American Journal of Neuroprotection and Neuroregeneration
Vol 5, 92-100, 2013

Motivated Lateralized Behaviour in the Rat: Role of the Ventral Hippocampus

Valeria A. Abrego, Silvia G. Ratti, and Edgardo O. Alvarez*

Laboratorio de Neuropsicofarmacología Experimental, Área de Farmacología, Facultad de Ciencias Médicas, Universidad Nacional de Cuyo, IMBECU-CONICET, Mendoza, Argentina

Lateralization of the brain in mammals has made animals more competitive during interaction with the environment in evolution. This property, first described in the 19th century in man, refers to the differentially modulation and control of some neuronal circuits of brain hemispheres on determined behavioural functions. In spite that lateralization has been described in several different animal species, still there are aspects not fully understood related to behavioural functions or identification of specific brain circuits involved. In the rat, coping behaviour is quite important for successful surviving; proper behavioural responses and careful analysis of spatial clues of the environment are needed. However, if lateralization mechanisms in the brain participate in these processes is still not known. In this work, exploratory lateralized responses and the possible role of the hippocampus as a probable lateralized structure were investigated. Intact and rats implanted with microinjection guide cannulae into the hippocampus were tested in three lateralized devices, the T labyrinth (TL), the multiple compartment labyrinth (MCL), and the double lateral hole-board labyrinth (DHBL). Decisions making to select left or right responses for seeking shelter (the TL), passing through left or right doors in a compartments in series (the MCL), and exploring left or right walls in a corridor (the DHBL) were investigated in these two groups of rats. Results show that intact rats presented lateralized exploratory behaviour with a left-bias in the DHBL but random responses in the other devices. This left preference observed in the DHBL was manifested in spending more time exploring the left wall of the DHBL than the right one. Hippocampus implanted rats retained the left-bias exploration in this device. Blocking the neural activity of left, right or both hippocampi with lidocaine in the implanted rats, the spontaneous left-bias exploration was nullified only when lidocaine was microinjected into the left hippocampus. Results suggest that the hippocampal structure is functionally lateralized for modulating exploration in novel environments emphasizing the hippocampus role on coping behaviour in the rat.

Keywords: Hippocampus, Lateralized Behaviour, Functional Laterality, Double Lateral Hole-Board Labyrinth, Multicompartment Labyrinth.

RESEARCH ARTICLE

1. INTRODUCTION

Laterality, sometimes referred to as brain functional asymmetry, is one of the most subtle neural mechanisms found in the mammalian brain.[19] This feature, considered a property of the brain, is linked to the existence of parallel neural circuits in each hemisphere differentially controlling specific physiological functions in the organism.[29] It is interesting to point out that the basic concept of laterality dates back to the 19th century, where 20 years before Paul Broca published his historical description of the left hemisphere dominance for speech,[10] the French neurologist Marc Dax emphasized the relationship between language and lesions of the left hemisphere,[36] giving rise to the idea of differential functional roles for the same paired structures in the brain. During the early part of the 20th century it was believed that this property was exclusively present in the human being.[37] However, evidence from studies in singing birds put forward that left-right paired nerves innervating the avian syrinx have different roles in the control of song production,[16, 22, 23] suggesting strongly that at least in some animals neural circuits also are lateralized. After that, an impressive amount of reports in different animal species including tadpoles, frogs, fishes, dogs, chickens, sheeps, rats, lizards and apes just to mention a few, have supported the concept of brain laterality in animals.[3, 9, 24, 33, 37, 39] All this evidence put on firm ground that lateralization is an advantageous mechanism that animals gained in evolution, thus, offering the possibility to

*Author to whom correspondence should be addressed.

ISSN:1947-2951
EISSN: 1947-296X

study in animal models some features of the complex lateralization mechanisms in man.[19]

It has been assumed that behavioural lateralization must depend on the differential activation of determined brain structures. Since in many cases, the lateralized behaviour exposed by animals is related to recognition of particular clues or spatial features of the environment,[6, 15, 25, 38] the hippocampus has been considered an interesting model for investigating the neural mechanisms of lateralized behavioural expression. In the past 20 years, sufficient evidence has been accumulated to consider the hippocampal structure a sophisticated neural organization implicated in memory, spatial recognition and navigation maps.[2, 7, 18, 30, 31, 35] Furthermore, the existence of specialized neurons in the hippocampus and parahippocampal structures, such as the place cells and head-direction cells[13, 21, 34] have provided a convenient theoretical background to explain possible modulation of lateralized behavioural expression in animals. Regarding the participation of the hippocampus in laterality, it has been found that in pigeons, lesioning the left but not the right hippocampus affected seriously the capacity to learn navigational maps.[15] This finding suggested that the left hippocampal circuits are lateralized to modulate homing behaviour in these birds. In rodents, hippocampal CA1 synapses receiving neuronal input from the right CA3 pyramidal cells are larger and have more post-synaptic density than those receiving input from the left CA3 cells.[32] On the other hand, proteomic analysis of left and right hippocampi isolated from the rat revealed higher abundance of metabolic enzymes related to cellular energy metabolism in the right than in the left hippocampus.[28] Furthermore, description about the differential expression of the NR2B subunits of the NMDA glutamic acid receptor in the mouse hippocampus and persistence of left-right asymmetry in a particular strain with inverse location of its internal organs,[17] suggest that at the molecular level the hippocampus appears to have the functional machinery supporting some lateralized behavioural expressions. Nevertheless, very little is known about specific behavioural expression and hippocampal activation in the rat. Furthermore, some other important behavioural expressions that might be lateralized, such as motivated exploration and coping behaviour, have not been studied in the rat. If these behavioural patterns depend on hippocampal functions is currently unknown. Thus, the objectives of this work were on one hand, to evaluate the existence of spontaneous exploratory lateralized behaviours, and on the other hand if these behaviours are modulated by the ventral hippocampus in the rat.

