Diadema, SP, Brazil) in their drinking water for four weeks (first week: 5%, second week: 10%, third and fourth weeks: 20%). The ETOH doses tested in preliminary experiments were based on previously published data (16), and those that produced consistent, repeatable responses were adopted for use in this study.

Measurement of respiratory parameters

Minute ventilation (V_E) was obtained by whole-body plethysmography (17). Unanesthetized rats were placed into a 5 L Plexiglas chamber at 25°C and allowed to move freely while humidified air circulated throughout the chamber. During each V_E measurement, the airflow was interrupted for a short time (~2 min), and the chamber remained closed. Breathing-related oscillations in pressure were detected by a differential transducer and amplified (MLT141 spirometer, Power Lab, AdInstruments, NSW, Australia). The recordings were saved and analyzed using PowerLab Chart5 software (AdInstruments, NSW, Australia). A volume calibration was performed during each V_E measurement throughout the course of the experiments by injecting a known air volume (1 mL) inside the chamber. The tidal volume (V_T) was calculated using a previously described formula (17). V_E was calculated as the product of V_T and respiratory frequency (f), and it is presented at the ambient barometric pressure and at body temperature and ambient pressure saturated with water vapor (BTPS). A gas-mixing pump (Cameron, Canada) allowed the chamber to be ventilated with different gas mixtures.

Experimental protocol

During the four weeks of exposure to ETOH or tap water, each animal was exposed to hypercapnia or hypoxia once at the end of each week according to an experimental protocol adapted from Sabino et al. (18). The animals were placed into a plethysmographic chamber and allowed to move freely for 40-50 min for acclimatization while the chamber was flushed with humidified air (21% O_2 , 79% N_2 , 1.2 L/min). After the baseline measurement of ventilatory parameters, each animal was subjected (30 min) to hypoxia (10% inspired O_2) or hypercapnia (7% inspired CO_2), and the

ventilatory parameters were measured at 10, 20 and 30 minutes after each exposure.

Statistical analysis

The results are expressed as the mean \pm standard error of the mean (SEM). The basal values and changes in f , V_T and V_E in response to hypoxia and hypercapnia were evaluated using two-way ANOVA followed by Tukey's post-hoc test. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Baseline ventilatory parameters

Figure 1 shows the baseline ventilatory parameters before hypoxia and hypercapnia. During the four weeks of the study, rats administered ETOH showed lower baseline values for f compared with the control tap water group ($F_{1,63} = 29.55$, $p < 0.05$). After the first week, experimental rats showed a higher V_T ($F_{1,63} = 8.03$, $p < 0.05$), whereas V_E

