Accepted Manuscript

Title: Acute ethanol and taurine intake affect absolute alpha power in frontal cortex before and after exercise

Authors: Dailson Paulucio, Bruno M da Costa, Caleb G. Santos, Bruna Velasques, Pedro Ribeiro, Mariana Gongora, Mauricio Cagy, Renato L. Alvarenga, Fernando AMS Pompeu

PII: S0304-3940(17)30588-8
Reference: NSL 32966

To appear in: Neuroscience Letters

Received date: 5-5-2017
Revised date: 4-7-2017
Accepted date: 14-7-2017

Please cite this article as: Dailson Paulucio, Bruno M da Costa, Caleb G.Santos, Bruna Velasques, Pedro Ribeiro, Mariana Gongora, Mauricio Cagy, Renato L.Alvarenga, Fernando AMS Pompeu, Acute ethanol and taurine intake affect absolute alpha power in frontal cortex before and after exercise, Neuroscience Lettershttp://dx.doi.org/10.1016/j.neulet.2017.07.026

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.
Acute ethanol and taurine intake affect absolute alpha power in frontal cortex before and after exercise

Dailson Paulucio\textsuperscript{1,2,7}, Bruno M da Costa\textsuperscript{1,2,5}, Caleb G. Santos\textsuperscript{1,6}, Bruna Velasques\textsuperscript{2,4}, Pedro Ribeiro\textsuperscript{2,3}, Mariana Gongora\textsuperscript{3}, Mauricio Cagy\textsuperscript{8}, Renato L. Alvarenga\textsuperscript{1,2} and Fernando AMS Pompeu\textsuperscript{1,2}.

\textsuperscript{1}Biometrics Laboratory, School of Physical Education and Sports, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil;
\textsuperscript{2}Postgraduate in Physical Education, School of Physical Education and Sports, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil;
\textsuperscript{3}Brain Mapping and Sensory Motor Integration Laboratory, Institute of Psychiatry, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil;
\textsuperscript{4}Neurophysiology and Neuropsychology of Attention Laboratory, Institute of Psychiatry of the Federal University of Rio de Janeiro (IPUB/UFRJ), Rio de Janeiro e RJ, Brazil;
\textsuperscript{5}Neuroscience Laboratory of Exercise, Institute of Psychiatry, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil;
\textsuperscript{6}Army Biology Institute, Brazilian Army, Rio de Janeiro, Brazil;
\textsuperscript{7}Department of physiology in soccer, Botafogo de Futebol e Regatas, Rio de Janeiro, Brazil;
\textsuperscript{8}Biomedical Engineering Program, COPPE, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil.

Corresponding Author:

Dailson Paulucio, Federal University of Rio de Janeiro, School of Physical Education and Sports – Biometrics Laboratory (LadeBio), Rio de Janeiro, Brazil.

E-mail: dailsonpaulucio@gmail.com
Highlights
- Taurine is used in the treatment of alcoholism and with ethanol through energy drinks;
- Little is known about the acute effects of taurine and possible modulations of mix with ethanol on cortical activity in frontal cortex.
- Ethanol reduces EEG activity, but this reduction is greater when taken with taurine;
- After exercise the EEG activity remains reduced in the taurine with ethanol treatment;

Abstract

Taurine and alcohol has been popularly ingested through energy drinks. Reports from both compounds shows they are active on nervous system but little is known about the acute effect of these substances on the frontal cortex in an exercise approach. The aim of this study was to determine the effects of 0.6 mL·dL⁻¹ of ethanol (ET), 6 grams of taurine (TA), and taurine with ethanol (TA+ET) intake on absolute alpha power (AAP) in the frontal region, before and after exercise. Nine participants were recruited, five women (22 ± 3 years) and four men (26 ± 5 years), for a counterbalanced experimental design. For each treatment, the tests were performed considering three moments: "baseline", "peak" and "post-exercise". In the placebo treatment (PL), the frontal areas showed AAP decrease at the post-exercise. However, in the TA, AAP decreased at peak and increased at post-exercise. In the ET treatment, AAP increased at the peak moment for the left frontal electrodes. In the TA+ET treatment, an AAP increase was observed at peak, and it continued after exercise ended. These substances were able to produce electrocortical activity changes in the frontal regions after a short duration and low intensity exercise. Left and right regions showed different AAP dynamics during peak and post-exercise moments when treatments were compared.

