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ROLE OF GABAERGIC SYSTEM IN THE REGULATION OF ANXIETY BEHAVIOR IN OFFSPRING

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ABSTRACT

Experimental studies have shown that GABA plays a significant role in dendritic development in the hippocampus, cortex and cerebellum, and is involved in anxiety and depression disorders. The current study was aimed at evaluating the effects of GABA agonist and antagonist on brain development and anxiety-like behaviors of mice and possible interactions of the Gabaergic system in the modulation of anxiety-like behaviors.

In the present study, treatment was done on pregnant female mice in late pregnancy (16 th to 18 th day of gestation) for 3 days with antagonist (bicuculline) or agonist (muscimol) of GABAA receptor. Used does were 10, 50, 100, 500 and 700 µg / kg for muscimol and 20, 70, 140, 500 and 800 µg / kg for bicuculline. Elevated plus maze and forced swimming tests were used for testing the anxiety and depression of offspring in adulthood.

The results showed that the percent of open-arm time (OAT%) and also the percent of open arm entries (OAE%) both decreased after being treated by muscimol and bicuculline which do not result in locomotor disability in the EPM and indicates that the anxiogenic-like effects are

increased in the adult mice on the elevated plus-maze (EPM). Overall, these results illustrate that excessive activation of GABAA receptors or their blockade increases the anxiogenic-like behaviors in adult mice on the EPM.

Keywords: Anxiety, GABAA-¬R, Brain Development, Mice

INTRODUCTION

Chemical communication between neurons is achieved though neurotransmitters which are known as the small molecules that are released from synaptic terminal. Their important role during embryonic life is the cell development **[7-9-41-54, 72]**.More recently, several neurotransmitters have been observed to regulate postnatal neurogenesis **[73]**. In epigenetic signaling processes which influence on the development of mammals' central nervous system (CNS), neurotransmitters are closely contacting with neural cells during CNS maturation **[63, 67]**. At developmental periods, Neurotransmitters can also be recognized as growth regulators **[2-18-19-22-33-41-51-54-56-57-81, 82]**. The primary inhibitory neurotransmitter in the vertebrates' central nervous system is γ - Aminobutyric acid (GABA), which is being used by one third of brain neurons, and makes GABA important in a wide range of physiological and psychological processes **[14]**. The two main types of this neurotransmitter's receptors are Ionotropic (GABAA and GABAC) and metabotropic (GABAB) receptors **[65]**. The induction of cortical neuronal migration is done by Endogenous GABA via multiple GABA receptors **[6, 8]**. GABA is a signaling molecule which influences on growth, migration and synaptogenesis of neural cells during brain development **[10, 74]**. Consequently, the development of neurons is influenced by GABA [42]. This neurotransmitter is also detected in several regions of the developing cortex **[41]**, and GABAergic fibers are available earlier, (at embryonic day (E) 13) in the brainstem, midbrain and interbrain of rodents. At E14, GABAergic cell bodies are observed in the lateral cortical anlage while at E16, they are found in the proencephalon and all cortical regions **[15, 74]**. Prior to synapse formation, GABA plays a significant role in neurons maturation and synapse communication, which illustrates an example of a molecule acting in different developmental stages **[74]**. Contrary to the previously established opinion which stated that the generation of neurons are limited to the embryonic period, it is now considered that the brain can adapt to the environmental stimuli and stress upon