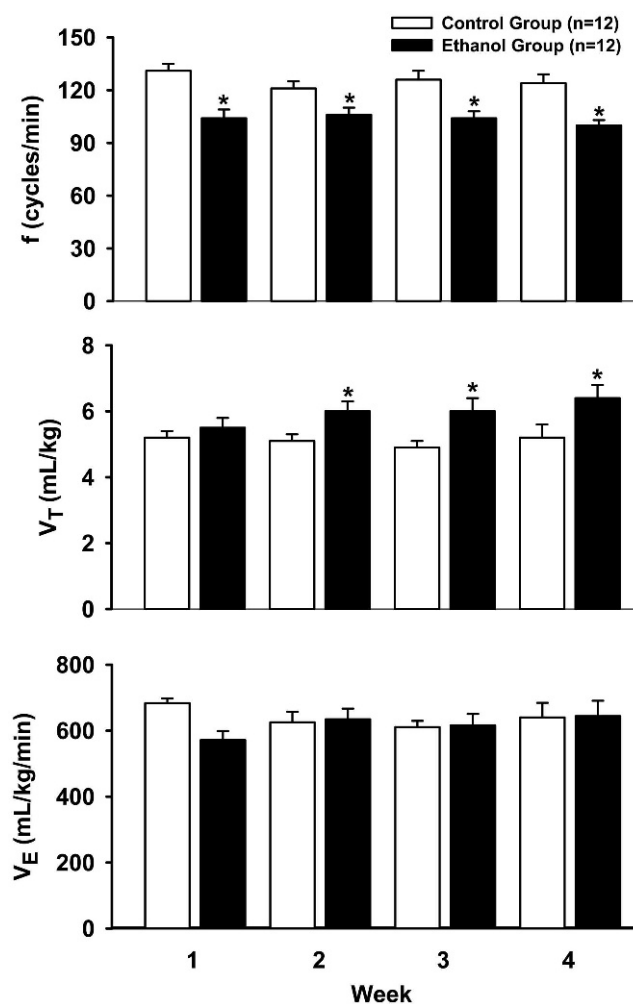


Figure 1 - Baseline values of respiratory frequency (f , top), tidal volume (V_T , middle) and minute ventilation (V_E , bottom) in rats receiving water (control group) or ethanol (ETOH) in increasing concentrations (first week: 5% v/v, second week: 10% v/v, third and fourth weeks: 20% v/v). Values are expressed as the mean \pm SEM. *, $p < 0.05$ (two-way ANOVA) compared to control rats.



remained unchanged during the experimental period ($F_{1,63} = 0.46, p > 0.05$) (Figure 1).

Ventilatory response to hypoxia

In general, during the four-week period, hypoxia caused a sharp (1.8-fold) increase in V_E that was maintained during the 30 minutes of hypoxia exposure. This increased V_E was due to increases in f and V_T .

Figure 2 shows the changes in ventilatory parameters in response to hypoxia during the first and second weeks. Rats that received ETOH exhibited a similar increase in f ($F_{1,30} = 1.42, p > 0.05$) and V_T ($F_{1,30} = 0.24, p > 0.05$). However,

the increase in V_E was attenuated in the ETOH group ($F_{1,30} = 7.81, p < 0.05$) compared with the control group (Figure 2). In the second week, the hypoxia-induced increase in V_T was similar in both groups ($F_{1,30} = 0, p > 0.05$); however, hypoxia-induced tachypnea ($F_{1,30} = 13.63, p < 0.05$) and the response of V_E to hypoxia ($F_{1,30} = 8.49, p < 0.05$) were lower in rats that received ETOH (Figure 2).

Figure 3 shows the changes in the ventilatory parameters in response to hypoxia during the third and fourth weeks of ETOH exposure. In the third week, the ETOH group showed attenuated increases in f ($F_{1,30} = 19.08, p < 0.05$) and