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 ventral hippocampus (HPC). Stereotaxic coordinates were: 3.3 mm rostro-caudal, 5.0 mm lateral and 4.0 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. The following experimental groups were formed:

(i) Intact animals ($n = 21$);

(ii) hippocampal implanted rats microinjected with lidocaine in the left hippocampus ($n = 21$);

(iii) hippocampal implanted rats microinjected with lidocaine in the right hippocampus ($n = 21$);

(iv) hippocampal implanted rats microinjected with lidocaine in both hippocampi ($n = 21$), and

(v) hippocampal implanted rats microinjected with saline in both hippocampi ($n = 23$).

2.3. Drugs

Lidocaine chlorhydrate 2.0% (AstraZeneca Laboratorios, Argentina), freshly prepared in saline before the onset of the experiments, was used. Saline solution was considered as control.

2.4. Intact Animals

A group of intact animals, rats not subjected to stereotaxic surgical procedures, were selected for one experiment. These animals were considered absolute controls. Animals subjected to stereotaxic surgery, implanted with guide cannulae and microinjected with saline solution only, were considered relative controls.

2.5. Testing Laterality Behaviours

Three devices were used in order to detect spontaneous lateralized exploratory behaviour in rats. Each one is based on different principles.

2.5.1. The "T" Labyrinth (TL, Fig. 1)

The TL is made of wood formed by two corridors (horizontal and vertical) disposed 90° each in a "T" shape (Fig. 1). Walls are 23 cm height and painted white. On each end of the horizontal corridor is a closed box with a single entrance representing a shelter to the rat. Each shelter has 20 cm depth and 12 cm wide. Total length of the horizontal corridor, including the shelters is 63.5 cm.

RESEARCH ARTICLE

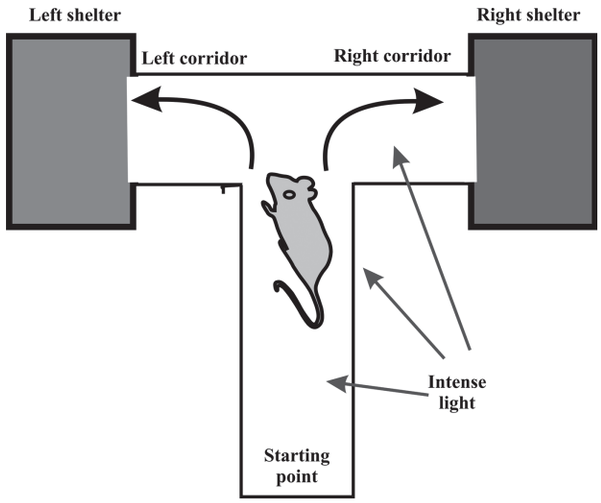

Fig. 1. *T* labyrinth (TL) schematic diagram. Animals are tested only once and put in the starting point (bottom in the drawing) at the beginning of the test. Spontaneous decisions are taken by rats at the end of the TL selecting the right or the left shelter. Additional details see Materials and Methods section.

The vertical corridor is 11.5 cm wide and 41 cm length. In the test, each rat is put in the opposed end of the vertical corridor under a fluorescent lamp illumination giving a light intensity of about 930 Lux. The avoiding response of the animal to the light is manifested by seeking refugee. At the crossing of the vertical and horizontal corridor, rats can choose to hide in the left or the right shelter. Position of the head of the animal in a rostro-caudal axis was taken as the reference direction (egocentric reference). Behavioural laterality was considered to be present when proportion of animals choosing the left or right shelter statistically outnumbers the proportion of animals choosing the opposite. All animals were exposed to the T Labyrinth only once in order to avoid habituation or other spatial clues interfering with the spontaneous behavioural selection of the shelters. Rats were tested individually and the time spent by animals in the labyrinth during the test was 15–90 sec. In rare occasions some rats remained in the initial site or did not choose any shelter during the test. These animals were discarded and not included in the results.

2.5.2. The Multiple Compartment Labyrinth (MCL, Fig. 2)

MCL is made of wood and is composed by a rectangular cage 39 cm wide, 70 cm length and 15 cm height. Inside there are a set of 4 compartments in series, each one with a single central entrance and facing a wall with two passing doors, one located to the left and the other one to the right of the entering animal (Fig. 2). Separation of the walls is 12 cm apart. At the beginning of the test, animals are put in one end of the labyrinth. Exploration is initiated by crossing over the central entrance. In order to continue exploration rats have to choose either walk through the left