Keywords: Alcohol; Amino Acids; Brain mapping; Frontal cortex; Electroencephalography; Physical exertion.

Introduction
The study of the physiological and neuronal mechanisms involved in the acute effects of drugs and supplements during exercise has been extensive in the recent years. Within this context, two compounds are the most commonly consumed supplements among athletes: taurine (TA) and ethanol. Although it is common to find reports in the literature about the effects of these substances on performance, little is known about their expression in the central nervous system (CNS), in particular concerning the mechanisms involved in the Electroencephalographic activity.

The quantitative electroencephalography (qEEG) is considered to be an essential tool for analyzing neurological disorders [15] and the acute effects of exercise [5, 6, 19]. Thus, alpha frequency (8-12Hz) has been investigated as an indicator of changes in brain activity reflecting cognitive, perceptual and motor functions [2, 14]. The electroencephalographic activity has also been analyzed during the acute effects of drugs, such as ET [16, 18]. Taurine demonstrates multiple cellular functions in central nervous system and has been used in the treatment of alcoholism [10, 34], but little is known about the acute effect of this substance on electrophysiological variables. Despite these substances being popularly consumed together with energy drinks, in order to increase the feeling of pleasure and reduce some side effects of ET [9], such as sleepiness, the impact of this combination on EEG activity remains unknown. Therefore, the objective of this study is to analyze the influence of TA and ET intake, administered both separately and together, on electrophysiological parameters (absolute alpha power over the frontal cortex), before and after exercise of moderate intensity. We hypothesized that both substance would change the frontal AAP dynamic, and when administrated together, the TA would modulate the ET effect.

**Methodology**

**Subjects**
Nine healthy, right-handed individuals of both sexes (5 women, 4 men; mean age 23.6, SD: 4.66), regular practitioners of physical exercise, non-smokers and non-athletes participated in the experiment. The sample size was defined based on previous qEEG studies that had a similar design [4, 20]. Individuals with liver disorders (as determined by the activity of the enzymes aspartate aminotransferase, alanine aminotransferase, direct and indirect bilirubin, alkaline phosphatase, gamma-glutamyl transferase and lactate dehydrogenase) or who were taking medications, as well as alcohol users with a weekly intake greater than fifteen or less than two alcohol servings were excluded from this study. The participants were asked to avoid physical activities with more than 5 metabolic equivalents (METs) and any food containing caffeine and taurine for 48 hours before the test. The Edinburgh inventory was applied to identify the hand laterality [22]. Volunteers signed an informed consent form. All experimental procedures were conducted according to the Declaration of Helsinki (1964) and approved by ethics committee on research of HCFF-UFRJ (03899312.5.0000.5257).

**Experimental Design**

This study lasted approximately five weeks for each participant. The experiment began with an anthropometric and an effort test. During each week, a particular intervention was applied (PL, TA, ET or TA+ET). The experimental design of these tests was in agreement with a specific treatment (Figure 1). Six grams of microcrystalline cellulose were diluted in 150 ml of orange juice (Clight®, 21 g.L\(^{-1}\)) used as the placebo (PL), while six grams of powdered taurine (TA) were used for the experimental solution. Ethanol (Orloff® vodka, 38% alcohol content) was administered in doses of 0.6 ml. kg\(^{-1}\), combined with the ingestion of orange juice in a proportion of 2:1 (juice: vodka).

The TA and PL solutions were ingested two hours before the exercise, while the ET was ingested thirty minutes before the exercise. As the peak plasma levels of TA [11]
occur about 2 hours after ingestion and the ET peaks 30 minutes after intake [1], in the TA+ET treatment, ET was ingested one hour and thirty minutes after the administration of taurine, and the exercise was performed thirty minutes after the ingestion of ET. Taurine administration followed a simple double-blind procedure. The EEG was recorded at the peak and post-exercise. Since the frontal region is related to executive functions, our aim was observe the frontal regions dynamics in order to evaluate cognitive alterations when subjects were under drug influence.

