Efectos de la estimulación eléctrica habenular en la modulación de respuestas emocionales en ratas Wistar

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Resumen

A pesar del amplio uso de la estimulación cerebral profunda para controlar patologías neurológicas y neuropsiquiátricas, su mecanismo de acción aún no es claramente conocido, y existen pocos estudios sistemáticos que relacionen la variación de parámetros de estimulación eléctrica (frecuencia, intensidad, duración del pulso) y la ejecución comportamental. La habénula es una estructura reguladora de respuestas emocionales diana en tratamientos para dolor crónico y depresión, pero la relación entre su estimulación crónica y el desempeño animal en pruebas conductuales no se ha establecido con claridad. Con el objetivo de evaluar el efecto emocional de la estimulación habenular crónica, en este estudio se utilizaron ratas Wistar que recibieron estimulación habenular a intensidad baja (10-80 μA) o alta (120-260 μA) y frecuencia baja (80-150 Hz) o alta (240-380 Hz): BIBF-AIBF-BIAF-AIAF, durante 15 minutos a lo largo de tres días consecutivos. Al cuarto día, se hizo la evaluación en un laberinto elevado en cruz y en campo abierto. Los resultados indican un efecto de tipo ansiolítico en el tratamiento BIAF, en comparación con BIBF y AIBF (aumento del número de entradas, porcentaje de tiempo en brazos abiertos y de la distancia recorrida en ellos), efecto que no se explica por cambios en la locomotricidad (distancia recorrida en los brazos cerrados y la exploración en el campo abierto). Se concluye que el parámetro *frecuencia* posee mayor impacto sobre el efecto comportamental que la *intensidad* –lo que puede explicar algunos hallazgos paradójicos previos–, que los parámetros utilizados no poseen efecto ansiogénico, y que los efectos potencialmente ansiogénicos de la estimulación a baja frecuencia y el papel de los sistemas dopaminérgicos y serotoninérgicos encontrados deben ser estudiados en futuras investigaciones.
Palabras clave: comportamiento emocional, estimulación eléctrica cerebral profunda, habénula, ratas.

Effects of electrical stimulation of the habenula on the modulation of emotional responses in Wistar rats

Abstract

Deep brain stimulation is a widely-used approach to the treatment of neurologic and neuropsychiatric diseases. However, its mechanisms remain unclear. There are few systematic studies relating variations on electrical stimulation parameters (frequency, intensity, pulse duration) and behavioral outcome. The habenula relates to emotional behavior and is a main target for chronic pain and depression stimulation treatment. The relation between habenular electrical stimulation and performance in behavioral tests has not been clearly defined. In order to assess the emotional effects of chronic habenular electrical stimulation, Wistar male rats were unilaterally implanted with electrodes aimed to the lateral habenula and assigned to low (10-80 μA) or high (120-260 μA) intensity and low (80-150 Hz) or high (240-380 Hz) frequency conditions: BIBF-AIBF-BIAF-AIAF. They received electrical stimulation 15 minutes/day for three consecutive days and on the fourth day were tested in the elevated plus maze and the open field. The results of these study show that BIAF stimulation has a possible anxiolytic-like effect when compared to BIBF and AIBF (increase in the percentage of open-arms time, entries into the open-arms and total-distance-run in the open-arms). This is not due to any changes in locomotion (total-distance-run and open field exploration). It is concluded that frequency is more important than intensity for behavioral modification. This could explain some previous inconsistent results. The data also suggest that these parameters of stimulation have no anxiogenic effects. The role for dopaminergic and serotonergic systems must be subsequently evaluated as well as potential anxiogenic-like effects of low frequency stimulation.
Key words: deep brain stimulation, emotional behavior, habenula, rats.

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Efeitos da estimulação elétrica habenular na modulação de respostas emocionais em ratos Wistar