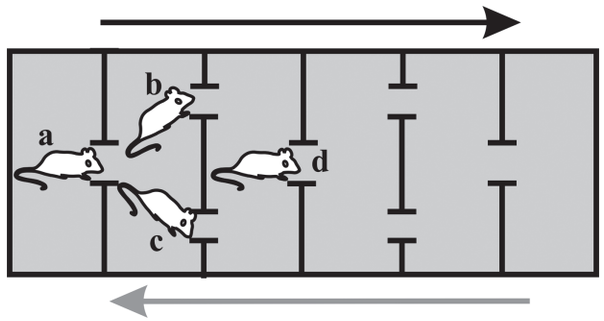

Fig. 2. Multiple compartment labyrinth (MCL) schematic diagram. Animals at the beginning of the test are put in the starting point (a). Once rats pass through the center door, there are two next doors positioned at the right (c) and the left (b) of the compartment. Animals have to choice any of them in order to follow the exploration. Exploration behaviour choosing any of the doors is evaluated in the "forward" and "backward" direction (represented by the arrows in the drawing) during the 5 min test. As explained in Materials and Methods section, the rostro-caudal axis of the animal is taken as reference to identify right versus left.

door or the right door. Whatever the selection of the door were, rats passing to the next compartment; face the same situation as before. Exploration can be performed in the initial-end and end-initial direction of the labyrinth. Thus, rats are able to perform many selections during the 5 min test. In this test the behavioural activity of the animals is driven only by exploratory motivation induced by novel environments.

Behavioural laterality was considered to be present when number of animals using left doors higher than right doors statistically outnumbers the opposite preference.

2.5.3. The Double Lateral Hole-Board Labyrinth (DHBL, Fig. 3)

DHBL is made of wood and is composed by a rectangular cage 39 cm wide, 70 cm length and 15 cm height. Inside there are two compartments disposed in 90° each. The first compartment (Initial) has 39 cm length and 15 cm wide with a central entrance to the second compartment (Corridor, Fig. 3). Corridor has 55 cm of length, 17 cm wide, and on its side walls there are 4 lateral holes, each 3 cm in diameter. In this test just like the MCL, behavioural activity of animals was driven only by exploratory motivation induced by novel environments. The following variables were measured:

(1) Corridor behavioural activity. All behaviours displayed by rats while they are in the corridor of the labyrinth, such as walking, rearing, head-dipping, and sniffing on the left or right side walls, including non-exploratory behaviours such as grooming and immobilization measured by a digital automatic counter (counting rate 2 counts/sec) monitored by an observer unaware of treatments.

(2) Initial Compartment behavioural activity. It is included in this measure all the behavioural activity displayed by rats while they were in this compartment. This activity

RESEARCH ARTICLE

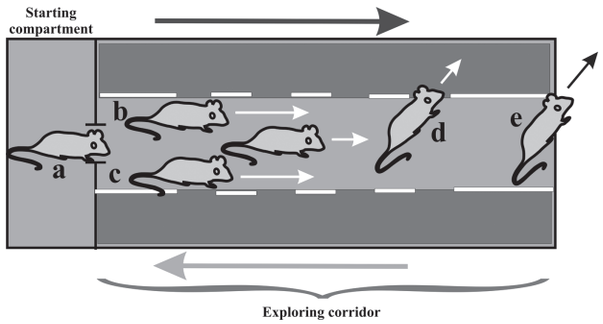

Fig. 3. Double Lateral Hole Board Labyrinth (DHBL) schematic diagram. Animals at the beginning of the test are put in the starting point (a). Once the rat pass through the corridor, can either move forward by the left (b), the right (c) or the center of the corridor. During exploration, animals can display lateral head-dipping on any hole in the left or right wall (d). At any position of the corridor (left or right) rats can rear (e). As in the MCL, exploration behaviour is evaluated in the "forward" and "backward" direction. For additional details, see Material and Methods section.

was not measured directly and was calculated by subtracting corridor behavioural activity counting from the total counting of the test (5 min = 600 counts).

(3) Lateralized exploration. It is included in this variable all behaviours related to exploration displayed when the animal chooses one side of the corridor as subject of exploration. Behaviours were: (i) walking nearby the left or right wall of the corridor, at constant speed, with vibrissae touching the wall; (ii) lateral head-dipping, and (iii) rearing against the left or right walls of the corridor. This score was measured in the same way than Corridor Behavioural Activity.

(4) Non-exploratory activity. It is included in this variable the following behaviours: (i) immobilization at any site of the corridor; walking at the center of the corridor not approaching to any side wall, and (iii) grooming. Its value was calculated by subtracting the lateralized exploratory activity from the corridor behavioral score.

In this test, behavioural laterality was considered to be present when the median of lateralized exploration on one side of the walls statistically outnumbers the opposite exploration.

2.6. Experiments

The following experiments were performed:

2.6.1. Experiment 1: Spontaneous Behavioural Patterns of Rats in Lateralized Environments and the Effect of Hippocampal Implantation Cannulae

In this experiment normal intact rats ($n = 24$) were studied in the three different labyrinths in order to detect spontaneous lateralized behaviours. In a separate group ($n = 23$), animals implanted in the ventral hippocampus were also subjected to the three tests. Animals during 5 min were tested only once in each labyrinth. Each test was separated by 24 h. Statistical comparisons in each variable were performed between both groups in order to evaluate possible differences due to the surgical and microinjections procedures.