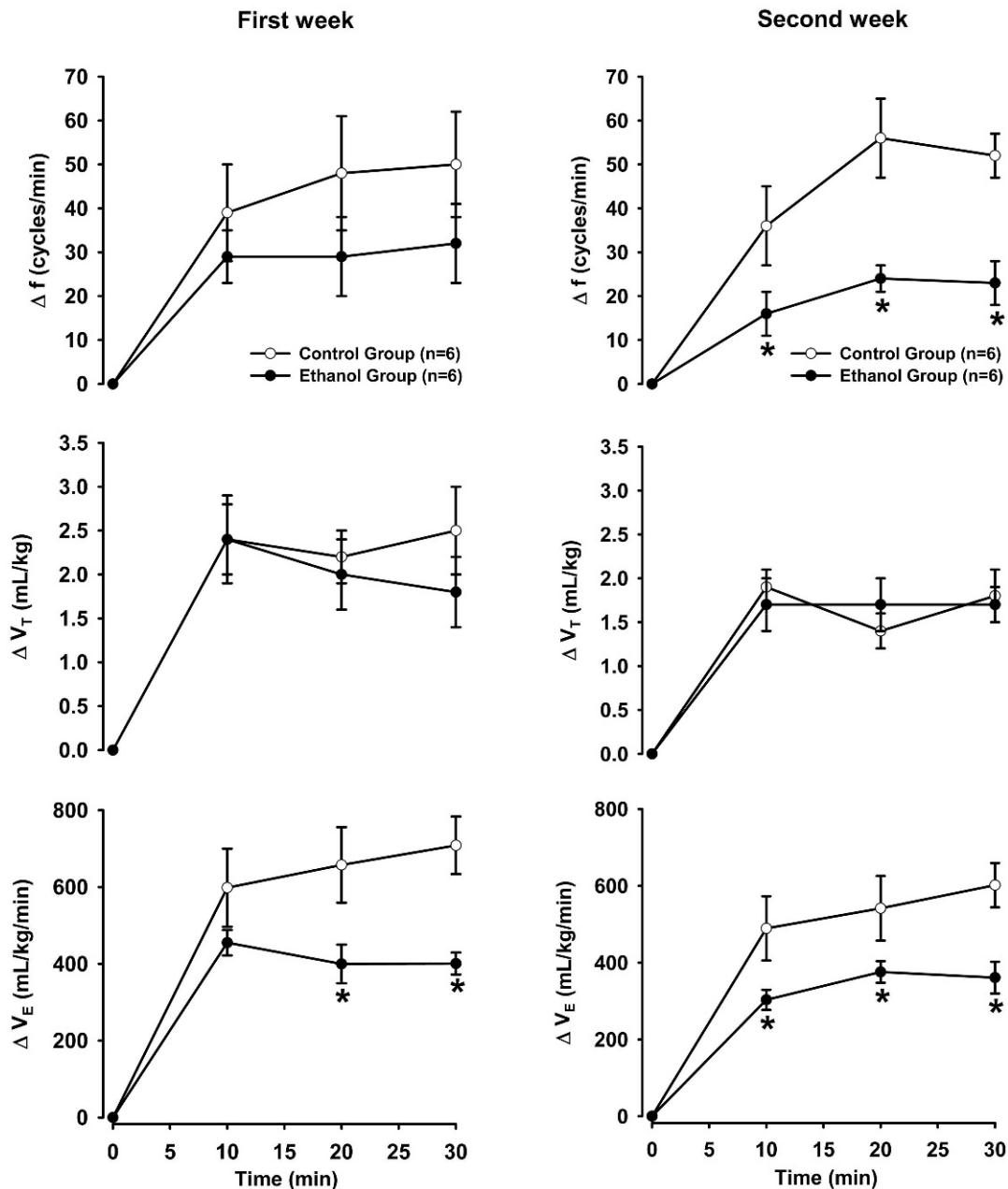


Figure 2 - Changes during the first (left panel) and second (right panel) weeks in respiratory frequency (f , top), tidal volume (V_T , middle) and minute ventilation (V_E , bottom) in response to 10% O_2 -mediated hypoxia in rats receiving water (control group) or ethanol (ETOH) in increasing concentrations (first week: 5% v/v, second week: 10% v/v, third and fourth weeks: 20% v/v). Values are expressed as the mean \pm SEM. *, $p < 0.05$ (two-way ANOVA) compared to control rats.