*Ergometric Protocol*

Metabolism was analyzed using open-circuit indirect calorimetry (Vista Mini-CPX®, Vacumed®). A cycle ergometer with an electromagnetic brake (Imbrasport®) was used for the effort test. A constant-load protocol (SWT) was adopted in the Ergometric tests. The resting and warm-up procedures used in the graded exercise test (GxT) were used in this protocol. An intensity 10% lower than the load of the anaerobic threshold was subsequently determined and maintained for 10 minutes at a rate of 60 revolutions per minute (RPM).

*Blood collection and biochemical analysis*

Serum and plasma (Fluoride/anticoagulated EDTA) were obtained from peripheral blood samples of subjects at three moments (at the beginning, thirty minutes after ET ingestion and immediately after the exercise) for each treatment (ET and TA+ET). Serum levels of liver enzymes were determined using the VITROS® Chemical System and plasma ET levels by Siemens Dimension® Series.

*Acquisition of EEG Signals*

The EEG signal was recorded using the BNT36 (EMSA). Twenty monopolar derivations were arranged on a lycra cap following the 10/20 International System [17].
Different cap sizes were used, according to the head circumference. The impedance of the electrodes was kept below 10 kΩ. The signal was analogically filtered between 0.1Hz (high-pass) and 100Hz (low-pass), and sampled at 400Hz. The Data Acquisition software from the Brain Mapping and Sensory Motor Integration Lab was employed with digital notch filter (60Hz).

Data processing and analysis

We applied visual inspection and independent component analysis (ICA) to remove possible sources of artifacts using routines developed by the Brain Mapping and Sensoriomotor Integration lab [23]. The data were collected using the averaged-auricular reference. Through visual inspection, all the trials, which clearly showed blinking and muscle-related artifacts, were rejected. Through ICA, the components that showed blinking and muscle-related artifacts were also removed. The Absolute Alpha value was based on the Auto Power Spectral Densities estimated via Bartlett Periodogram. Hence, Absolute Alpha was obtained as the mean value of the Power Density along all frequency bins within 8Hz and 12Hz, for each considered electrode.

Data Analysis

In order to analyze the absolute alpha power, a two-way ANOVA was used (treatment vs. moment). When an interaction between factors was found, a one-way ANOVA (Bonferroni post-hoc) was applied to each treatment for all frontal electrodes. The Bonferroni post hoc analysis was also determined for multiple comparisons between treatments. Descriptive statistics was used with the mean ± standard error (SE) (p ≤ 0.05).

Results

The results were divided in blood ethanol content and the electrocortical variables from three areas of the frontal cortex.
Blood ethanol content

When investigating the ET concentration in the plasma in ET and TA+ET treatments, a significant increase was seen for both treatments, compared to the Baseline values. However, there was no significant difference between treatments when specific moments were compared (ET Baseline vs. TA+ET Baseline; ET peak vs. TA+ET peak; ET post-exercise vs. TA+ET post-exercise) (Table S1).

Effects of substances on the Electrophysiological Parameter

When analyzing the Absolute Alpha Power, the two-way ANOVA (treatment vs. moment) demonstrated an interaction between factors for all frontal electrodes [Fp1(F=22.23; p=0.001); Fp2(F=22.17; p=0.001); F3(F=16.53; p=0.001); F4(F=16.153; p=0.001); F7(F=41.09; p=0.001); F8(F=17.39; p=0.001)]. In order to examine in details the interaction between moment and treatment, a one-way ANOVA was performed for each treatment (PL, TA, ET and TA+ET). The results will be described according to the different regions of the frontal cortex.

A significant difference was found for Fp1 and Fp2, for PL, TA and TA+ET at the post-exercise moment when compared to the baseline (Fp1 p=0.001; Fp2 p<0.05); difference was also found for PL and TA between post-exercise and peak moments (Fp1 p=0.001; Fp2 p=0.001). However, the TA treatment also showed a significant difference for Fp2 between baseline and peak moments (p=0.001). For Fp1, in the ET treatment, a significant difference was observed at the peak moment, when compared to baseline (p=0.002) and post-exercise (p=0.004) (Figure 2).