2.6.2. Experiment 2: Role of Hippocampus on the Spontaneous Lateralized Behaviour

In this experiment, hippocampal implanted rats were microinjected with 2 μg/μl of lidocaine into the left, right or both hippocampi in order to evaluate the effect of partial or total blocking of hippocampal neuronal activity on the expression of exploratory behaviour of rats. Groups were:

(i) Saline-Saline rats ($n = 23$), considered control;

(ii) Lidocaine into the left and saline into the right hippocampus rats ($n = 21$);

(iii) Saline into the left and lidocaine into the right hippocampus rats ($n = 21$), and

(iv) Lidocaine into both hippocampi rats ($n = 21$). Dose of 2 μg/μl of lidocaine was used since in previous studies in similar conditions this dose was found to effectively block the neural activity of restricted regions of the brain.[3] Behavioural laterality was tested in the same experimental conditions that in Experiment 1.

2.7. Statistics

Comparisons between left and right behavioural activity of animals in the different groups were performed by the non parametric test of Wilcoxon.[11] Comparisons of behavioural activity displayed between two different groups were performed by the Mann-Whitney Test.[11] Multiple comparisons between the lidocaine groups were performed by the non parametric Test of Dunn.[12] Comparisons of the significance of proportions of animals choosing left versus right in the TL, and in the MCL tests were analyzed by the Binomial distribution for events with equal probability (The sign Test).[11] A probability less than 0.05 was considered significant.

2.8. Care of Animals

The present experimental protocol was revised and approved by the Comité Institucional de Cuidado de Animales de Laboratorio (Institutional Committee of Care and Welfare of Experimental Animals) of the Faculty of Medical Sciences, Universidad Nacional de Cuyo (CICUAL).

3. RESULTS

3.1. Experiment 1

The behavioural parameters in the lateralized environments of intact and hippocampal-implanted rats are shown in Table I.

In the TL control intact animals did not show any preference for the left or right shelter. Implant and handling procedures did not affect this behaviour (Table I, (A)).

RESEARCH ARTICLE

Table I. (A) The T labyrinth. (B) The multiple compartment labyrinth.

(A)

Group	Shelter choice [Expressed as number of of animals choosing left or right shelter.]		Left versus Right
	Left	right	
Intact rats	10	14	n.s.
HPC-implanted rats	12	11	n.s.
Intact versus Implanted rats	n.s.		

(B)

Group	Doors choice during exploration [Expressed as number of animals using one door more frequently than the other. Four rats using the same number of left and right doors were excluded.]		Left versus Right
	Left door > right door	Right door > left door	
Intact rats	7	14	n.s.
HPC-implanted rats	11	11	n.s.
Intact versus Implanted rats	n.s.		

In the MCL, proportion of control intact rats using left doors more often than right doors during exploration were statistically the same that proportion of animals using the inverse. Implant and handling procedures did not affect this behaviour (Table I, (B)).

In the DHBL control intact animals displayed about the same behavioural activity in the corridor than the initial compartment of the labyrinth (Table II). The hippocampal implanted rats, instead showed an increased total behavioural activity in the initial compartment compared to that of the the corridor of the labyrinth (Table II, $p < 0.01$). When behavioural corridor and initial compartment scores were compared between intact and hippocampal-implanted rats, significant differences were found (Table II, $p < 0.01$). In the spontaneous exploration of the corridor, control intact rats showed longer exploration of the left side of the labyrinth which was statistically significant from that of the right side (Table II, $p < 0.01$). This behaviour was conserved in the hippocampal implanted rats but their scores were lower than the intact rats (Table II). Out of the total exploratory activity displayed by control rats in the corridor, about half of the activity was inverted in non exploration behaviours; hippocampal implanted rats conserved this ratio but with lower scores (Table II).

3.2. Experiment 2

The behavioural parameters in the TL of hippocampal-implanted rats and microinjected with saline or lidocaine are shown in Figure 4. Treatment with 2 μg/μl of lidocaine into the left, the right or both hippocampi did not affect the random selection of shelters in rats (Fig. 4). Proportions of selection of right or left shelters were statistically similar to that of saline-treated rats.

The behavioural parameters in the MCL of hippocampal-implanted rats and microinjected with saline or lidocaine are shown in Figure 5. When animals were microinjected into the left or both hippocampi with lidocaine, proportion of rats selecting right or left doors were not different from saline-treated rats (Fig. 5). However, when lidocaine was microinjected into the right hippocampus, proportion of animals selecting left doors over right doors was significantly decreased ($p < 0.01$, Fig. 5).

The behavioural parameters in the DHBL of hippocampal-implanted rats and microinjected with saline or lidocaine are shown in Figure 6. Saline-treated rats spend more time in the initial compartment than in the corridor (Fig. 6(A)). This behavioural pattern was not modified when left or right hippocampus was microinjected with lidocaine. However, when both hippocampi were injected with lidocaine, a significant increase in the corridor behavioural activity with a decrease in the initial behavioural activity was observed (Fig. 6(A)). When rats were in the corridor, part of the activity they showed corresponds to non-exploratory behaviours. This non-exploratory activity was not affected when the lidocaine treatment was applied into the left or the right hippocampus. Nevertheless, when lidocaine treatment was applied to both hippocampi, a significant increase in this behaviour was found (Fig. 6(A)).

When left exploration activity was compared with right exploration activity in the same animals, control animals conserved the significant left biased exploration (Fig. 6(B)). Application of lidocaine into the left hippocampus was effective to block this left biased exploration (Fig. 6(B)); however, when the lidocaine treatment was applied to the right hippocampus, the left exploratory preference was conserved (Fig. 6(B)). Application of lidocaine into both hippocampi, also blocked the natural tendency to left exploration of treated rats (Fig. 6(B)).