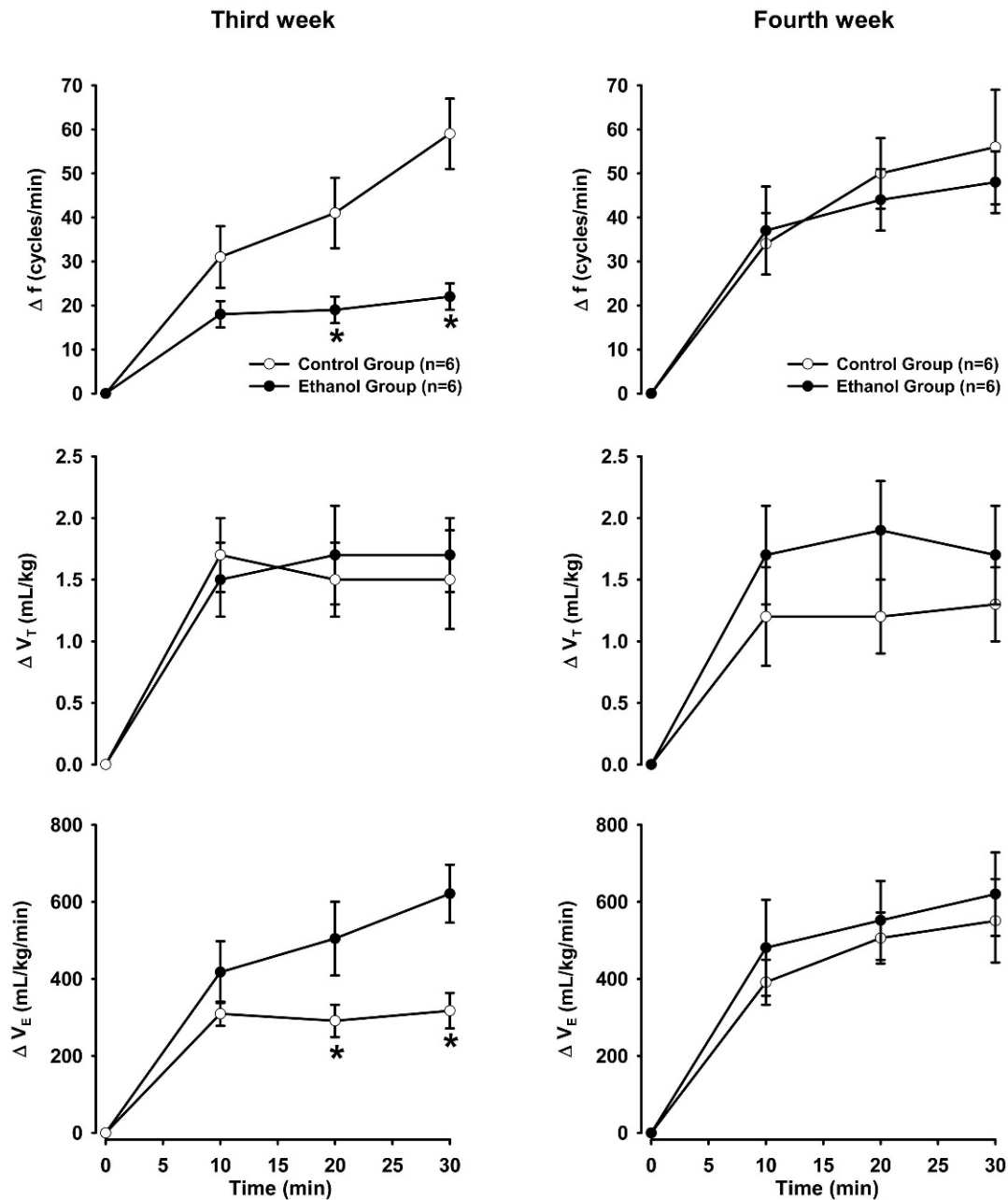


Figure 3 - Changes during the third (left panel) and fourth (right panel) weeks in respiratory frequency (*f*, top), tidal volume (*V_T*, middle) and minute ventilation (*V_E*, bottom) in response to 10% O₂-mediated hypoxia in rats receiving water (control group) or ethanol (ETOH) in increasing concentrations (first week: 5% v/v, second week: 10% v/v, third and fourth weeks: 20% v/v). Values are expressed as the mean ± SEM. *, *p* < 0.05 (two-way ANOVA) compared to control rats.

V_E ($F_{1,30}=9.51, p<0.05$). In contrast, the changes in *V_T* ($F_{1,30}=0.05, p>0.05$) were similar in the ETOH and control groups (Figure 3). In the final week, hypoxia caused similar increases in *f* ($F_{1,30}=0.14, p>0.05$), *V_T* ($F_{1,30}=1.23, p>0.05$) and *V_E* ($F_{1,30}=0.28, p>0.05$) in both groups.

Ventilatory response to hypercapnia

Similar to hypoxia, the typical hypercapnia-induced hyperventilation response was generally observed in all groups. Hypercapnia caused a sharp (2.5-fold) increase in *V_E* that was maintained during the 30 minutes of hypercapnia. This increase in *V_E* was due to increases in *f* and *V_T*.

Figure 4 shows the changes in ventilatory parameters in response to hypercapnia during the first and second weeks. In the control and ETOH groups, hypercapnia caused similar increases in *f* ($F_{1,30}=3.85, p>0.05$ and $F_{1,30}=2.38, p>0.05$, respectively), *V_T* ($F_{1,30}=3.96, p>0.05$ and $F_{1,30}=0.05, p>0.05$, respectively) and *V_E* ($F_{1,30}=0.12, p>0.05$ and $F_{1,30}=0.48, p>0.05$, respectively) (Figure 4). Figure 5 shows the ventilatory parameters in response to hypercapnia during the third and fourth weeks. In the control and ETOH groups, hypercapnia caused similar increases in *f* ($F_{1,30}=1.90, p>0.05$ and $F_{1,21}=0.83, p>0.05$, respectively), *V_T* ($F_{1,30}=1.32, p>0.05$ and $F_{1,21}=0.16, p>0.05$, respectively)