In the PL treatment, for F7 electrode, a significant difference was found at the peak (p=0.001) and post-exercise (p=0.001) moments, when they were compared to baseline. Moreover, for F8 electrode, a significant difference was observed at the peak
moment when compared to the post-exercise ($p=0.041$). In the TA treatment for both electrodes, a significant difference was observed at the post-exercise moment when compared to baseline and peak ($p=0.001$). In addition, for F8 electrode, a difference was seen between the baseline and peak moments ($p=0.048$). In the ET treatment, for F7 electrode significant difference was found between peak moment and baseline ($p=0.001$) and post-exercise and baseline ($p=0.023$). In the TA+ET treatment, a significant difference was observed between all moments for F7 ($p=0.001$); for F8, a significant difference was reported for peak ($p=0.011$) and post-exercise ($p=0.002$) moments when compared with baseline (Figure 3).

For the F3, Fz and F4 electrodes, a significant difference was found in the PL and TA treatments at the post-exercise moment, compared to the baseline and peak moments ($p<0.05$). Moreover, we also found a significant difference for F4, in the TA, when peak moment was compared to baseline ($p=0.001$). In the ET treatment, a significant difference was observed when peak moment was compared to baseline for F3 ($p=0.011$) and Fz ($p=0.003$) electrodes. In the TA+ET treatment, a significant difference was found to peak and post-exercise moments when compared to baseline for F3 ($p=0.011$ and $p=0.002$, respectively), Fz ($p=0.001$) and F4 ($p=0.001$) electrodes (Figure 4).

**Discussion**

This study aimed to analyze the influence of TA and ET intake, administered both separately and together, on electrophysiological parameters, before and after exercise of moderate intensity. We have observed that the substances used influence the AAP, as predicted by our hypothesis and these finding were be discussed in the following areas of the frontal cortex:

*Anterior prefrontal cortex (FP1 and FP2)*
The anterior prefrontal cortex, corresponding to Brodmann area 10, is one of the areas involved with planning, organization and motor control. This area also plays an important role in sensory integration, mnemonic information and intellectual function [13]. The two investigated electrodes showed a similar pattern in the PL treatment, that is, an AAP decrease after exercise. As previously mentioned, AAP is inversely proportional to brain activity, i.e., the decrease AAP implies the increase in brain activity. In a previous study, Yanagisawa et al. [35] found that increased brain activity in this region after exercise of short duration and moderate intensity interferes in the improvement of some cognitive functions. On the other hand, in the TA treatment, AAP increased post-exercise at both electrodes and it decreased at peak moment for Fp2.

Some reports claim that this amino acid may act as an inhibitory neurotransmitter in the CNS, because of the allosteric increase of GABA\textsubscript{A} receptors [33]. However, in certain instances, TA may increase the activity of glutamate receptors, by regulating the cytoplasmic and intramitochondrial calcium homeostasis [7, 8, 27]. The AAP decrease in the TA at peak, in the right region may be involved in some specific functions, such as planning and decision-making. The electrocortical activity differed between Fp1 and Fp2 in the ET treatment: AAP was higher at peak for Fp1 electrode, while no difference between moments was found for Fp2. Our findings are in agreement with other data, which found a cortical activation decrease of the left prefrontal cortex, with acute effect of ET [31].

Acute ingestion of ET may cause deficits in cognitive activities related to the prefrontal cortex, such as performance decrease in partial recognition tasks and planning [30], and a decline in decision-making [12]. The TA+ET intake increased the AAP at peak for both electrodes, and this increase continued even after exercise ended. Very little EEG data has been collected about the acute effect of TA, both administered alone or together.
with ET. However, the alpha variation between moments in each specific treatment may support the increased efficiency of the anterior cortex region.

_Inferior Frontal Gyrus – IFG (F7 and F8)_

There is evidence that the IFG is related to functions such as working and episodic memory, behavioral inhibition and visuospatial aspects [25, 29]. We saw for both electrodes, AAP decrease after exercise in the PL. Moraes et al [20] investigated the effect of an acute bout of exercise on a cycle ergometer at full intensity and found no AAP significant difference in post-exercise when compared to the baseline. It seems that the intensity of exercise can be an important factor in the change of this variable. By contrast, in the TA, an AAP increase at post-exercise was found for both electrodes; also AAP decrease at peak compared to the baseline moment only for the F8 electrode. As the acute effect of TA tends to activate the right hemisphere (F8) at peak, its intake may be directly involved in the modulation of specific functions, such as behavioral and motor inhibition, and decision-making [3].