Resumo

Apesar do amplo uso da estimulação cerebral profunda para controlar patologias neurológicas e neuropsiquiátricas, seu mecanismo de ação ainda não é claramente conhecido e existem poucos estudos sistemáticos que relacionem a variação de parâmetros de estimulação elétrica (frequência, intensidade, duração do pulso) e a execução comportamental. A habênula é uma estrutura reguladora de respostas emocionais específicas em tratamentos para dor crônica e depressão, mas a relação entre sua estimulação crônica e o desempenho animal em testes comportamentais não foi claramente estabelecida. Com o objetivo de avaliar o efeito emocional da estimulação habenular crônica, neste estudo foram utilizados ratos Wistar que receberam estimulação habenular de intensidade baixa (10-80 μA) ou alta (120-260 μA) e frequência baixa (80-150 Hz) ou alta (240-380 Hz): BIBF-AIBF-BIAF-AIAF, durante 15 minutos ao longo de três dias consecutivos. No quarto dia, foi feita a avaliação em um labirinto em cruz elevado e em campo aberto. Os resultados indicam um efeito de tipo ansiolítico no tratamento BIAF, em comparação com BIBF e AIBF (aumento do número de entradas, porcentagem de tempo em braços abertos e da distância percorrida neles), efeito que não se explica por mudanças na locomotividade (distância percorrida nos braços fechados e a exploração no campo aberto). Conclui-se que o parâmetro "frequência" tem mais impacto sobre o efeito comportamental do que a "intensidade" – o que pode explicar algumas descobertas paradoxais prévias –, que os parâmetros utilizados não tenham efeito ansiogênico, e que os efeitos potencialmente ansiogênicos da estimulação de baixa frequência e o papel dos sistemas dopaminérgicos e serotoninérgicos encontrados devem ser estudados em pesquisas futuras.
Palavras-chave: comportamento emocional, estimulação elétrica cerebral profunda, habênula, ratos.

INTRODUCTION

Deep Brain Stimulation (DBS) is one of the most developed techniques over the last years which have a promising potential for future treatment of many neurological and neuropsychiatric conditions such as Parkinson disease (Borgonovo et al., 2017; Arocho-Quinones, Hammer, Bock, & Pahapill, 2017; Fukaya et al., 2017; Birchall et al., 2017), distonia and diskynesia (Almeida et al., 2017; Cif & Coubes, 2017; Lumsden, Kaminska, Ashkan, Selway, & Lin, 2017; Ostrem et al., 2017; Toda, Saiki, Nishida, & Iwasaki, 2016; Kim, Chang, Jung, & Chang, 2015), epilepsy (Cukiert & Lehtimaki, 2017; Dalkilic, 2017; Klinger & Mittal, 2016; Krishna, Sammartino, King, So, & Wennberg, 2016), obsessive compulsive disorders (Choudhury, Davidson, Viswanathan, & Strutt, 2017; Dell'Osso, Cremaschi, Oldani, & Carlo, 2017; Mulders et al., 2016; Coenen et al., 2016; Sturm et al., 2003), anxiety (Chang et al., 2012; Bewernick et al., 2010; Clark et al., 2009; Castelli et al., 2006), aggressiveness (Golden et al., 2016; Harat, Rudas, Zielinski, Birska, & Sokal, 2015; Faria, 2013; Howland, 2013), chronic pain (Ray & Burton, 1980; Plotkin, 1982; Li et al., 2017; Boadas-Vaello, Homs, Reina, Carrera, & Verdu, 2017), obesity (Dupre, Tomycz, Oh, & Whiting, 2015), depression (Bewernick et al., 2017; Birchall et al., 2017; Accolla et al., 2016; Bergfeld et al., 2016; Sourani, Eitan, Gordon, & Goelman, 2012), and schizophrenia (Nicolaidis, 2017; Agarwal, Sarris, Herschman, Agarwal, & Mammis, 2016; Salgado-Lopez et al., 2016; Bakay, 2009), among others.

The origin of brain stimulation goes back possibly to the year 46 of our era when Scribonius Largus proposed to set electric fishes (*Torpedo nobiliana*) over the head of patients with migrain and epilepsy (Schwalb & Hamani, 2008). At the end of the XIX century, Gustav Fritsch and Eduard Hitzig began the era of the electric brain stimulation, when they provoked muscular contractions in anesthetized dogs, as a consequence of the electrical stimulation of the cerebral cortex. Equally well known are the works developed in humans in the mid XX century by Wilder Penfield that led to the development of motor and sensory maps of the cerebral cortex, and without doubt, constituted the initial bases for the actual DBS (Murrow, 2014).

In the modern age, Lawrence Pool first used electrical brain stimulation as a therapy in 1948, which successfully treated symptoms of depression and anorexia in a patient with Parkinson disease by implanting an electrode in the caudate nucleus (Rosenow, Mogilnert, Ahmed, & Rezai, 2004). The awareness about the serious secondary effects associated to the pharmacological treatments, such as L-Dopa, the technological advance, and the knowledge of the physiology of the basal ganglia (impulsed largely by the study on animal models), widely encouraged brain stimulation for the treatment of Parkinson and other associated motor problems from the XX century (Roth, Flashman, Saykin, & Roberts, 2001; Schwalb & Hamani, 2008).