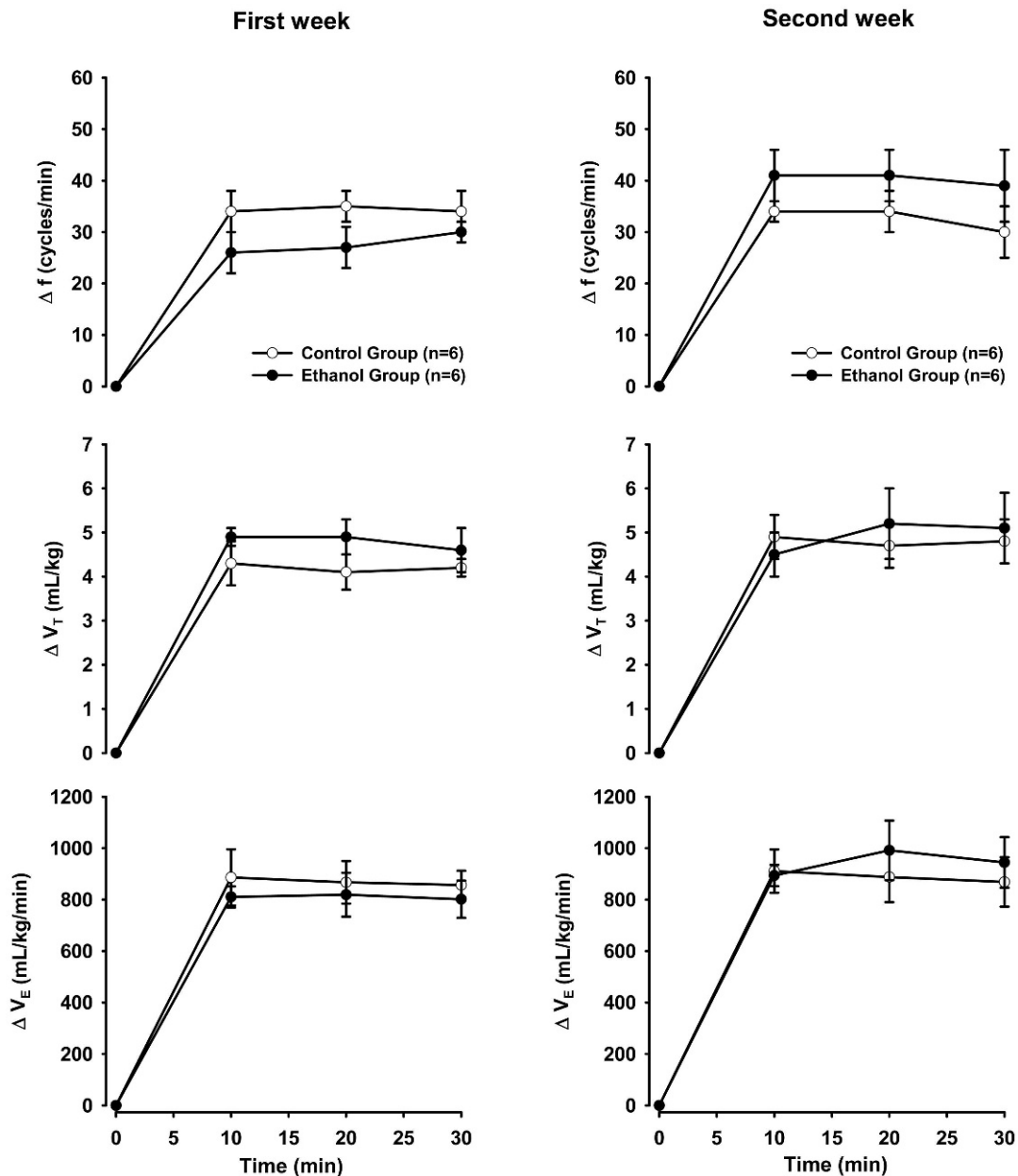


Figure 4 - Changes during the first (left panel) and second (right panel) weeks in respiratory frequency (f , top), tidal volume (V_T , middle) and minute ventilation (V_E , bottom) in response to 7% CO_2 -mediated hypercapnia in rats receiving water (control group) or ethanol (ETOH) in increasing concentrations (first week: 5% v/v, second week: 10% v/v, third and fourth weeks: 20% v/v). Values are expressed as the mean \pm SEM.

and V_E ($F_{1,30} = 1.45$, $p > 0.05$ and $F_{1,30} = 0.28$, $p > 0.05$, respectively).