Electrocortical activity differences between F7 and F8 were highlighted in the ET treatment. An AAP increase occurred at peak and continued at post-exercise for F7, but no difference was found for F8. The electrocortical activity of the left IFG is directly involved in specific functions of language and selective attention [24, 28], and could be affected by ET intake, according to our results. When substances are ingested in an associated manner, F8 inhibitory effect maximized, while for F7 this only occurred at peak, and this was seen through an AAP increase compared with the results of the single treatments.

_Superior Frontal Gyrus (F3, Fz and F4)_
The Superior Frontal Gyrus, corresponding to area Brodmann 8, is involved in working memory, data recovery, planning, visual-motor attention and motor learning [21, 26]. The AAP decreased after exercise at F3, Fz and F4 in the PL treatment. Moraes et al [20] found no significant difference in alpha behavior, at the post-exercise moment when compared to the baseline moment. However, the different results in alpha activity after exercise can also be explained by differences between studies methods.

In the TA treatment, we found for the F3 and FZ electrodes similar results to electrodes located in the left hemisphere (Fp1 and F7), that is, AAP increase after exercise. In turn, AAP at the F4 electrode followed the same pattern of the right hemisphere electrodes (Fp2 and F8). An AAP decrease at peak and an AAP increase at post-exercise. The activation of the right area of the Superior Frontal Gyrus is associated with some specific functions, such as maintenance and response selection [26]. Furthermore, in the ET treatment, an AAP increase at peak compared to baseline moment also occurred for the F3 and FZ electrodes. This acute effect of ET may interfere with sensorimotor integration processes, since the left Superior Frontal Gyrus is directly involved in this function [32]. In the TA+ET treatment, an AAP increase was found at peak and continued post-exercise for F3 and F4.

Conclusion

According to our results in the PL treatment, it can be concluded that electrocortical activity increases after an exercise of short duration and low intensity. We also conclude that the electrocortical activity increases after TA intake at its peak in the right hemisphere of the frontal cortex, while after ET intake there is a decrease in the left hemisphere at the peak. The intake of this amino acid, however, causes the decrease of electrocortical activity after exercise. When ET and TA were administered together, the frontal electrocortical activity decreases at peak moment and continued decreasing even
after the exercise ended. Thus, the used substances affect the AAP in frontal cortex before and after exercise, supporting our hypothesis and highlighted the TA modulation over the ET effect. Although this study has shown interesting results, some limitations are listed as follows: 1) the small amount of ethanol administered in comparison to other studies and 2) The analysis of taurine serum levels could improve explanation of the results.

List Captions

Figure 1 – Schematic timeline illustration in different treatments. A – Placebo treatment; B – Taurine treatment; C – Ethanol treatment; D – Taurine+Ethanol treatment.

Figure 2 – Absolute alpha power of anterior prefrontal. Values are expressed as means and standard error. * Significant difference (p ≤ 0.05).

Figure 3 – Absolute alpha power of inferior prefrontal gyrus. Values are expressed as means and standard error. * Significant difference (p ≤ 0.05).

Figure 4 – Absolute alpha power of superior frontal gyrus. Values are expressed as means and standard error. * Significant difference (p ≤ 0.05).

Supplementary material

Table S1 – Plasma ethanol concentration

Acknowledgments

The authors would like to thank the Institute of Biology of the Brazilian Army (Instituto de Biologia do Exército Brasileiro - IBEX) for the help with the biochemical analyses. Professor Fernando A.M.S. Pompeu received funding from the Rio de Janeiro Research Foundation (Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro - FAPERJ).

References


[27] E. Trenkner, A. el Idrissi, C. Harris, Balanced interaction of growth factors and taurine regulate energy metabolism, neuronal survival, and function of cultured mouse cerebellar cells under depolarizing conditions, Advances in experimental medicine and biology 403 (1996) 507-517.


Figure 2

Fp1

Absolute Alpha Power (µV)

- Placebo
- Taurine
- Ethanol
- Taurine + Ethanol

Baseline | Peak | Post-exercise

Fp2

Absolute Alpha Power (µV)

- Placebo
- Taurine
- Ethanol
- Taurine + Ethanol

Baseline | Peak | Post-exercise