Another therapeutic area that has been benefited by the use of DBS, different to the treatment of motor symptomatology, is the control of chronic pain (Schwalb & Hamani, 2008; Boccard, Pereira, & Aziz, 2015; Cruccu et al., 2016). Nevertheless, despite the wide diffusion of the technique, the mechanism by which it works is not clear. It has been identified that high-frequency stimulation may have a superior

effect than low-frequency stimulation, but it is unclear if the effect is given by the depletion of the neurotransmitter, associated to the induction of a high rate of shooting or by the increasing release of the neurotransmitter. In fact, neurosurgery for the implant of DBS electrodes is performed with the patient awake, in such a way that the effectiveness of the stimulation is evaluated by adjusting the best parameters of frequency, intensity, and inter-pulse interval for each case. As far as it is known, the neurobiological long-term effects about processes such as *up-regulation* or *down-regulation* of receptors, the variation in plasticity associated with the increase (or decrease) of neurological activity or possible lesions dealing with the changes in the dynamic synapse in different regions of the brain have not been investigated (Udupa & Chen, 2015). In this way, studies are needed to determine the mechanism by which DBS exerts its effects.

The use of the electrolytic technique, as a tool to determine the role of different structures in certain behaviors, has demonstrated that the lesions over the habenula alter various affective and behavioral processes, mainly emotional, such as pain (Li et al., 2017; Margolis & Fields, 2016; Shelton, Becerra, & Borsook, 2012), reinforcement (Borsook et al., 2016; Baker, Raynor, Francis, & Mizumori, 2017; Borsook et al., 2016; Jean-Richard Dit & McNally, 2014), synaptic plasticity in a long term depression (Lecourtier et al., 2006), and behaviors related to adiction (Velasquez, Molfese, & Salas, 2014; Lecca, Meye, & Mameli, 2014; Yadid, Gispan, & Lax, 2013; Baldwin, Alanis, & Salas, 2011).

Studies on habenular damage indicated that the lesion of certain efferent paths of the habenula (mostly the retroflex fascicle) causes an increase of anxiety and locomotion in rats, as well as a rise in the plasmatic corticosterone levels and grooming behavior (Murphy, DiCamillo, Haun, & Murray, 1996). Likewise, it is interesting that after a habenular lesion the ability to react adequately to stress is lost, even though some authors have found contradictory results (Amat et al., 2001; Thornton & Bradbury, 1989; Hennigan, D'Ardenne, & McClure, 2015). These apparently contradictory results could explain that the habenula has an important role in the evaluation of reinforcement of various stimuli (Bromberg-Martin & Hikosaka, 2011). In fact, the activation of the habenula changes the dopaminergic and serotoninergic activity (Yang, Hu, Xia, Zhang, & Zhao, 2008). The differential activation of each of these systems could occur depending on their activity pattern.

In relation with anxiety, it has been demonstrated that the inactivation of the habenula causes a reduction of the rat`s anxiety during the elevated plus maze (Gill, Ghee, Harper, & See, 2013), but to date there are no reports of the effect of the increase of habenular activity on this type of behavior. Thus, the objective of this study is to determine if different patterns of lateral habenula stimulation lead to alterations in behaviors associated to anxiety and locomotion. For this purpose, and looking for a systematic study, two parameters of the stimulation were selected: frequency and intensity. Taking into consideration previous reports about DBS (Arocho-Quinones et al., 2017; Kim et al., 2016; Li et al., 2016), it was decided to divide the frequency parameter into two levels: high and low, determining a cut-off point of 150 Hz. In the same way, the intensity parameter was separated into two levels: high and low, determining a cut-off point of 100uA (Yeomans, 1990).

METHOD

Experimental design

Four experimental groups and one control group were used, without repeated measures. The experimental groups received unilateral DBS on the habenula, with a variation in the intensity: low (10 – 80 μA) or high (120 – 260 μA) and in the frequency: low (80 – 150 Hz) or high (240 – 380 Hz).

Subjects

26 male Wistar rats, weighing between 250 and 350 g were used. They were raised at the Charles River Institute and kept at the Neuroscience and Behavior Laboratory of the Universidad de los Andes. The animals were housed in home boxes under controlled lighting conditions (light-dark cycle 12:12h, light started at 5:00PM), temperature (23ºC±2), relative humidity maintained (57%), attenuation of external noise, and with free access to water and food throughout the experiment. All the animals were manipulated three days before the start of the experiments, for ten minutes each day, in order to familiarize them with the investigator, the processes, and the experimental room.