DISCUSSION

In this study, we tested the hypothesis that chronic ETOH exposure increases peripheral and central chemoreflex sensitivity. Surprisingly, the obtained data do not support our hypothesis because a reduced ventilatory response to hypoxia was observed, indicating decreased peripheral chemoreflex sensitivity. In addition, we found an unaltered ventilatory response to hypercapnia in rats administered ETOH, indicating unchanged central chemoreflex sensitivity. Such findings indicate that the autonomic control

changes observed during chronic ETOH intake are complex and that the well-documented elevation in sympathetic tonus (responsible for ETOH-induced hypertension) may not involve increased chemoreflexes.

Moreover, this study also provides evidence indicating that chronic ETOH intake reduces f , with little effect on V_E , due to a higher V_T . Similarly, V_E has been observed to be normal in subjects who were acutely exposed to ETOH (14,15). Therefore, the results indicate that pulmonary ventilation remains unchanged in both humans and experimental animals following exposure to ETOH.

Conversely, when ETOH is administered during the perinatal period, a marked decrease in spontaneous

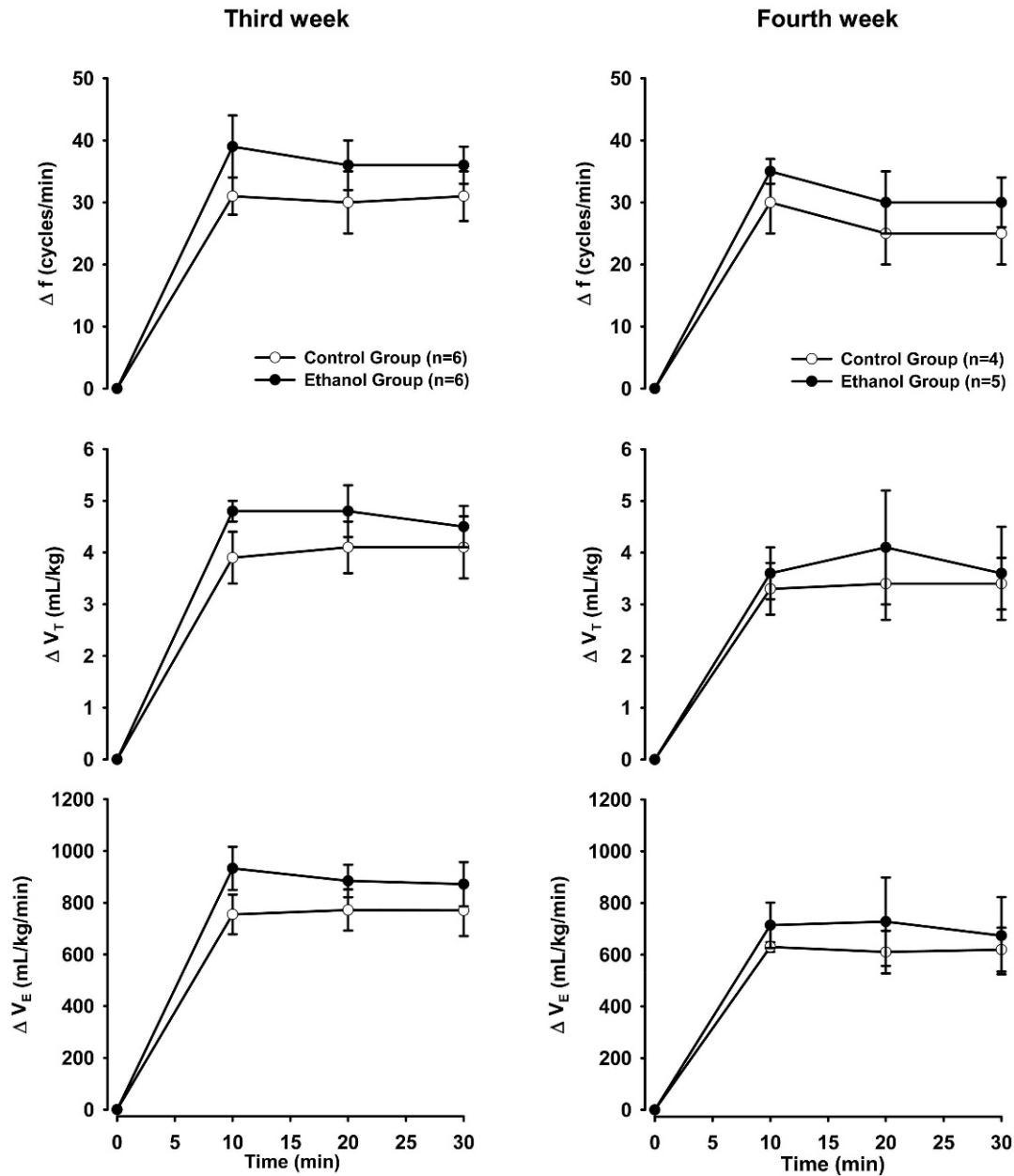


Figure 5 - Changes during the third (left panel) and fourth (right panel) weeks in respiratory frequency (f , top), tidal volume (V_T , middle) and minute ventilation (V_E , bottom) in response to 7% CO_2 -mediated hypercapnia in rats receiving water (control group) or ethanol (ETOH) in increasing concentrations (first week: 5% v/v, second week: 10% v/v, third and fourth weeks: 20% v/v). Values are expressed as the mean \pm SEM.

respiratory frequency and V_E can be observed in 3- to 4-week-old rats, whereas V_T remains unchanged (19). The differences between our results and those of Dubois and colleagues (19) may be due to the periods of ETOH exposure examined, the concentration of ETOH or the age of the animals. Nevertheless, it is worth noting that both studies showed a decrease in spontaneous f after ETOH exposure.

As previously mentioned, this study showed that chemoreflex sensitivity was attenuated in response to hypoxia until the third week of ETOH exposure. These results indicate that the chemoreflex, which is a sympathoexcitatory reflex, may not be involved in the increased sympathetic or decreased parasympathetic activities and

