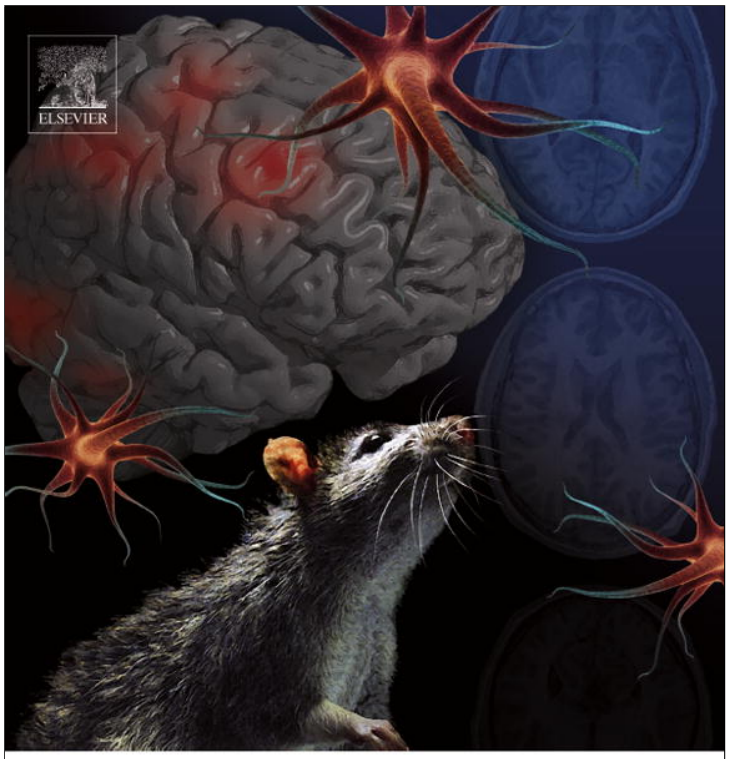

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Research report

Functional lateralization of the baso-lateral amygdala neural circuits modulating the motivated exploratory behaviour in rats: Role of histamine

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ABSTRACT

Functional laterality appears to be present in many brain functions in man and animals. The existence of paired neural circuits which act differentially to modulate a specific behavioural function seems to be an evolutionary successful strategy in animal evolution. In spite of many examples described in mammals, birds and other vertebrates and invertebrates, still its intrinsic mechanism is not completely understood. In this work the participation of the baso-lateral amygdala (BLA) on lateralized motivated exploratory behaviour and the possible influence of histamine neurons in these mechanisms were studied in rats. Different groups of animals under xylacine–ketamine anesthesia were implanted with microinjection guide cannulae into the right or left BLA. 72 h after implantation, animals were tested in hole-board cage (OVM) with a novelty object positioned in the center of the arena, as a model of exploration of a non-conflictive environment, and 24 h later they were tested in the Elevated Asymmetric Plus Maze (APM) as a model of conflictive exploration. In the day of the experiment, lidocaine was applied into the left, or right BLA in order to block the electrical activity of BLA neurons. Saline in the contralateral BLA was considered control. Results showed that exploratory activity in the OVM was significantly inhibited when lidocaine was microinjected into the left BLA, and no changes were observed when lidocaine was applied into the right BLA. When histamine was microinjected into the right BLA and lidocaine into the contralateral BLA, head-dipping, rearing, and focalized exploration behaviour were significantly inhibited. In the APM, lidocaine treatment increased equally the exploration of the "single wall" and "high and low walls" arms of the labyrinth, independently if blocking of electrical activity of the BLA neurons was performed in the left or right amygdala. Histamine treatment inhibited significantly exploration of the lesser fear-inducing arms of the labyrinth but its effect was more pronounced when histamine microinjection was in the left BLA. In conclusion, present evidence support the lateralized participation of the amygdala on exploratory behaviour and histamine neurons appear to mediate part of these differential modulations.

1. Introduction

Brain functional lateralization or brain laterality was proposed about 146 years ago, when the French neurologist Jean P. Broca, after post-mortem examination of several of his patients suffering conspicuous speaking alterations described brain lesions in restricted regions of the left hemisphere, concluding that man speaks with the left hemisphere [9]. In spite that Broca's conclusion was very outstanding, suggesting a complete new aspect of the complex functioning of the brain, the scientific community thought that this was a rare finding in the very abundant physiological functions of man. However, research during the last part of the 20th century provided substantial evidence that the "rarity" appears to be a more common mechanism in brain functions that it was ever thought [12,38,41,48,49]. For instance, evidence has been found that the right parietal lobe in man process preferentially conceptual decisions of numbers instead of names in the same conditions of attention of tasks [11]. Using an ingenious experimental setup of the rubber hand illusion test in healthy adults, a right-hemispheric dominance was described for sense of body ownership [33]. Investigating the nature of the hemispheric specialization of brain activity during rhythm processing, functional magnetic resonance data showed that neural activity of left perisylvian cortices was higher than the contralateral structures in subjects with musical training [28]. This lateralization was not valid only for the musical tones, since stimulation with binaural linguistic sounds to normal subjects a predominant and consistent leftward functional asymmetry of the primary auditory cortex was found [50,51]. On the other hand, monitoring electromyographic activity from the zygomatic major and the corrugators supercilii muscle regions of the face of normal subjects exposed to happy or angry facial expres-

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sions, electrical activity was significantly greater in the left side of the face suggesting that the right brain hemisphere is predominantly involved in the control of spontaneous evoked emotional responses [17]. Finally, lateralization in its most evident feature is found in handedness of humans where approximately 90% of human population is right-handed implicating a differential motor specialization of the brain left hemisphere [27,37,46,50].