changing its structural and physiological characteristics **[73]**. 18.1% incidence of anxiety disorders indicates them to be a very common type of psychiatric disorders **[48]**. Since Depression is also an important problem of the modern society, it is predicted by the World Health Organization (WHO) that on 2030, HIV and depression would be the first two leading cause of disease burden worldwide respectively **[60]**. The Invention and development of new drugs necessites better recognition of the pathophysiology of depression **[12]**. Different neurotransmitters including GABA, serotonin, dopamine and glutamate are now proposed to influence on the pathophysiology/control of depression and anxiety, while their receptors are being considered as potential targets in drug design **[17- 26-28-38-39-53-64-70,71]**. More specifically, many observations have been done on GABAergic processes, which focused on its role in these disorders including the higher level of GABA concentration in plasma and cerebrospinal fluid in stressful and anxious conditions, activation of GABAA in stress, and changing in GABAA g2 subunit expression upon stressor exposure **[1-17- 20-21-50-77, 80]**. Furthermore, GABAA affect anxiety, while antidepressant drugs may affect interneuron functioning **[1-17- 20-21-50-77, 80**]. In suicide victims, GABAA receptor expression is abnormal **[61, 62]**. Finally, it seems that GABAA and dopamine activity may influence amygdala functioning **[59]**, while in the brain regions which are interrelated with depression, mutual innervations are available between GABAA and 5HT processes **[17]**. Whereas it was observed that the neurotransmitters and neural systems are interacted **[9, 68]**, the current study was aimed at evaluating the effects of GABA agonist and antagonist on brain development and anxiety-like behaviors of mice and possible interactions of the Gabaergic system in the modulation of anxiety-like behaviors.

MATERIALS AND METHODS

Animals

Adult NMRI mice were purchased from the animal house of Pasteur Institute of Iran which were kept in standard polycarbonate cages in a room with a 12: 12 h light/dark cycle (lights on at 8: 00 A .M.), and controlled temperature (23 ± 1◦ C) which had free access to food and water. At all stages of experiments, these conditions were remained as the standard housing condition. Having accustomed to the new animal holding room within 2 weeks, male and female mice were kept together one-by-one in a cage to facilitate the mating. Successful mating was verified via the presence of vaginal plug and

which was referred as gestational day 0 **[30]**. Having identified a pregnant female, it was removed from the breeding cage and housed individually in a standard cage. The normal delivery conditions were available for all pregnant mothers and the delivery day was referred as postnatal day (PND) 0. On 21 day, the mothers were kept away from the infants and in this way the infants were weaned and then were housed with same sex littermates (5 animals per cage). The experimental protocol was approved by the Research and Ethics Committee of Science and Research Branch, Azad University, Tehran.

Chemicals

Muscimol and bicuculline were purchased from Sigma Chemical Co., St. Louis, MO. Muscimol was dissolved in sterile 0.9% saline while bicuculline was dissolved in a minimal volume of diluted acetic acid (1 drop; 5 ml by Hamilton micro- syringe 10 ml) which made a 5 ml volume of saline that was diluted to the required volume with vehicle. Before administration, Drug solutions were freshly prepared. During the experiment, Control animals received saline or vehicle.

Treated Groups

The Pregnant female mice in late pregnancy were treated for 3 days from 16th to 18t day of gestation with the antagonist or agonist receptor GABAA in 10, 50, 100, 500 and 700 µg / kg Muscimol and 20, 70, 140, 500 and 800 µg / kg Bicuculline. Havinbg reached the postnatal adulthood(at 60-70 days) by elevated plus maze (EPM) and forced swimming test (FST), the tests were done on Offsprings due to anxiety and depression Pregnant females were divided into the following groups.

A) Control Group: the control group only received saline .

B) Experiment Group1: to test the effects of GABAA receptor agonist on anxiety behavior;

5 groups of mice received Intraperitoneal injection (IP) at different doses of10, 50, 100, 500 and 700 µg / kg Muscimol with an insulin syringe on 18-16 days of pregnancy.

C) Experiment Group 2: to test the effects of GABAA receptor antagonist on anxiety behavior;

5 groups of mice received Intraperitoneal injection (IP) at different doses of 20, 70, 140, 500 and 800 µg / kg Bicuculline with an insulin syringe on 18-16 days of pregnancy.

D) Experiment Group3: to test the effects of GABAA receptor agonist on depression behavior,

5 groups of mice received Intraperitoneal injection (IP) at different doses of10, 50, 100, 500 and 700 µg / kg Muscimol with an insulin syringe on 18-16 days of pregnancy.

E) Experiment Group4: to test the effects of GABAA receptor antagonist on depression behavior,

5 groups of mice received Intraperitoneal injection (IP) at different doses of 20, 70, 140, 500 and 800 µg / kg Bicuculline with an insulin syringe on 18-16 days of pregnancy.