Materials

Deep Brain Stimulation. An S88X pulse stimulator (GRASS) was used to perform the brain stimulation, with four pulse control parameters (frequency, duration, repetitiveness in trains, and inter-pulse interval) for two independent outputs of current stimulation.

Elevated plus maze. In order to evaluate the anxiety levels of the animals, an elevated plus maze was used, which is a wooden artifact with two 1 m length perpendicularly intercrossed arms. Two of them are enclosed by walls of 50 cm in height (closed arms) and the other two bareheaded with a border of 1 cm in height to avoid the fall of the animals (open arms). The complete apparatus is raised to a height of 50 cm from the ground. The experiments were recorded

and digitized for further analysis. For the behavioral record, each arm was virtually divided into 10 cm squares.

Open field. This test consists of a cubic dark acrylic box of 60 cm per side, without the top cover. Experiments were recorded and digitized for later registration. For the behavioral record, the floor of the open field was virtually divided into squares of 10 cm on each side.

Procedure

The subjects were randomly assigned to one of the five groups, to receive DBS in the habenula, with different frequency and intensity parameters. The characteristics of the stimulation comprised low (10 – 80μA) and high (120 –260μA) intensities, and low (80 – 150 Hz) and high (240 – 380 Hz) frequencies. In this way, the following groups were conformed: low intensity – high frequency (LILF, n=5), high intensity – low frequency (HILF, n=5), high intensity – low frequency (HILF, n=5), and high intensity – high frequency (n=5). Additionally, a control group (n=6) was created, in which subjects underwent the same procedures but did not receive any stimulation. Table 1 represents the assignation of the subjects.

Table 1
Assignment of subjects to experimental groups

Group	Intensity	Frequency
LILF, n=5	Low (10-80 μA)	Low (80-150 Hz)
HILF, n=5	High (120-260 μA)	Low (80-150 Hz)
LIHF, n=5	Low (10-80 μA)	High (240-380 Hz)
HIHF, n=5	High (120-260 μA)	High (240-380 Hz)
Control group, n=6	-	-

The intracerebral electrodes for the stimulation were placed through traditional stereotaxic surgery. For this, animals were anesthetized with an intraperitoneal injection of pentobarbital (Penthal 64,8 mg/Kg) and set in the stereotaxic structure; lidocaine (2%) was used as a local anesthetic. During the surgery, vital signs were monitored and body temperature was maintained with a thermic blanket. The scalp was removed and the skull exposed. A 0.5 mm hole was made with a moto-tool, through which the electrode was inserted unilaterally with a 20º inclination. The final location of the electrode was AP: -3.6mm, ML: 0.7 mm, and DV: 5.0 mm according to Paxinos`s and Watson`s (2006) atlas, taking the bregma point as a reference. The electrode was fixed to the skull with two screws and dental acrylic. Once the surgery had finished, the animals were treated with an anti-inflammatory analgesic (Meloxicam 1mg/Kg) and antibiotic (Enrofloxacina 10 mg/Kg). All of the animals had a post-surgery recovery period of 6 days.

For the stimulation, each animal was taken from its habitat box and placed in a stimulation box. After a minute familiarization, the stimulation began with the frequency and intensity previously assigned to the subject, depending on the group it belonged to. Stimulation lasted 15 continuous minutes per day, during three consecutive days. Twenty-four hours after the last stimulation, the animals were transported to experimental rooms for the behavioral assessment. Due to the high sensitivity of the elevated plus maze to changes in the anxiety state of the animals, this test was always performed before the open field test. Figure 1 presents a scheme of the procedure.

During the behavioral evaluation phase, each animal was placed in the center of the elevated plus maze facing one of the closed arms and was permitted to explore freely for five minutes. During this period, the number of entries and the time spent in each arm and center was quantified. In addition, the frequency and duration of the behaviors such as grooming, stretching, lifting, and looking down were also recorded, by the edge of the open arms.

After the exploration time in the elevated plus maze, the animal was taken to another experimental room for the evaluation in the open field. Each subject was placed in the center of the field facing one of the walls and allowed to explore freely for five minutes. The time spent in the periphery (defined as a corridor of 10 cm wide from any of the walls) and in the center of the field was registered. Always, after finishing the test, the device was cleaned with alcohol 10% to avoid olfactory clues.