During some time there was the belief that brain laterality was a property exclusively of humans, since the most conspicuous representing functional characteristics were language and handedness, considered to be unique to the human species [1,41,45,48]. However, increasing evidence after description about innervations of the avian syrinx by right/left nerves fulfilling different roles on the song production [31,32], it become clear that lateralization processes appeared to be basic functional mechanisms operating in the brain. Lateralization has been found in a great variety of animal species. Thus, just only to cite a few examples, it has been reported that chicks with lesions of right hippocampus failed to find food hidden beneath sawdust by ground-scratching when the correct position of the food was indicated by a landmark [46]; pigeons with left ablation of the hippocampal structure were impaired to orient its way back to their home site [20]; correlative analysis between behavioural and neurochemical measures in brain marmosets revealed the importance of the left hemisphere in a predator confrontation experimental model [16]; tadpoles of five different species preferentially approached and positioned themselves to a mirror located on their left side, looking at the image with the monocular field of their left eye [7], and using the same technique of the mirror, fish also revealed a preferential use of the monocular visual field of the left eye to look its image [42]. The evident presence of lateralized responses in animals such as frogs, fishes, birds, non human and human mammals strongly suggest this mechanism is a general strategy in life evolution. Extensive reviews about lateralization covering several species with discussion about its possible evolutionary meaning have been published elsewhere [23,38,41,48,49].

The most basic characteristic in brain lateralization is the existence of paired neural circuits in the brain hemispheres exerting a differential control on some determined functions. However, the brain has many paired nuclei and neural areas but not all of them appear to be lateralized, indicating that the mere fact of being a paired structure is not enough to justify lateralization. Thus, Broca's and Wernicke's areas in frontal and parietal lobes of humans [9,46], hippocampal neural structures [34], the intraparietal sulcus of the parietal regions [11], perisylvian brain areas [28], primary auditory cortex [50], motor cortex neurons [44], and the amygdala complex [6,19,25,26,35] have been described as brain circuits involved in lateralization.

In animals, successful interaction with a changing environment requires versatile exploratory behaviours and threat-evaluating mechanisms in order to cope and show appropriate responses. In a different and more sophisticated context, this is also true for man. One neural circuit known to process fearful characteristics and incentivating aspects of environment is the amygdaloid structure [8,13,25]. The amygdala, composed by at least four major nuclei (lateral, baso-medial, baso-lateral and central) has extensive reciprocal projections with the multiple sensory neocortex, frontal lobes, ventral striatum, hypothalamus, midbrain reticular formation and brain stem [13,36]. All these brain structures are important to coordinate and generate plastic behavioural responses. Although it has been described that lateralization of the amygdaloid structure affects many complex functions in animals and humans, such as pain detection [25], fear discrimination [22], and provoking also changes in morphology and number of astrocyte neurons [26], still the intrinsic nature of the laterality mechanisms in this brain region are not clear. For instance, if right or left amygdala has some particular differential role in modulation of some basic behavioural expression such as those of exploring novel environments has not been fully investigated. Also, synaptic physiology involved in the lateralization mechanisms as evidenced as participation or identification of their neurotransmitters is not fully understood. Although some evidence has been found for some neurotransmitters in the amygdaloid structure possibly involved in lateralization processes [43], the role of neuromodulators and neurotransmitters still remain unclear.

Our laboratory has been working in the physiological role of histamine on cognitive processes in the brain [4] and it was of interest to study its possible participation on lateralization mechanisms in selected brain circuits of the limbic system. Thus, the purpose of this work was on one hand, to evaluate the possible lateralization of the neuronal circuits of the baso-lateral amygdala on the spontaneous exploratory behaviour in the rat, and on the other to define the possible role of histamine as neuromodulator of these lateralization processes.

2. Materials and methods

2.1. Animals

Male rats of a Holzman-derived colony, weighing 250–300 g, 90 days old and maintained in thermoregulated (22–24 °C) and controlled light conditions (06:00 on–20:00 h off) were used. Standard rat chow and water were available ad libitum.

2.2. Implantation procedures

Animals were anesthetized with a mixture of 3.75 mg/ml of xylacine and 62.5 mg/ml of ketamine in saline solution injected i.p. and bilaterally implanted with guide steel cannulae (23 gauge, 15 mm length) into the right and left basolateral nucleus of the amygdaloid complex (BLA). The baso-lateral nuclei were selected because their neuronal networks are involved in associative functions for external stimuli related to threatening situations [13] or learning under stressful conditions [2,29]. Stereotaxic coordinates were: 1.2 mm rostro-caudal, 1.0 mm lateral and 4.5 mm vertical. Bregma was considered the "zero" reference point. After implantation, rats were given one single shot of penicillin G in order to avoid any skin infection, caged individually and allowed to rest for at least 72 h before they were tested in the behavioural experiments.

2.3. Drugs

Lidocaine chlorohydrate 2.0% (AstraZeneca Laboratorios, Argentina), and histamine dihydrochloride (HA, Sigma Chemical Co., U.S.A.), freshly prepared in saline before the onset of the experiments, were used. Saline solution was considered control.