Elevated Plus-Maze

As previously described, EPM test, as an assay for the anxiety, was performed. In short, the EPM was a "+"-shaped device, made of wood, elevated up to 50 cm height above the floor. This device is consisted of acentral platform (5 cm × 5 cm), two open arms (30 cm × 5 cm), and two equal closed (30 cm × 5 cm × 15 cm) arms opposite to each other with an open roof. The animals were put separately in the middle of the EPM, confronting each of the open arms and they were explored freely for 5 minutes. Measurements were done on the following: (a) duration on the open arms, (b) duration on the closed arms, (c) number of entries into the open arms, and (d) number of entries into the closed arms within 5-min test. A mouse on either the open or closed arms (spent time) when all four paws were in the respective arm. For Analyzing, the percent of open-arm time [OAT% (time in open arm/time in open + closed arm) × 100], and the percent of open arm entries [OAE% (number of open arm entries/number of open arm + closed arm entries) × 100] were defined that was used as the anxiety measure. The total number of open arms entries, as well as the total number of closed arm entries was defined as an indicator of general locomotor activity (LMA) **[30]**.

Forced Swimming Test (FST)

The forced swimming test (FST) is one of the most frequently used tools for assessing antidepressant activity. Mice were individually put into the transparent glass cylinders (Height: 25 cm, Diameter: 10 cm), filled with water to 15 cm height and kept at 25 C. Between each test, fresh water replace to the filled water. In last 4 min out of the 6 min testing duration, the immobility was recorded. At the end of swimming session, the animals were removed from the cylinder, dried with towels, and placed gently near an electric heater for 15-30 min. When mouse stopped trying and floated motionless on the water and just had few movements that were necessary for keeping the head above the water, they are considered as immobile. Reducing the immobility duration indicates the antidepressant-like effects **[27]**.

Statistical Analysis

Since the data had normal distribution and homogeneity of variance were obvious, one-way ANOVA was used for comparing the effects of different doses of drugs with

vehicle. P< 0.05 between experimental groups at each point was the indicator of statistically significant difference.

RESULTS

Effects of GABAA Receptor Agonist on Anxiety Behavior

Figure 1 shows the effects of administration (IP injections) at different doses of muscimol (10, 50, 100, 500 and 700 μg /kg) on anxiety behaviors in the EPM. The one-way ANOVA revealed that OAT% (P< 0.05), OAE% (P < 0.05) were decreased at 10, 50, 100, 500 and 700 μg / kg muscimol which indicates the anxiogenic-like response induction via muscimol while no changes were observed in the locomotor activity (P > 0.05).

Effects of GABAA Receptor Antagonist on Anxiety Behavior

Figure 2 shows the effects of administration (IP injections) at different doses of Bicuculline (20, 70, 140, 500 and 800 μg / kg) on anxiety behaviors in the EPM. The one-way ANOVA revealed that OAT% (P< 0.05), OAE% (P < 0.05) were decreased at 20, 70, 140, 500 and 800 μg / kg Bicuculline which is an indicator of anxiogenic-like response induction of via Bicuculline, while no changes were observed in the locomotoractivity(P > 0.05).

Effects of GABAA Receptor Agonist on Depression Behavior

Figure 3 shows the effects of administration (IP injections) at different doses of muscimol (10, 50, 100, 500 and 700 μg / kg) on depression behavior in the FST. In our experiment, no significant effect was observed on depression behavior in the FST. The one-way ANOVA does not show significant effects on the immobility time in the FST.

Effects of GABAA Receptor Antagonist on Depression Behavior

Figure 4 shows the effects of administration (IP injections) at different doses of Bicuculline (20, 70, 140, 500 and 800 μg / kg) on depression behavior in the FST. In our experiment, no significant effect was observed on depression behavior in the FST. The one-way ANOVA does not show significant effects on the immobility time in the FST.