After the behavioral evaluation, the animals were anesthetized with a mixture of ketamine/xylazine (90mg/Kg + 10mg/Kg) and perfused with 300mL of saline solution (0.9%), followed by 300mL of paraformaldehyde (4%). Brains were extracted, stored in paraformaldehyde (4%) for four days, and processed for histological use in order to verify the location of the electrodes, coronal slices of 30μm thickness were made in a vibratome (VIBRATOME 1500). These cuts were subsequently marked with Nissl coloration by cresyl violet. The data corresponding to the animals that showed locations of the stimulation point different from the desired one were removed from the study.

Ethical considerations

All the procedures performed in this work were approved by the Institutional Committee for the Care and use of Laboratory Animals (CICUAL) of Los Andes University and were aligned with the ethical and legal standards required for research with laboratory animals in Colombia (Law 84 of 1989 and resolution No. 8430 of 1993 Ministry of health). Agreements of the Universal Declaration of Animal Rights were proclaimed by the International League of Animal Rights, Geneva, Switzerland (1989), and the ethical principles of the

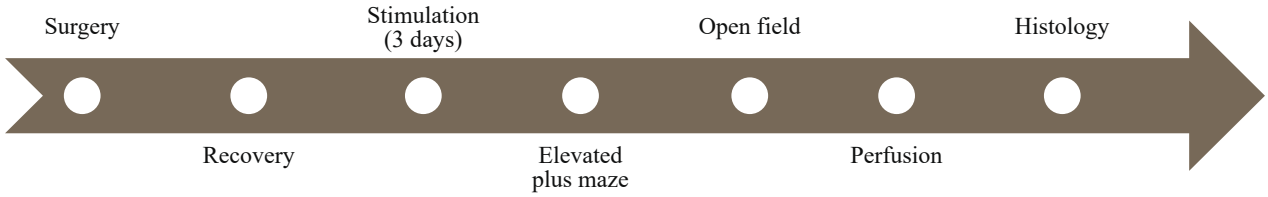


Figure 1. Experimental procedure

animal experimentations announced by the *International Council for Laboratory Animal Science* (ICLAS).

Data analysis

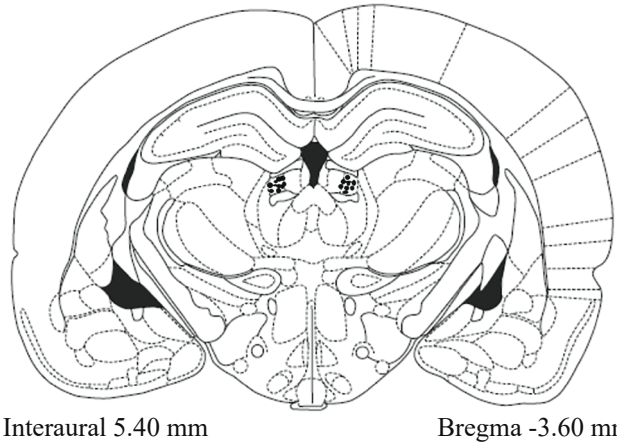
Normality tests (Shapiro-Wilk) were approved for all the measurements, which permitted the realization of a one-way analysis of variance. When it was necessary, a *post hoc* analysis was made, using Tukey test. In all of the cases, the alpha was established as 0.05.

RESULTS

The results of the study are presented below, reporting initially the histological findings and later the emotional and motor behavior ones.

Histology

Figure 2 indicates the location of the electrodes. The animals that did not have the electrode in the desired place were removed from the study.



Interaural 5.40 mm Bregma -3.60 mm

Figure 2. Points of arrival of the electrodes. Adapted from Paxinos and Watson, 2006.

Elevated plus maze

In relation to the number of entries to the open arms, ANOVA showed significant differences between the effects of the types of stimulation ($F_{[4,21]}$=6,71; P<0,001). Post hoc comparison of the mean among the groups (Tukey) indicated that the animals from LIHF entered more to the open arms of the maze than the animals of HILF and LILF (P<0,05). No effect was found on the number of entries to closed arms (figure 3-B), ($F_{[4,21]}$=0,91; P=0,475).

In relation to the percentage of entries to the open arms (figure 3-A), the ANOVA showed that there were significant differences between the effects of the stimulation ($F_{[4,21]}$=3,06; P=0,039). The post hoc comparison of the means of the groups (Tukey) showed that the animals of the LIHF group entered more into the open arms of the maze than the animals of the HILF group (P<0,05).