2.4. Experimental schedule

Three experiments were performed.

2.4.1. Experiment 1

Effect of lidocaine (Lid) administration into the right or left BLA on motivated exploration induced by exposition to a novel non-conflicting environment.

In this experiment the possible differential influence of the neural circuits of the right or left BLA on processing input from an unfamiliar environment with a low degree of exploratory conflict [3,40] was studied. Several groups of animals were microinjected into the right or left BLA with 0.2, 2 and 20 μg lidocaine and saline in the contralateral BLA. Thus, 6 groups were generated: lidocaine into the left BLA, saline into the right BLA (1) $Lid0.2_L/Sal_R$, $n = 12$; (2) $Lid2_l/Sal_R$, $n = 12$; (3) $Lid20_l/Sal_R$, $n = 12$; lidocaine into the right BLA, saline into the left BLA; (4) $Sal_l/Lid0.2_R$, $n = 12$; (5) $Sal_L/Lid2_R$, $n = 11$; (6) $Sal_L/Lid20_R$, $n = 13$. Saline into both BLA, was considered control (Sal/Sal, $n = 11$). On the day of the experiment (72 h after the implantation procedure), at 10:00–12:00 h the different groups were microinjected into the BLA with lidocaine and saline. Each animal was carefully restrained for microinjection of 0.5 μl of saline solution with or without lidocaine into each baso-lateral nucleus. Injection took about 15–20 s and rats were not stressed, remaining passive until procedure was ended, as previously reported in similar experiments [2]. Five minutes afterwards, animals were individually exposed to a 5 min exploration of an automatic behavioural activity detector (OVM, Optovarimex), enriched with a circular tube rack put in the center of the arena as a novelty object, as already described previously [3]. In order to test the approximate duration of the local anesthetic lidocaine effect into the BLA, separate groups of rats were microinjected at time zero into both BLA nuclei with 2 μg dose of lidocaine and tested 5 ($n = 13$), 15 ($n = 7$) and

25 min ($n = 7$) after injections in the OVM for a 5 min test. Behavioural activity in saline injected animals was considered control ($n = 12$).

2.4.2. Experiment 2

Effect of lidocaine and histamine (HA) administration into the right or left BLA on motivated exploration induced by exposition to a novel non-conflicting environment.

In this experiment, the possible influence of HA in the right or left BLA neuronal nuclei on motivated exploration induced by exposition to a novel non-conflicting environment was studied. Since the interest was to evaluate the single stimulation of HA on the BLA neurons, at the time of HA administration the neuronal activity of the contralateral nucleus was blocked by a 2 μg dose administration of lidocaine. A dose–response curve of histamine of 9, 45 and 90 nmol/nucleus was done. Thus, there were 6 experimental groups: lidocaine into the left BLA and HA into the right BLA: (1) $Lid_L/HA9_R$, $n = 10$; (2) $Lid_L/HA45_R$, $n = 13$; (3) $Lid_L/HA90_R$, $n = 14$. Lidocaine into the right BLA and HA into the left BLA; (4) $HA9_L/Lid_R$, $n = 13$; (5) $HA45_L/Lid_R$, $n = 10$; (6) $HA90_L/Lid_R$, $n = 13$. Lidocaine/saline combination was considered the relative control (Lid_R/Sal_L, $n = 16$; Sal_R/Lid_L, $n = 15$). Microinjection procedures were identical to those described in Section 2.4.1.

2.4.3. Experiment 3

Effect of lidocaine administration into the right or left BLA on motivated exploration induced by exposition to a novel conflicting environment.

In this experiment the possible differential influence of the neural circuits of the right or left BLA on processing input from an unfamiliar environment with a high degree of exploratory conflict [3,40] is studied. The elevated asymmetric plus maze (APM) was used as a model of a conflictive exploratory environment [39,40]. Lidocaine groups were: saline into the left BLA, lidocaine 2 μg into the right BLA ($n = 16$); lidocaine 2 μg into the left BLA, saline into the right BLA ($n = 15$). Saline into the left/right BLA was considered control ($n = 11$). Microinjection procedures were identical to those described in Section 2.4.1.

2.4.4. Experiment 4

Effect of lidocaine and histamine (HA) administration into the right or left BLA on motivated exploration induced by exposition to a novel conflicting environment.

In this experiment, the possible influence of HA in the right or left BLA neuronal nuclei on motivated exploration induced by exposition to a novel conflicting environment was studied. Six experimental groups were formed: lidocaine 2 μg into the left BLA, HA into the right BLA: (1) $Lid_L/HA9_R$, $n = 10$; (2) $Lid_L/HA45_R$, $n = 13$; (3) $Lid_L/HA90_R$, $n = 14$, and lidocaine 2 μg into the right BLA, HA into the left BLA; (4) $HA9_L/Lid_R$, $n = 13$; (5) $HA45_L/Lid_R$, $n = 10$; (6) $HA90_L/Lid_R$, $n = 13$. Lidocaine into the left BLA, saline into the right BLA ($n = 15$), and lidocaine into the left BLA, saline into the right BLA ($n = 16$) were considered relative controls. Microinjection procedures were identical to those described in Section 2.4.1.