DISCUSSION

In this study, we tried to find out the effect of perinatal neuronal challenge on the anxiety-related behaviors in mice. Our findings show that GABAA receptors' perinatal exposure to agonist and antagonist decrease both OAT% and OAE%, without causing locomotor disability in the EPM which indicates the increasing anxiety in adult mice on the EPM. Recent studies suggest that GABA is the first neurotransmitter which becomes functional in

the developing networks which supplies much of the initial excitatory drive **[74]**. The important developmental role of GABA is that it is produced in many brain regions **[10, 74]**. The other characteristic of GABA is that it is the main fast-acting inhibitory neurotransmitter in the adult neocortex **[25, 49]**, which makes a variety of trophic influences during neural development **[5, 51]**. In final week of gestation, GABA expressed close to the destinations of migratory neurons which stimulates the neuronal migration **[6-52]**. During rapid growth, in the immature mammalian brain, the transient blockade of glutamate N-methyl-d-aspartate (NMDA) receptors, as well as the excessive activation of GABAA receptors may result into neuronal apoptosis [44]. So agonists of GABAB receptors may result into apoptotic response **[43]**. Since ethanol has NMDA antagonist **[55, 24]** and GABAA-agonist properties, we can conclude that superimposing the neurodegeneration patterns resulting from the use of NMDA antagonists and GABAA agonists that is similar to the neurodegeneration pattern caused by ethanol **[43]**. Ethanol is an interfering agent with neurotransmitter systems, and damage neurons in the developing brain which result in neurobehavioral and psychiatric disturbances that is manifested at various ages (even to adulthood) **[44]**. In young adult rats at late gestational period, the GABAA agonist, Diazepam causes neurobehavioral effects **[47]**. In newborn rats, one week after being treated with muscimol, we can see the significant cell death in the dentate gyrus (DG) and hippocampus, which is a GABA-A R agonist **[68, 69]**. Meanwhile Muscimol increases the cell death at different regions of the hippocampus **[68]**. GABA-R antagonist induces stress, and File and Lister **[32]** have showed that several behavioral signs of anxiety were induced by subconvulsive doses of picrotoxin (PT) which interrelated to increasing corticosterone levels. Moreover, injections of GABAA-receptor antagonists such as PT and bicuculline into the dorsomedial hypothalamus of rats result into a variety of physiological responses such as increased heart rate and plasma catecholamine levels, increased locomotion activity and anxiogenic-like effects **[45- 75,76]**. Perinatal exposure to PT may also interfere with normal male masculinization, instead of increasing anxiety in male rats **[78]**. Bicucullin, GABAA receptor antagonist, induces cell death in slice cultures **[34-40, 66]**. The studies suggested that exposure to bicuculline resulted in prominent CA1, CA2 and CA3 cell death in hippocampal slice cultures **[34-40, 83]**. The extensive CA1 cell

death and further lesion spread is a feature that is commonly observed in human hippocampal sclerosis associated with epilepsy **[11, 13]**. During late embryonic stages, GABA promotes dendritic development in hippocampus, cortex and cerebellum **[4-16-23, 58]**, which triggers dendritic proliferation **[35]** and maturation **[29]** as well as synapticintegration **[36, 37]** during adult neurogenesis. The adrenal and gonadal hormones' balance are influenced by prenatal stress at important stage of fetal hypothalamic segregation **[79]**. Several neural structures are involved in the anxiety disorders which include hippocampus, amygdala, hypothalamus and septum. Impairment in neurons could be involved in the pathology of anxiety disorders **[46]**. The effective drugs in anxiety, such as ethanol and benzodiazepines influences on dorsal hippocampus **[3, 31]**. Impaired neural structures associated with anxiety could lead to increasing the anxiogenic-like behaviors in adult mice on the EPM. In conclusion, since in late embryonic stages, GABA cultivated dendritic development in hippocampus, cortex and cerebellum **[4-16-23, 58]**, having activates the GABAA receptor by its agonist, Muscimol may increasing cell death at different regions of the hippocampus **[68]**. Bicuculline, as GABAA receptor antagonist, has been used in studies to induce cell death **[34-40, 66]** and the hippocampus is involved in the anxiety disorders **[3, 31]**.

CONCLUSIONS

The excessive activation of GABAA receptors or the blockade of GABAA receptors in offspring may have resulted in the induction of cell death at the neural structures of anxiety at late embryonic stages which finally increases anxiogenic-like behaviors.