ANOVA showed that there was a stimulation effect on the percentage of time the rats spent in the open arms ($F_{[4,21]}$=4,43; P=0,01) (figure 3-C). Post hoc comparison of the mean of the groups (Tukey) showed that the animals of the LIHF group remained more in the open arms than the ones from the HILF and LILF groups (P<0,05). In relation to the total distance traveled within the open arm (figure 3-D), the ANOVA showed a significant effect of the stimulation ($F_{[4,21]}$=4,88; P=0,01). Post hoc comparison of the mean of the groups showed that the animals of the LIHF group traveled more distance than the animals of the HILF and LILF groups (P<0,05, see table 2 for the statistical results of all measures).

Open field

The performance of the subjects in the open field showed that there were no significant differences in locomotion, measured in terms of the total distance traveled, obtained from the total number of crosses made ($F_{[4,21]}$=0,57; p=0,642). No significant differences were found for the percentage of time spent in the center or for the time spent in grooming ($F_{[4,21]}$=0,87; p=0,501, $F_{[4,21]}$=1,44; p=0,26, respectively, see figure 4 and table 3 for the statistical results of all measures).

DISCUSSION

In this study the effect of the sub-chronic electrical stimulation of the lateral habenula on anxiety levels and the locomotor response in Wistar rats was evaluated. Animals were tested in the elevated plus maze and open field after the