Once experiments were over, animals were sacrificed by exposition to ether excess. Brain was dissected out and put in 10% formaldehyde during at least 48 h. Histological inspection of brain sections verified if the site of implant was into the baso-lateral amygdala. Those animals that the site of microinjection was not located in the right, or the left amygdala or both of these nuclei were discarded. Out of the total animals used, approximately 20% were discarded for displaced implants, infections or other reasons. No significant differences were found in the proportion of discarded animals due to any of the exclusion criteria just mentioned.

2.5. Behavioural measures

Spontaneous exploration was studied in two experimental conditions: OVM as a non-conflictive exploratory environment, and APM as a conflictive exploratory environment. Details of devices have been described elsewhere [39,40].

2.5.1. OVM

Three variables were selected because of its close relation to motivated exploratory activity:

i) Head-dipping, counted as frequency of head dips into any of the four holes of the OVM hole-board when this animal behaviour lasted at least 2 s.
ii) Rearing, counted as frequency of animal's rears, standing still on his rear feet and leaned on the walls of the OVM hole-board cage, sniffing to the air for at least 2 s.
iii) Focalized exploration, measured by the time in seconds the animal sniffs, touches with its front feet, climbs over the tube rack or explores the holes of the rack.

2.5.2. APM

Exploratory activity, expressed as walking at constant rate sniffing the floor of the arm; standing still, sniffing a localized spot of any arm wall; rearing on any wall; extension of the animal's head at the end of the maze's arm, sniffing to the air in any direction, was considered motivated exploration and was recorded in seconds.

All behavioural tests were applied to single animals, with a total duration of 5 min and separated 24 h each test from the other. Exploratory activity was filmed with a digital video camera, and recorded in a DVD player/recorder Phillips, model DVDR3455H. Measurements of behavioural scores were performed at later time by trained investigators unaware of treatments.

2.6. Statistical methods

For evaluation of the significant difference of the medians between experimental groups, the Non Parametric Multiple Comparisons Test of Dunn was used [18], since variables were found not to follow the normal distribution. All results are expressed as the median ± standard error of the median. A probability value of less than 0.05 was considered statistically significant.

2.7. Animal care

Experimental protocol was revised and approved by the Institutional Committee of Laboratory Animal Care of the Faculty of Medical Sciences (Comité Institucional de Cuidado y Uso de Animales de Laboratorio, CICUAL), Universidad Nacional de Cuyo, Mendoza, Argentina.

3. Results

3.1. Experiment 1

The administration of lidocaine into the left BLA affected only head-dipping and focalized exploration in the lidocaine-treated rats. These two behaviours, considered an approximate index of motivated exploration [1], were affected in opposite direction. Significant decrease in the head-dipping score and a corresponding increase in the focalized exploration were observed (Fig. 1A, $p < 0.01$ and $p < 0.05$ respectively). When lidocaine was applied into the right BLA, the only score modified was the focalized exploration that was significantly increased compared to control ($p < 0.05$, Fig. 1B). Comparing the behavioural activity of left versus right BLA, only head-dipping was significantly lower in the left BLA lidocaine-treated rats compared to the right BLA lidocaine-treated animals ($p < 0.05$, Fig. 1A and B).

In order to estimate approximately the duration of the lidocaine treatment, dose of 2 μg of lidocaine, considered a working intermediate dose, was administered into both BLA nuclei of different groups of animals. Rats in the OVM were tested 5 up to 25 min after lidocaine microinjection. As shown, at 25 min still a significant increase in focalized exploration, indicating a lidocaine effect was still observed ($p < 0.01$ versus time zero, Fig. 1C). Behavioural activity of Saline-injected animals at time "zero" was considered the reference basal activity score.

3.2. Experiment 2

Animals that were injected with lidocaine into the left BLA and with increasing doses of histamine into the right BLA showed significant decrease in the behavioural scores of all three motivated exploratory behaviours compared to the relative control of lidocaine into the left BLA and saline into the right BLA (Fig. 2A). This effect was more evident with the higher histamine dose. When histamine was microinjected into the left BLA, a significant decrease in all three behavioural scores was observed ($p < 0.01$ for all three motivated explorations, Fig. 2B). Comparison of the animal's behavioural activity of left versus right BLA stimulation with histamine while the contralateral BLA was blocked with lidocaine, significant differences in rearing and focalized exploration were found. At doses of 9 and 45 nmol of histamine, animals stimulated with histamine into the right BLA, rearing was significantly less inhibited than when the stimulation was in the contralateral BLA (Fig. 2A and B, $p < 0.05$). In focalized exploration, only animals with the dose of 9 nmol of histamine into the right BLA decreased less the score than the contralateral stimulation (Fig. 2A and B, $p < 0.05$).

3.3. Experiment 3

The motivated exploration in a conflictive environment of animals microinjected into the left or right BLA is shown in Fig. 3.