Table II. The double lateral hole board labyrinth.

Group	Behavioural activity				Non exploratory
	Corridor	Initial compartment	Lateralized exploration		
			Left side	Right side	
Intact rats $n = 24$	308 ± 14.6	284.5 ± 15	$81 \pm 7.4^{(b)}$	63.5 ± 6.9	144 ± 14
HPC-implanted rats $n = 23$	$224.5 \pm 18.9^{(a)}$	375.5 ± 18.7	$64.5 \pm 5.4^{(b)}$	48.0 ± 5.2	91.5 ± 12
Intact versus HPC-implanted rats	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$

Behavioural scores expressed as the median ± standard error of the median. (a) $p < 0.01$ Corridor versus Initial Compartment; (b) $p < 0.01$ versus Right side.

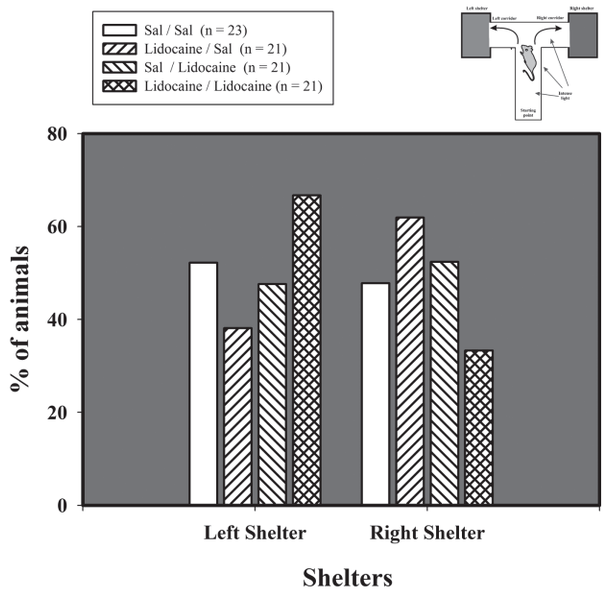

Fig. 4. Percentage of animals choosing left or right shelter in the TL, after they are microinjected into the hippocampus with saline and/or lidocaine.

4. DISCUSSION

It is interesting to note that most of laterality research in humans and animals has been interpreted as that lateralization somehow involves the entire brain hemisphere for some specific behavioural function.[8, 14, 19, 26, 29, 37] Although it is practical considering that brain circuits in one half of the central nervous system might process functions in this way, it is known that not all brain nuclei are lateralized to differentially influence behavioural processes. Thus, the search for identifying nuclei or specific brain circuits in one hemisphere presenting lateralization is a reasonable logical step after detection of the lateralized behaviour.

In this work all three experimental setups used have in common the exploration behaviour of the rat. In one instance, exploratory behaviour is related to an avoidance response seeking for shelter (the *T* labyrinth, TL); in the others the investigation of surroundings is motivated by novelty (the multiple compartments, MCL and the double lateral hole-board labyrinths, DHBL). These behavioural expressions are considered spontaneous and natural responses in rats. In Experiment 1, the TL and the MCL, designed to detect left versus right making decisions did not show any evidence that intact rats have some lateralized preferences in these environments (Table II, (A) and (B)). Possibility that TL and MCL are not sensitive enough to detect biased behaviours is not likely, since in one preliminary testing in adult rats bearing 6-OH-dopamine chemical lesions in the left corpus striatum and stimulated with apomorphine, animals showed a right-bias selection of the shelter in the TL (results not shown). On the other hand, in Experiment 2 when the right hippocampus was blocked by lidocaine and left hippocampus injected with saline, rats significantly prefer passing through right doors rather than left doors during exploration of the MCL (Fig. 5). These data support the utility of the TL and MCL for evaluating lateralized exploratory behaviours in rats. Thus, the most reasonable conclusion regarding intact rats is that in the TL and MCL environments lateralized spontaneous exploration behaviours are not present. Instead, in the DHBL, intact animals showed a clear and significant left-biased exploration (Table II, Experiment 1). It is interesting to note that rats prefer to spend more time exploring the left wall than the right wall. Since walls (left and right) are geometrically identical and mirror images of each other, what it appears to be lateralized is the exploratory motivation related to the place-novelty. Another interesting feature is that intact rats dedicate about the same behavioural activity to the corridor and to the initial compartment (Table II, Experiment 1), and when animals are in the corridor, they spend about the same time in exploration and in non-exploratory activity (Table II, Experiment 1). These data suggest that intact rats perceive each compartment of the DHBL with about the same relative attraction. As shown in Table II, hippocampus implanted rats show a modification in these behavioural parameters, suggesting that bearing the cannulae and microinjections procedures inflict some irritatory effect. Nevertheless, the general relationship between the behavioural parameters observed in the intact animals is maintained in the implanted rats. It is not likely that implants by themselves can exert general unspecific effects, since in TL and MCL, no behavioural differences were found between intact and implanted rats during testing (Table II, (A) and (B)). This evidence support the concept that implantation procedure imposed to animals does not alter general brain functions.