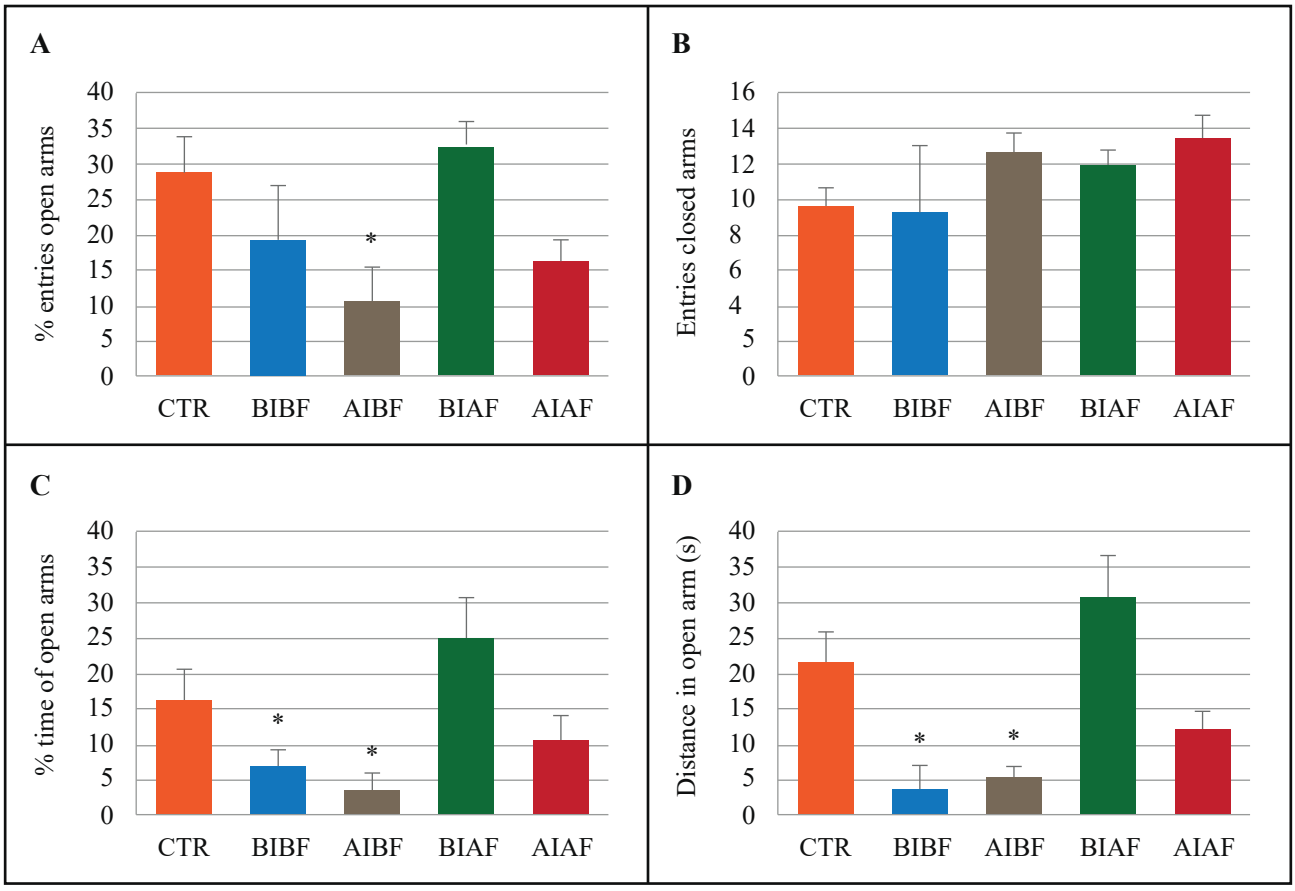


Figure 3. (A) Entries to the open arms of the elevated plus maze. (B) Entries to the closed arms. (C) Percentage of time spent in the open arms. (D) Total distance covered within the open arm. (*) Difference in relation to the group LIHF (P<0,05).

Table 2
Statistical results for the behaviors in the elevated plus maze

Behavior	F[4,21]	p
Entries to the open arm	6.709	< .001*
Percentage of entries to the open arm	3.058	.039*
Percentage of time in the open arm	4.430	.009*
Distance traveled in the open arm	4.880	.006*
Entries to closed arm	0.912	.475
Percentage of time in the closed arm	4.430	.009*
Distance traveled in the closed arm	0.590	.672
Percentage of time in the center	2.420	.080
Grooming time	1.191	.343

electrical stimulation of the lateral habenula during fifteen minutes for three consecutive days. The lateral habenula has been linked with many emotional and cognitive processes (Ootsuka & Mohammed, 2015; Hong & Hikosaka, 2008; Hikosaka, Sesack, Lecourtier, & Shepard, 2008; Chan et al., 2017; Moreines, Owrutsky, & Grace, 2017; Baker et al., 2016; Baker, Oh, Kidder, & Mizumori, 2015; Zhao, Zhang, Yang, & Rusak, 2015; Lecourtier, Neijt, & Kelly, 2004). However, although many studies have been carried out using the electrical stimulation of this structure as a methodology, there is still no consensus on the most effective type of stimulation. For example, some authors

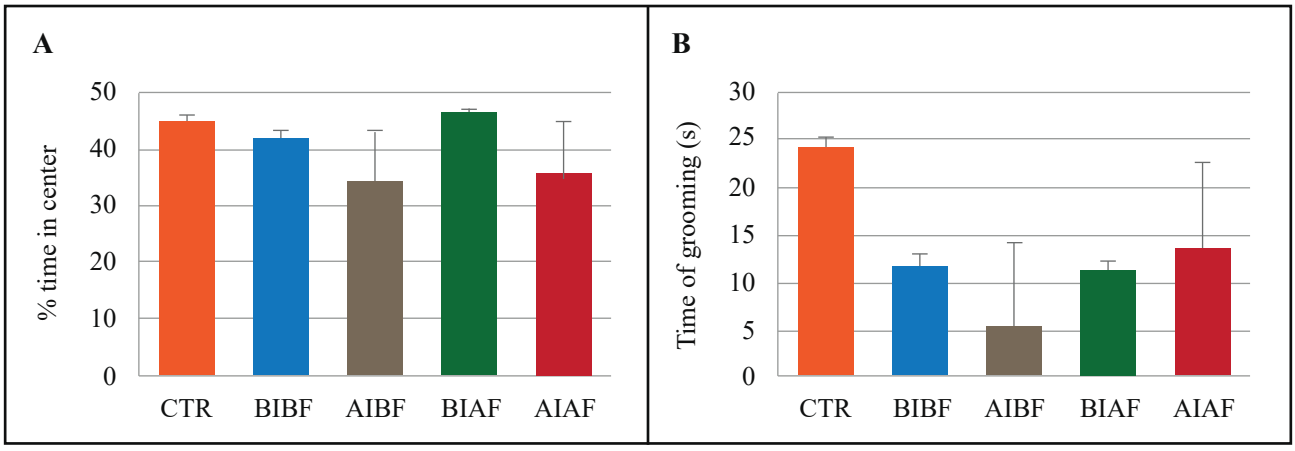


Figure 4. (A) Percentage of permanence in the central area of the open field. (B) Time for grooming in the open field.