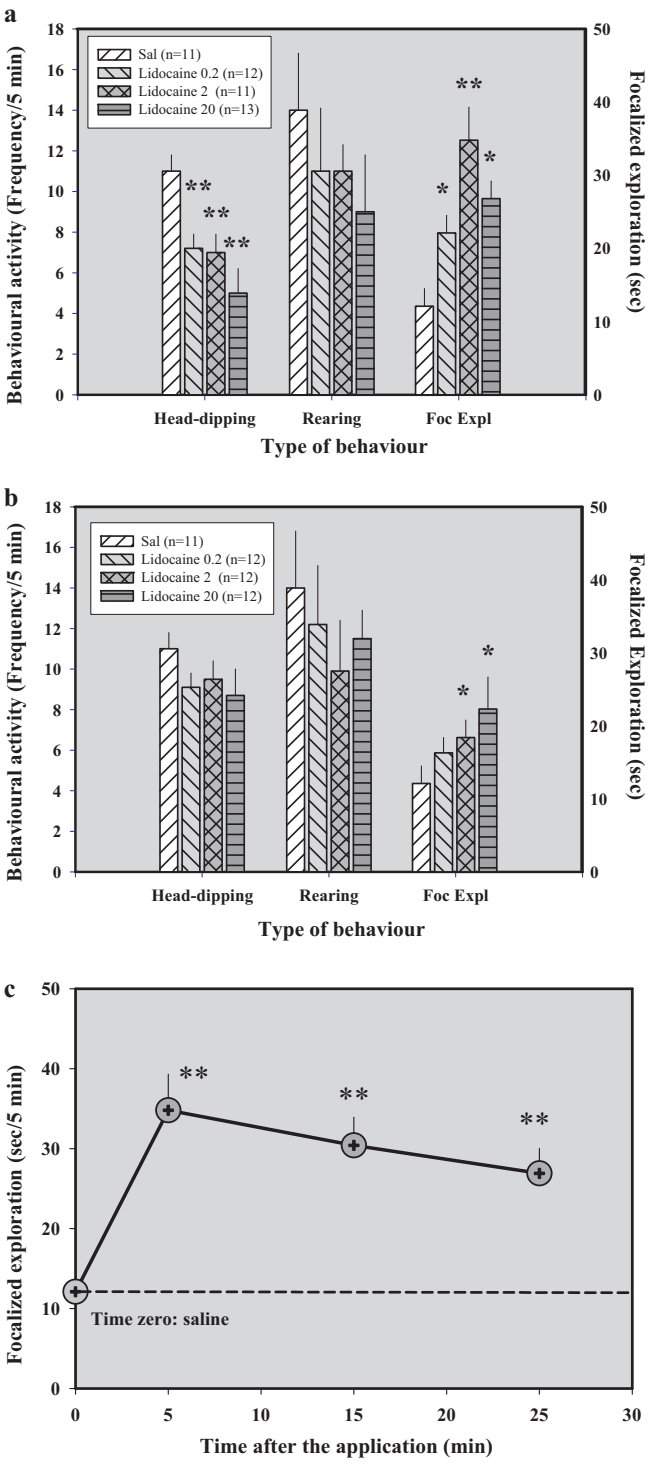

Fig. 1. Exploratory activity in non-conflictive environments of rats microinjected with lidocaine into the left or right baso-lateral amygdala (BLA). (A) Left ABL: head-dipping and rearing behaviours measured as frequency (left ordinate axis), and focalized exploration measured in seconds (right ordinate axis). Foc Expl = focalized exploration. Statistical comparisons: $^{*}p < 0.05$ versus saline group (Sal); $^{**}p < 0.01$ versus saline group. Comparisons of left versus right ABL: doses of 0.2 and 2 μg of lidocaine for head-dipping $p < 0.05$; 20 μg of lidocaine $p < 0.01$. Rearing and Foc Expl, n.s. (B) Right ABL: $^{*}p < 0.05$ versus saline group. (C) Left and right ABL: $^{**}p < 0.01$ versus time zero. Time "zero" means the moment that lidocaine or saline was microinjected into the amygdala. The behavioural activity showed at "zero" represents really the activity displayed by control animals 5 min after injection. All animals (lidocaine and saline treated rats) were tested for 5 min. Number of animals: 0 min ($n = 11$); 5 min ($n = 13$); 15 min ($n = 7$); 25 min ($n = 7$).