RESEARCH ARTICLE

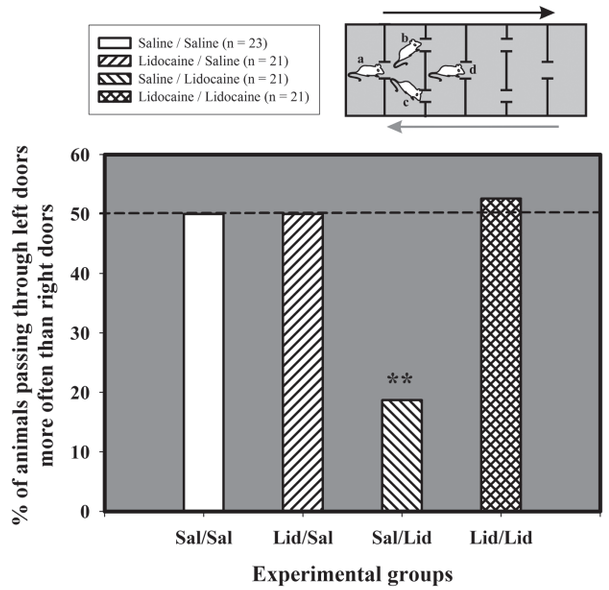

Fig. 5. Percentage of animals choosing to pass through left doors more often than right doors in the MCL, after they are microinjected into the hippocampus with saline and/or lidocaine. $^{**}p < 0.01$ versus 50% (dashed line).

Histological inspection of brain sections of rats consistently revealed that the site of microinjection was located into the ventral hippocampus. In this region the principal neuronal target was the CA_2–CA_4 pyramidal neurons. Thus, results of the present work can be interpreted as proper consequence of chemical stimulation or inhibition of hippocampal neurons.

Experiment 2 shows, in agreement with results observed for intact and implanted rats in Experiment 1, no evidence of lateralization when left, right or both hippocampi were blocked with lidocaine and measured by the TL test (Fig. 4). These results give support to the concept that hippocampus is not involved in decision making behaviour in the environment represented by the TL. In contrast, choice preference of rats for passing through the left or right doors in the MCL of rats was significantly affected when the right hippocampus was blocked by lidocaine (Fig. 5). This evidence strongly suggest that left and right hippocampal neural circuits co-participate modulating the multiple choices the animal has to perform during exploration of the MCL. Nevertheless, no evidence of hippocampal lateralization is collected from experiments with the MCL.

As shown in Figure 6(A), no lateralization was apparent when rat behavioural activities of Corridor, Initial Compartment and Non Exploration of the DHBL were examined after lidocaine treatment in the left or the right hippocampus. However, hippocampal neural circuits are involved in the expression of these behaviours because lidocaine application to both hippocampi was able to change these behavioural activities (Fig. 6(A)). Since

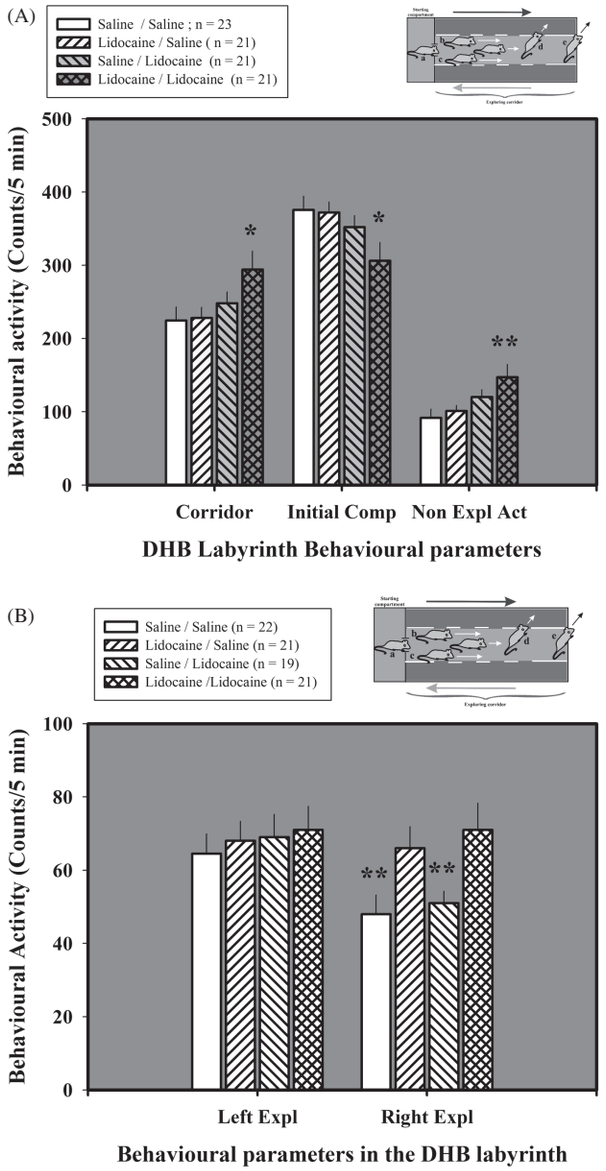

Fig. 6. Behavioural parameters displayed by rats in the DHBL after they are microinjected into the hippocampus with saline and/or lidocaine. (A) Motivational parameters: Initial Comp = Initial compartment; Non Expl Act = Non exploratory activity. $^{*}p < 0.05$ versus Saline/Saline group; $^{**}p < 0.01$ versus Saline/Saline group. (B) Lateralized exploration parameter: Expl = exploration. $^{**}p < 0.01$ versus left exploration in the respective group.