Table 3
Statistical results for the behaviors in the open field

Behavior	F[4,21]	*p*
Entries to the central area	0.853	.051
Total distance traveled	0.569	.642
Percentage of time spent in the central area	0.867	.501
Number of liftings	1.528	.232
Time of liftings	1.517	.235
Frequency of grooming	0.590	.674
Time spent in grooming	1.440	.257
Frequency of freezing	0.570	.688
Time of freezing	0.365	.831

such as Li, Zuo, Fu et al (2016; 2017) reported positive results with the electrical stimulation using a frequency of 130 Hz and an intensity if 150 µA (equivalent in this study to group HILF), while with low frequencies (eg. 80 HZ) and the same intensity do not presented results (equivalent to the same group in these study).

In this sense, these results indicate that the stimulation of the habenula with high frequency (240-380 Hz) induces clear behavioral effects by decreasing anxiety in the elevated plus maze. The stimulation of the same brain structure at low frequencies (80-150 Hz) did not induce changes. These data contrast with the reports of Hedt and Ressler (2006) and Gill et al. (2013), who described emotional changes associated with habenula lesions, including reduction of anxiety responses in rats with habenula inactivation. Their results, despite the use of a different methodology allow determining that the habenula has an important role in the generation and expression of emotional reactions. Perhaps the stimulation, to which our animals were subjected, caused an inhibitory effect on habenular eferences, either by a depletion phenomenon of neurotransmitters or by a decrease in the expression of receptors in postsynaptic structures. Unfortunately, these results do not allow us to determine the mechanism that causes this effect.

Song et al. (2017) recently reported that the complete lesion of the lateral habenula facilitates the learning of the active avoidance response and the extinction of fear (2017). These results would seem to contradict ours; nonetheless, it could be proposed that either the stimulation performed in the habenula had long-term effects (perchance mediated by processes of desensitization of receptors in target structures) or that the stimulation carried out generates a state of neural "fatigue" (possibly due to depletion of neurotransmitters), which remains for a few minutes after the stimulation has ended and stimulates the effect of the injury performed by Song et al. (2017). In any case, the realization of new experiments to determine the mechanism of action is required.

The results reported here, induced by the stimulation of the habenula, seem to be restricted to the emotional sphere,

since no changes were found in the motor function, evaluated in the elevated plus maze, by analyzing the total distance covered in the closed arm, as in the open field. In this sense, the data found in this study are in line with those reported by several authors in relation to increases in motor function associated with habenula lesions (Han, Jin, Song, Wang, & Zhao, 2014; Murphy et al., 1996; Wickens & Thornton, 1996). In this study, no alterations were found in grooming behavior, neither in the elevated plus maze and in the open field. According to the reports of Murphy and collaborators (1996), a decrease of these behaviors would be expected, since they found them elevated after the injury of the habenula.

There are many reports that correlate changes in the cerebral levels of serotonin (mainly in the amygdala and prefrontal cortex) with an increase in anxious responses in the elevated plus maze (John & Currie, 2012, two, by Andrade, & Graeff , 2010, Moraes, Bertoglio, & Carobrez, 2008, Faria et al., 2006, Lin & Parsons, 2002, Setem, Pinheiro, Motta, Morato, & Cruz, 1999, Andersen & Teicher, 1999, Maisonnette, Morato, & Brandao, 1993, Motta, Maisonnette, Morato, Castrechini, & Brandao, 1992). Because the activation of the habenula has a facilitating effect on the release of serotonin (Rolls, 2017, Lim et al., 2015, Vadovicova, 2014, Pobbe & Zangrossi, Jr., 2010, Yang et al., 2008), it would be feasible to suppose that the effect of the increment in the time spent in the open arms of the elevated plus maze, as well as the increase of the total distance traveled within the open arms, is an adaptation effect mediated by serotonin.

Finally, despite that differences in relation to the control group were not found, it is evident that the groups receiving low frequency stimulation showed a marked decrease in the exploration of the open arms, in terms of the percentage of permanence ($\overline{x}$[CTR]=16,7; $\overline{x}$[BIBF]=6,2; $\overline{x}$[AIBF]=3,6), number of entries ($\overline{x}$[CTR]=4,0; $\overline{x}$[BIBF]=1,2; $\overline{x}$ [AIBF]=1,6), and the total distance covered in them ($\overline{x}$ [CTR]=21,7; $\overline{x}$[BIBF]=3,6; $\overline{x}$[AIBF]=5,0). The exploration of smaller frequencies is recommended to confirm if they could increase these differences, constituting a possible anxiety-type effect.

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