ABBREVIATIONS

BMC, bicucullinemethochloride ; CNS, central nervoussystem; DG, dentate gyrus; EPM, elevated plus-maze; FST, forced swimming test; GABA, γ -Aminobutyric acid; IP, Intraperitoneal injection; LMA, locomotor activity; NMDA, N-methyl-d-aspartate; OAE, open arm entries; OAT, open-arm time; PND, postnatal day; PT, picrotoxin; WHO, World Health Organization.

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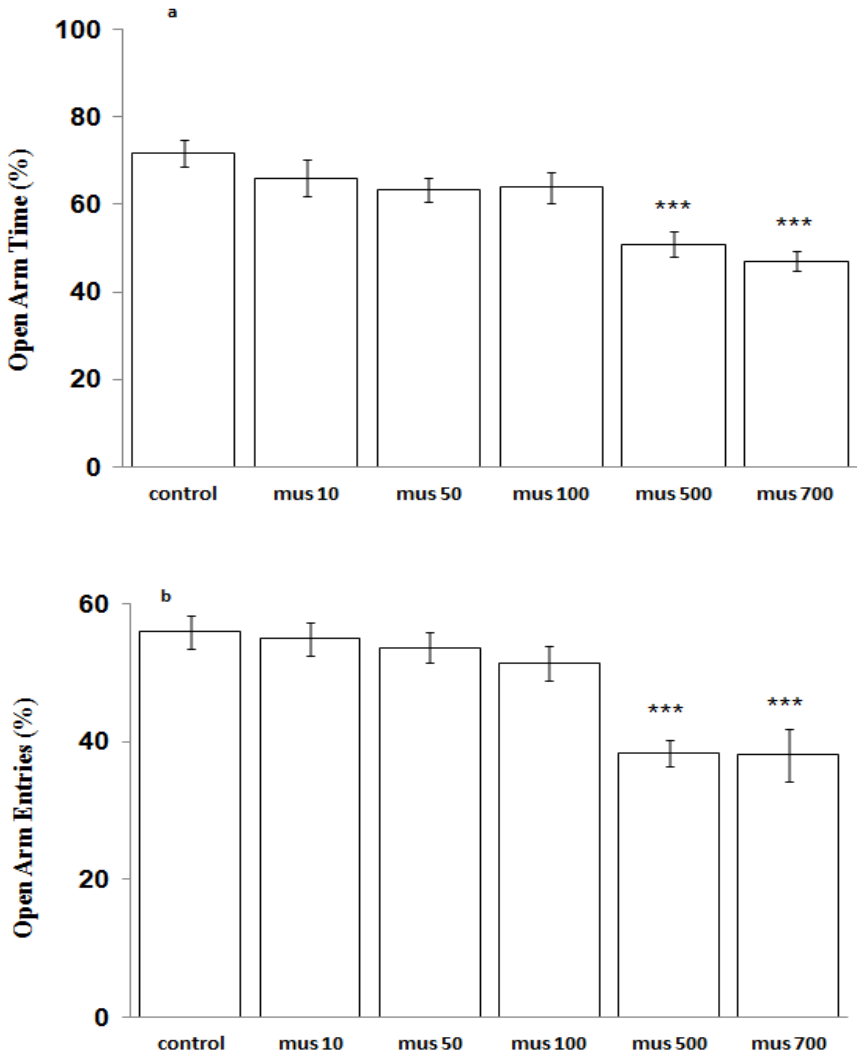