Corridor and Initial Compartment behaviours are related to animal's choice decision to enter or not to the corridor, evidence suggests that this brain process is not depending on a differential activity of left or right hippocampus. It is quite possible that the increase in the Corridor score of animals treated with lidocaine in both hippocampi might be related to a decrease of the emotionality associated to the unknown corridor, since non exploratory activity was increased in these animals.[1, 27] However, as

RESEARCH ARTICLE

shown in Figure 6(B) left-biased exploration of the corridor is lateralized, and depending on the left hippocampus, because lidocaine treatment in the left but not in the right hippocampus blocked the left tendency to explore the walls of the corridor. This evidence is suggesting that the hippocampal neural circuits appear to be specialized in the evaluation of the significance of input information, thus controlling the "intensity" of the exploratory analysis of the environment. This is the basis for considering the hippocampus as part of a brain novelty motivation system,[1,4,5] and as data from Figure 6(B) show, it appears that this function is lateralized to the left hippocampus. These results put into evidence the complexity of the hippocampal structure that in addition to its well known influence in learning and memory processes, it is also modulating motivation mechanisms. It is worthwhile to note that the present data pointing to the left hippocampus is in agreement with those obtained in pigeons where also the left hippocampus was critically involved in the spatial memory task.[15] Since animals were tested only once in the DHBL, and the environment and conditions of the test were not stressful, memory clues are not involved. Hippocampal neurons activity quite likely is related to exploratory motivation processes.

In humans, the left hippocampus has been described as functionally involved in verbal and contextual memory processing.[20] Unfortunately, these complex brain functions, in particular those related to verbal communication cannot be studied in animals such as the rat not possessing language abilities. Nevertheless, left-right asymmetry in hippocampal circuitry involved in learning and working memory has been found in the *inversus viscerum* mouse, which was accompanied with right isomerism of the synaptic distribution of the $\varepsilon 2$ subunit of the glutamatergic receptor.[17] However, since these animals have a randomized laterality distribution of their internal organs, the physiological significance of these observations regarding functional laterality is not clear. In the rat, proteomics analysis of the hippocampus has shown quantitative differences in about eighty proteins between the right and the left hippocampus. A higher abundance of metabolic enzymes related to energy metabolism were found in the right than in the left hippocampus.[28] The implication of these observations regarding our results is to be determined. It is interesting to note that in a previous study of our laboratory on the baso-lateral amygdala, using the same implantation techniques, the lidocaine treatment in the left amygdala but not in the right significantly inhibited exploration of an unknown environment.[3] This evidence suggests that both components of the limbic system (amygdala and hippocampus), known to be intercommunicated themselves, follow a common lateralized influence on exploratory activity.

Acknowledgments: Present work was supported by the Secretaría de Ciencia, Técnica y Postgrado of the Universidad Nacional de Cuyo, and by the Consejo de Investigaciones de la Universidad del Aconcagua. Authors also are indebted to Dr. Sebastian Casas and Dr, Roberto Yunes for allowing testing their corpus striatum lesioned animals in our lateralized tests.

References and Notes

1. E. O. Alvarez and P. A. Alvarez, Motivated exploratory behaviour in the rat: The role of hippocampus and the histaminergic neurotransmission. *Behav. Brain Res.* 186, 118 **(2008)**.
2. E. O. Alvarez and A. M. Banzan, Effects of localized histamine microinjections into the hippocampal formation on the retrieval of one-way active avoidance response in rats. *J. Neural Trasm.* 101, 201 **(1995)**.
3. E. O. Alvarez and A. M. Banzan, Functional lateralization of the baso-lateral amygdala neural circuits modulating the motivated exploratory behaviour in rats: Role of histamine. *Behav. Brain Res.* 218, 158 **(2011)**.
4. E. O. Alvarez and A. M. Banzan, Neurochemistry of exploratory behaviour: Role of histamine and glutamic acid neuronal circuits. *Int. J. Neuroprotection Neuroregeneration* 4, 130 **(2008)**.
5. E. O. Alvarez, M. B. Ruarte, and A. M. Banzan, Histaminergic systems of the limbic complex on learning and motivation. *Behav. Brain Res.* 124, 195 **(2001)**.
6. R. J. Andrew, L. Tommasi, and N. Ford, Motor control by vision and the evolution of cerebral lateralization. *Brain Lang.* 73, 220 **(2000)**.
7. V. P. Bingman and G. Yates, Hippocampal lesions impair navigational learning in experienced homing pigeons. *Behav. Neurosci.* 106, 229 **(1992)**.
8. B. Bonati, D. Csermely, P. López, and J. Martin, Lateralization in the escape behaviour of the common wall lizard (Podarcis muralis). *Behav. Brain Res.* 207, 1 **(2010)**.
9. B. Bonati, D. Csermely, and R. Romani, Lateralization in the predatory behaviour of the common wall lizard (Podarcis muralis). *Behav. Proccesses* 79, 171 **(2008)**.
10. P. Broca, Perte de la parole, ramollissement chronique, et destruction partielle du lobe antérieur gauche du cerveau. *Bull. Soc. Anthropol.* 2, 235 **(1861)**.
11. W. J. Conover, Practical Nonparametric Statistics, 3rd edition, John Wiley and Sons, New York **(1999)**.
12. O. J. Dunn, Multiple comparisons using rank sums. *Technometrics* 6, 241 **(1964)**.
13. H. Eichenbaum and N. J. Cohen, Representation in the hippocampus: What do the hippocampal neurons code? *Trends Neurosci.* 11, 244 **(1988)**.
14. A. G. Frasnelli, B. Maccagnani, L. J. Rogers, and G. Vallortigara, Behavioural and electrophysiological lateralization in a social (Apis mellifera) but not in a non-social (Osmia cornuta) species of bee. *Behav. Brain Res.* 206, 236 **(2010)**.
15. A. Gagliardo, P. Loalè, F. Odetti, V. P. Bingman, J. J. Siegel, and G. Vallortigara, Hippocampus and homing in pigeons: Left and right hemispheric differences in navigational map learning. *Eur. J. Neurosci.* 13, 1617 **(2001)**.
16. I. George, Hemispheric asymmetry of songbirds, edited by K. Hugdahl and R. Westerhausen, The Two Halves of the Brain: Information Processing in the Cerebral Hemispheres, The MIT Press, Cambridge, Massachusetts **(2010)**, pp. 91–120.
17. K. Goto, R. Kurashima, H. Gokan, N. Inoue, I. Ito, and S. Watanabe, Left-right asymmetry in the hippocampal circuitry impairs spatial learning and working memory in *iv* mice. *Plos One* 5, 1 **(2010)**.
18. T. Hartley, C. M. Bird, D. Chan, L. Cipolotti, Husain, F. Vargha-Khadem, and N. Burgess, The hippocampus is required for short-term topographical memory in humans. *Hippocampus* 17, 34 **(2007)**.
19. K. Hugdahl and R. Westerhausen, The Two Halves of the Brain: Information Processing in the Cerebral Hemispheres, edied by