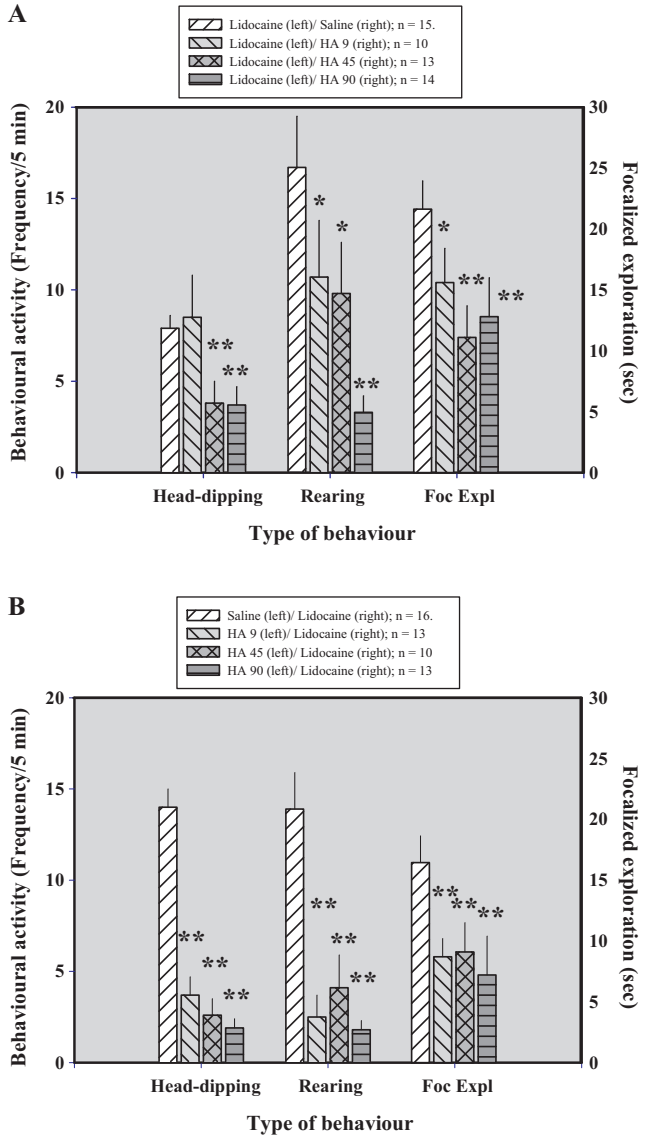

Fig. 2. Exploratory activity in non-conflictive environments of rats microinjected with 2 μg of lidocaine and histamine into the baso-lateral amygdala. (A) Left ABL (lidocaine)/right ABL (histamine): $^{*}p < 0.05$ versus lidocaine (left ABL)/saline (right ABL) group, considered control; $^{**}p < 0.01$ versus control. Comparisons of left versus right ABL: *Head-dipping*: doses of 9 and 45 nmol of histamine, $p < 0.05$ versus contralateral ABL. *Rearing*: doses of 9 and 45 nmol of histamine, $p < 0.05$ versus contralateral ABL. *Focalized exploration*: dose of 9 nmol of histamine, $p < 0.05$ versus the contralateral ABL. (B) Left ABL (histamine)/right ABL (lidocaine): $^{**}p < 0.01$ versus saline (left ABL)/lidocaine (right ABL) group, considered control.

Exploration time of the most fear-inducing arm (no walls, NW) and the less fear-inducing arm (two high walls, HH) were not affected by the lidocaine treatment in the left or right BLA (Fig. 3). However, in the other two maze arms (single wall arm, SW and high and low walls arm, HL), considered intermediate stimuli for fear [3,40] the exploration score was affected in opposed directions by the lidocaine treatment. Lidocaine microinjection into the left BLA increased motivated exploration of the SW arm, while decreasing exploration of the HL arm in the right BLA lidocaine treated rats (Fig. 3).

3.4. Experiment 4

Stimulation of the right BLA with increasing doses of histamine, and lidocaine in the left BLA, consistently decreased the explo-

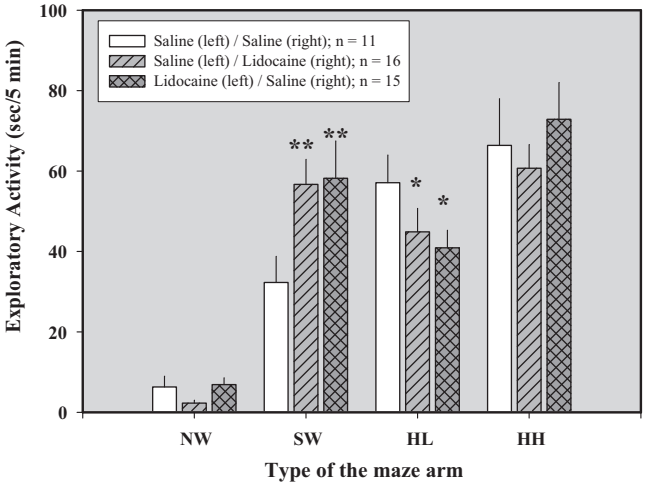

Fig. 3. Motivated exploration in a conflictive environment of rats microinjected into the baso-lateral amygdala with 2 μg of lidocaine. $^{*}p < 0.05$ versus saline (left ABL)/saline (right ABL) group, considered control; $^{**}p < 0.01$ versus control. NW: no walls arm, SW: single wall arm, HL: one high and one low wall, HH = two high walls arm.

ration scores of the SW and HH arms of the APM labyrinth (Fig. 4A). When the histamine stimulation was performed in the left BLA and lidocaine in the right BLA, the only exploration score that was not affected was that of NW arm. Significant decrease in exploration was observed in the other three labyrinth arms (Fig. 4B). Comparison of the animal's behavioural activity of left versus right BLA stimulation with histamine while the contralateral BLA was blocked with lidocaine, significant differences in the exploratory score of the single wall arm (SW) were found. At doses of 9 and 45 nmol of histamine, inhibition of exploration was more marked in those animals stimulated with histamine in the left BLA than the right BLA (Fig. 4A and B, $p < 0.05$).

4. Discussion

Histological inspection of brain sections of rats consistently revealed that the site of microinjection was located into the baso-lateral amygdala. Thus, results of the present work can be interpreted as proper consequence of chemical stimulation or inhibition of amygdala neurons. However, due to the close nearby proximity to the other neuronal divisions of the BLA, it was not possible to distinguish if behavioural effects observed, can be attributed to the excitation of the posterior, anterior or ventral portions of the BLA. Since sites of microinjections tended to be located ventrally in the amygdaloid zone, the central nucleus considered to be the neural circuit mediating the common output of the amygdala [13], possibly was not stimulated. Lidocaine microinjection used to block the electrical activity simulating a chemical "silence" of neuronal activity was effective even at the lowest dose used (Fig. 1A). The time lapse of 10 min was appropriate to block the neuronal activity and measuring the behavioural responses in animals, since the apparent half-life of the localized application of the anesthetic in the brain extended beyond the 30 min for the 2 μg dose (Fig. 1C). Thus, results can be interpreted as behavioural responses of the animals after single contralateral remaining neuronal activity of the BLA.