consequent hypertension observed in rats after ETOH exposure (2,3). In agreement with this notion, ETOH was reported to have an inhibitory effect on chemoreflexes by Sun and Reis (20), who showed that the increased blood pressure and vasomotor neuronal activity (located in the rostroventrolateral area of the medulla oblongata) in response to intracarotid cyanide administration were markedly attenuated following acute ETOH exposure.

Another reflex evaluated in this study was the central chemoreflex, which has been evaluated based on the ventilatory response to hypercapnia (15,21-24). We found that chronic ETOH exposure did not alter the increases in f , V_T or V_E induced by hypercapnia. Similarly, previous



studies performed in humans have also shown that hypercapnia-induced hyperventilation is not affected by acute ETOH exposure (14,15). Reconciling the available data, it appears that the central chemoreflex is not involved in the autonomic changes (including hypertension) induced by ETOH during acute or chronic ETOH exposure.

Thus, clinical evaluations of alcoholics should focus on the autonomic (including blood pressure) and endocrine changes caused by ETOH consumption but may not necessarily need to examine chemoreflex sensitivity. In fact, it has been documented that abstinent alcoholic subjects may show abnormal respiratory events during sleep; however, such changes are unlikely to be related to changes in chemoreflex sensitivity (25).

In conclusion, our results indicate that the chronic administration of ETOH to rats does not increase peripheral or central chemoreflex sensitivity. Therefore, chemoreflex sensitivity may not be related to the autonomic, neuroendocrine and cardiovascular changes observed following chronic ETOH exposure.

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■ AUTHOR CONTRIBUTIONS

Sabino JP and Lopes da Silva A collected the data presented in this manuscript. Resstel LB and Glass ML provided valuable insights regarding data interpretation and revised the manuscript. Branco LGS was the mentor of Sabino JP and was responsible for the conception and development of the study.

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