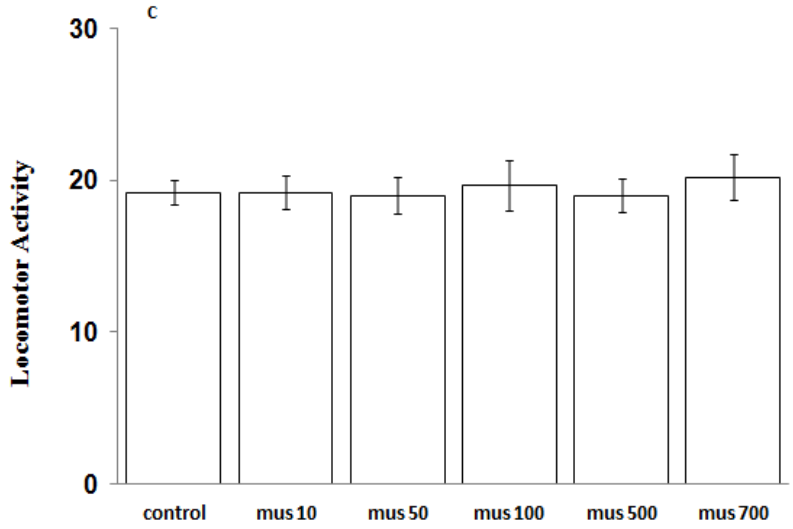
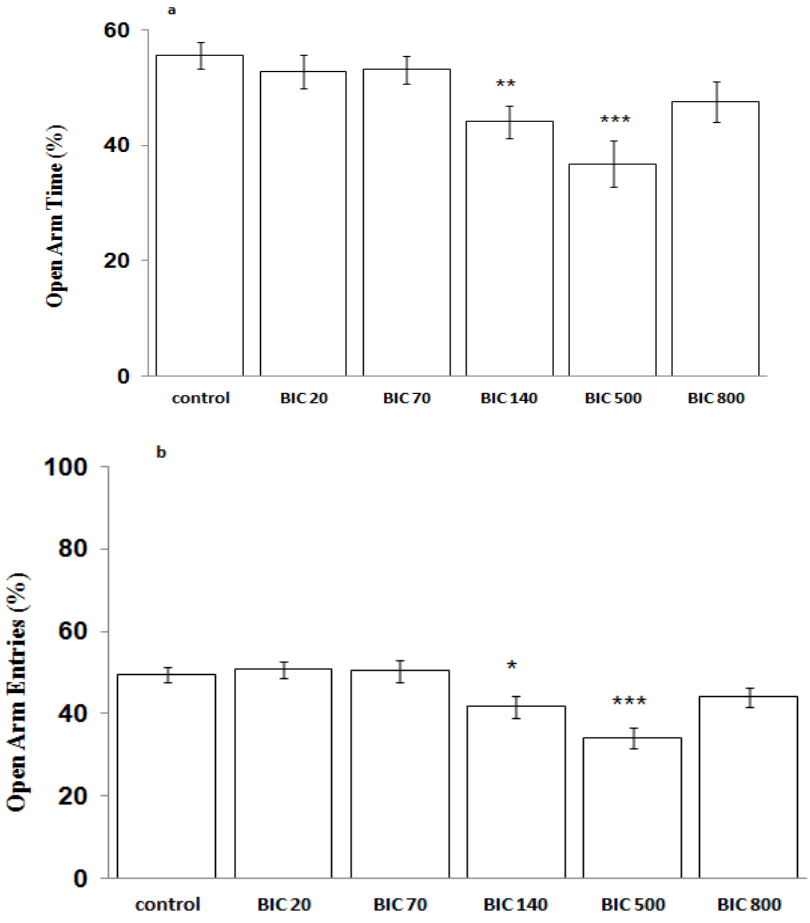


Figure 1: Effects of administration (IP injections) of muscimol the EPM. Mice were treated with either saline or with of muscimol (10, 50, 100, 500 and 700 µg / kg). Each bar is mean± SEM. N = 15. *P < 0.05 and **P < 0. 01, ***P<0.001 when compared to the control group.(One-way ONOVA)