4.1. Experiment 1

Out of the three behaviours related to motivated exploration measured in the OVM as a model of non-conflictive environment [39], only head-dipping showed characteristics of lateralized

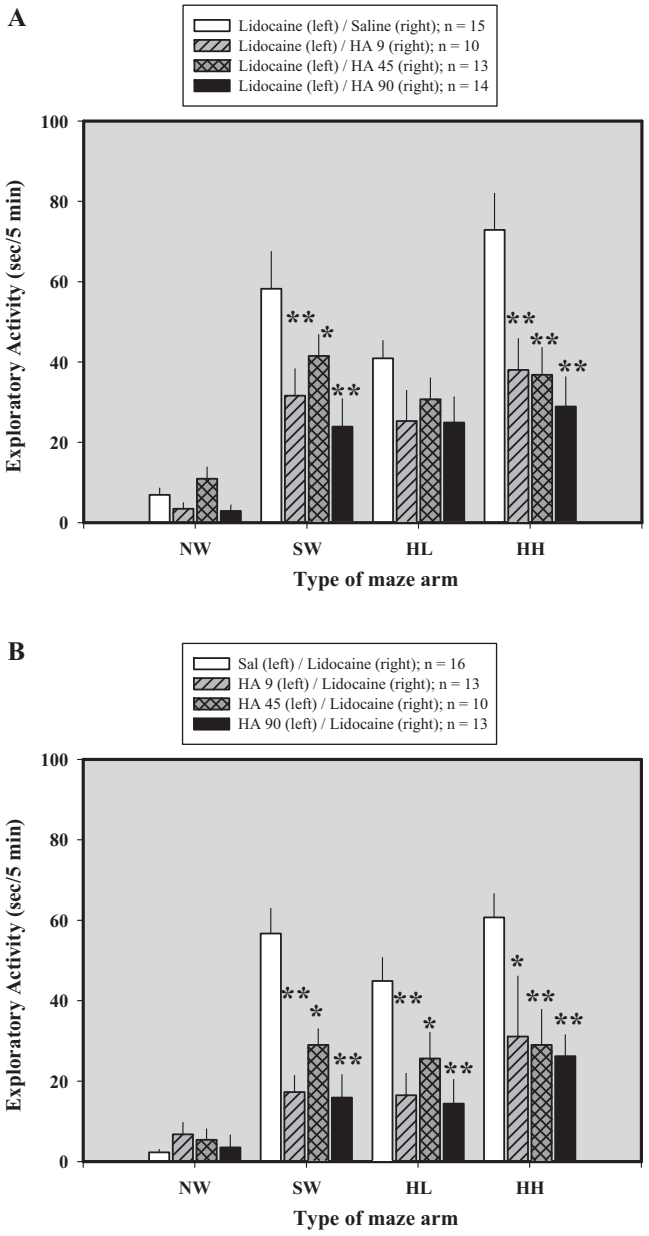

Fig. 4. Motivated exploration in a conflictive environment of rats microinjected into the baso-lateral amygdala with 2 μg of lidocaine and histamine. (A) Left ABL (lidocaine)/right ABL (histamine): $^{*}p < 0.05$ versus lidocaine (left ABL)/saline (right ABL) group, considered control; $^{**}p < 0.01$ versus control. Comparisons of left versus right ABL: *SW arm*: doses of 9 and 45 nmol of histamine, $p < 0.05$. (B) Left ABL (histamine)/right ABL (lidocaine): $^{*}p < 0.05$ versus saline (left ABL)/lidocaine (right ABL) group, considered control; $^{**}p < 0.01$ versus control.

behaviour (Fig. 1A and B). These data suggest that the neural circuits of the left BLA are involved in modulation of lateralized expression of this behaviour. Blocking the neuron activity of the left BLA provokes a decreasing exploration of novel ground stimuli represented for the holes in the floor of the OVM. This evidence is in agreement with description that the left brain hemisphere appears to be more competent with recognition of unfamiliar environments in fish [42], or discriminating objects between other distracting stimuli, such as the case of attacking prey in toads [38] or pecking grains in a distracting background in chicks [5]. Since lidocaine treatment affects all the neural activity in the range of diffusion, it is not possible with this experiment to identify the neurotransmitters and neurons regulating this modulation. Other additional feature seen in this experiment is that both BLA in different degrees facilitate

the focalized exploration (Fig. 1A and B). Head-dipping in this test is the only behaviour having a slight fearful component because deepness and darkness of holes offer some uncertain to exploration, giving to the animal some degree of conflict [39,40]. Present data suggest that left BLA is subserving an important influence to lateralized modulation of behaviour, in agreement with findings of other authors who after inducing dopamine depletion in the right BLA a lateralized "anxiolytic" effect in a conflictive environment was found in rats [43]. Thus, evidence from experiment 1 supports the notion of lateralization of the amygdala in agreement with studies performed in humans and animals [14,22,30,43].

4.2. Experiment 2

As shown in Fig. 2, histamine treatment was effective to modify all three motivated exploratory behaviours in the OVM test. The consistent effect observed was behavioural inhibition of all exploratory parameters, in agreement with the inhibitory role that already was described for the imidazolamine on other cognitive functions [4,24,29]. Two particular findings regarding these results are worthwhile to point out:

i) Out of the two motivated exploratory behaviours affected by the lidocaine treatment (Fig. 1), both head-dipping and focalized exploration showed a significant lower exploration score when 9 or 45 nmol of histamine was applied to the left BLA, compared to right BLA.
ii) Rearing, in experiment 1 was not affected by the lidocaine treatment but the unilateral administration of 9 or 45 nmol of histamine to right or left BLA provoked different behavioural responses (Fig. 2A and B). At the same dose of histamine, the stimulation of the left BLA was more effective to inhibit rearing than stimulation of the right BLA (2.5 ± 1.2 s versus 10.7 ± 3.1 s, $p < 0.05$, left BLA versus right BLA, 9 nmol dose of histamine, Fig. 2A and B). These results suggest that histamine fibers are modulating the neural activity of the BLA neurons, and this regulation is left lateralized. Puzzlingly, regarding rearing behaviour it calls the attention that lidocaine treatment alone did not put into evidence any signs of lateralization (Fig. 1), while treatment with lidocaine and histamine showed that rearing was lateralized (Fig. 2). A possible explanation might be linked to the electrical properties of the histaminergic neurons exerting a basal tonic modulation to the BLA neurons [10,21]. The unilateral local injection into the BLA of exogenous histamine provokes an abrupt input burst of activity to the BLA histamine-sensitive neurons, which is not counterbalanced by the contralateral neuronal activity. In these conditions, BLA neuron responses can induce evident lateralized behavioural responses in the animal. These results suggest that the intrinsic neuron activity of the BLA on rearing might be lateralized.

4.3. Experiment 3

The APM represents a useful model for studying conflictive exploration in rats as it has been previously discussed in detail elsewhere [39,40]. Thus, environment novelty as exploratory attractor to the animal is mixed with motivational and fear-inducing aspects of the exploratory stimulus. Lidocaine treatment in the BLA affected selectively exploration of the "SW" (single wall) and "HL" (high and low walls) arms of the labyrinth (Fig. 3). These arms represent decreasing stimulus "strength" of the fearfulness and motivational aspects of exploration [39,40]. As shown in Fig. 3, the lidocaine treatment into both BLA increases and decreases uniformly exploration of the "SW" and "HL" arm, respectively. This evidence suggests that modulation of the motivated exploration in the APM by the BLA appears not to be lateralized.

4.4. Experiment 4

As shown in Fig. 4, combination of lidocaine and histamine treatment was effective to decrease significantly exploration scores in the "SW", "HL", and "HH" arms of the APM. In spite of variations of the exploratory activity in animals stimulated with histamine, a statistical difference was found between the score of right- and left-histamine stimulated rats in the SW arm. This result suggests that histamine neurons modulating the expression of behaviour can affect both BLA neuronal circuits, but showing some lateralization in processing some particular stimuli.

Considering all these data, several interesting conclusions are evident. The baso-lateral amygdaloid nucleus appears to be a highly specialized neural circuit, showing a conspicuous and selective stimulus-discriminating function. Thus, in the non-conflictive environment (the OVM), considered a relative non-threatening stimulus to exploration, the BLA neurons showed a marked lateralized exploratory modulation (Fig. 1), whereby histamine fibers appear to be part of the modulatory control (Fig. 2). Meanwhile, in the conflictive environment (the APM), a rather threatening intensity stimulus to exploration, the same regional neuronal network respond in concerted parallel action with some discrete lateralized control to determined stimuli (Fig. 4A and B, the SW arm). Thus, the BLA neurons appear to have two distinct internal mechanisms discriminating intensity of environmental stimuli; one mediating a differential activation of left and right BLA, and the other one involving a selected activation of both amygdalae. It is not surprising that BLA neuronal networks can show such complexity. It has been described that neuronal activity of BLA neurons of left and right counterparts depends on the type of the stimulus and of the sex of the animal [43]. Electrophysiological studies aimed to analyze the pain related neuroplasticity and the amygdala response showed that after induction of a pain stimulus in rats, neurons of the right amygdaloid central nucleus, but not the left, developed increased electrical activity and evoked responses [25]. It is not possible with the present experimental data to discern the intrinsic complex synaptic relationships in the BLA that could be mediating the behavioural responses found in this study. In spite that many workers using different experimental paradigms support the idea that the right amygdala appears to be the important lateralized neural circuit [25,26,35,47], in our work both amygdalae show some type of specific lateralized aspect (Figs. 1 and 2). Blocking completely the neural activity of all neurons in the circuit with lidocaine (experiment 1) suggest that the left BLA has a regulatory lateralized function. In agreement with this evidence, inactivating just one BLA with lidocaine and stimulating with histamine the contralateral BLA, the left BLA appears to be the primary modulator (experiments 2 and 4). Our results support the general idea of a differential specialization of the brain hemispheres related to the coping behaviour, where approach and withdrawal processes appear to be selected modulated by left and right counterparts of the brain [15]. In conclusion, present data give an additional support to the physiological role of histaminergic neurons in modulation of behaviour and its participation in the lateralized control of motivated exploration.

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