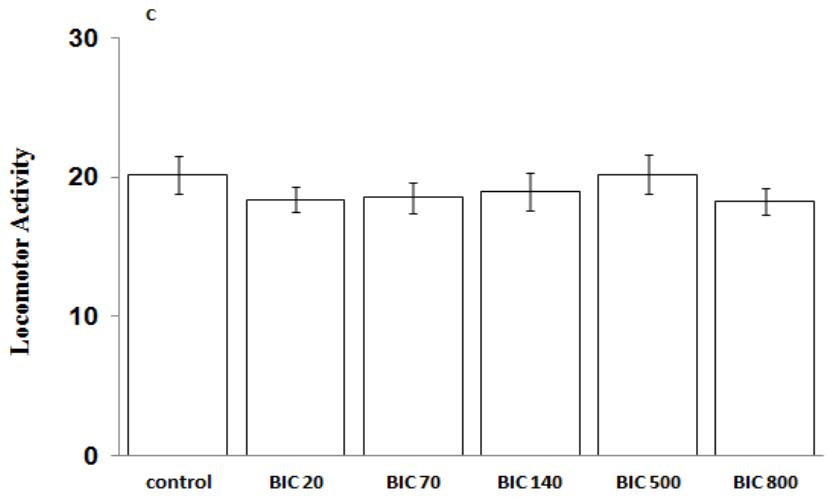


Figure 2: Effects of administration (IP injections) of Bicuculline on the EPM. Mice were treated with either saline or with of Bicuculline (20, 70, 140, 500 and 800 µg / kg). Each bar is mean± SEM. N = 15. *P < 0.05 and **P < 0. 01, ***P<0.001 when compared to the control group. (One-way ANOVA)

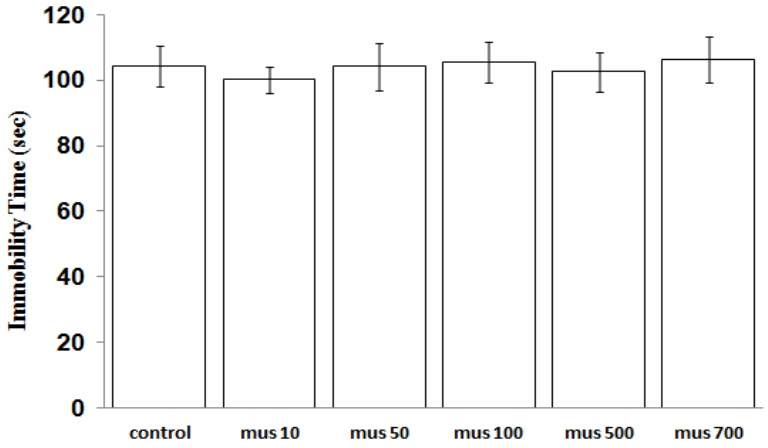


Figure 3: Effects of administration (IP injections) of muscimol on depression behaviors in the FST. Mice were treated with either saline or with of muscimol (10, 50, 100, 500 and 700 µg / kg). ***The one-way ANOVA does not show significant effects on the immobility time in the FST .***

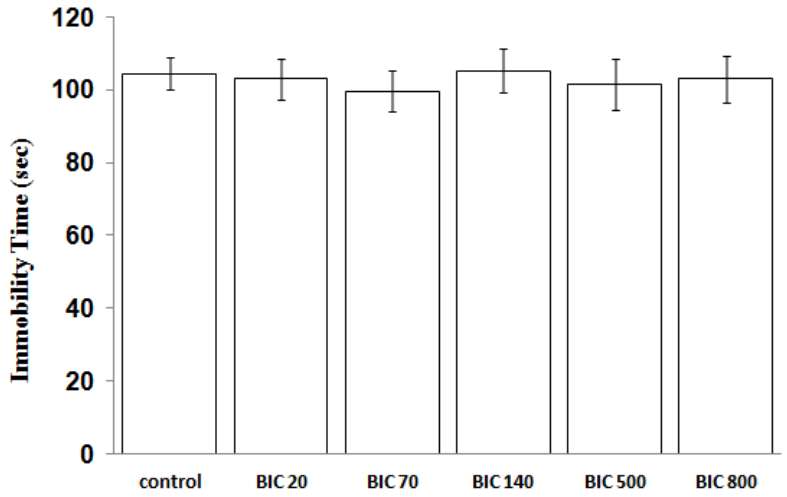


Figure 4: Effects of administration (IP injections) of Bicuculline on depression behaviors in the FST. Mice were treated with either saline or with of Bicuculline (20, 70, 140, 500 and 800 µg / kg). ***The one-way ANOVA does not show significant effects on the immobility time in the FST***