RESEARCH ARTICLE

K. Hugdahl and R. Westerhausen, The MIT Press, Cambridge, Massachusetts **(2010)**.

20. G. Kalpouzos and L. Nyberg, Hemispheric asymmetry of memory, edited by K. Hugdahl and R. Westerhausen, The two Halves of the Brain: Information Processing in the Cerebral Hemispheres, The MIT Press, Cambridge, Massachusetts **(2010)**, pp. 498–530.

21. R. U. Muller, J. B. Ranck Jr, and J. S. Taube, Head direction cells: Properties and functional significance. *Curr. Op. Neurobiol.* 6, 196 **(1996)**.

22. F. Nottebohm and M. E. Nottebohm, Left hypoglossal dominance in the control of canary and white-crowned sparrow song. *J. Comp. Physiol.* 108, 171 **(1976)**.

23. F. Nottebohm, Neural lateralization of vocal control in a passerine bird. *J. Exptl. Zool.* 177, 229 **(1971)**.

24. S. G. Ratti, P. Rabello, M. C. Tavares, C. Tomaz, and E. O. Alvarez, Evaluación de un inventario modificado para determinar habilidad manual en monos: Comparación y análisis evolutivo con la destreza humana. *Rev. Méd. Univ.* 3 http://revista.medicina.edu.ar/Vol03_01/index.php, **(2007)**, pp 1–13.

25. L. J. Rogers and J. M. Anson, Lateralisation of function in the chicken forebrain. *Pharmacol. Biochem. Behav.* 10, 679 **(1979)**.

26. L. J. Rogers, Hand and paw preferences in relation to the lateralized brain. *Phil. Trans. R. Soc. Biol. Sci.* 364, 943 **(2009)**.

27. M. B. Ruarte, A. G. Orofino, and E. O. Alvarez, Hippocampal histamine receptors and conflictive exploration in the rat: Studies using the elevated asymmetric plus-maze. *Br. J. Med. Biol. Res.* 30, 1451 **(1997)**.

28. A. Samara, K. Vougas, A. Papadopoulou, E. Anastasiadou, N. Baloyanni, E. Paronis, G. P. Chrousos, and G. T. Tsangaris, Proteomics reveal rat hippocampal lateral asymmetry. *Hippocampus* 21, 108 **(2011)**.

29. A. M. Schaafsma, B. J. Riedstra, K. A. Pfannkuche, and A. Bouma, Groothuis TGG. Epigenesis of behavioural lateralization in humans and other animals. *Phil. Trans. R. Soc. B* 364, 915 **(2010)**.

30. Y. Shen, S. M. Specht, I. Saint Ghislain, and R. Li, The hippocampus: A biological model for studying learning and memory. *Prog. Neurobiol.* 44, 485 **(1994)**.

31. D. F. Sherry, L. F. Jacobs, and S. J. C. Gaulin, Spatial memory and adaptive specialization of the hippocampus. *Trends Neurosci.* 15, 298 **(1992)**.

32. Y. Shinohara, H. Hirase, M. Watanabe, M. Itakura, and M. Takahashi, Left-right asymmetry of the hippocampal synapses with differential subunit allocation of glutamate receptors. *Proc. Natl. Acad. Sci. USA* 105, 19498 **(2007)**.

33. V. A. Sovrano, Visual lateralization in response to familiar and unfamiliar stimuli in fish. *Behav. Brain. Res.* 152, 385 **(2004)**.

34. A. Speakman and J. O'Keefe, Hippocampal complex spike cells do not change their place fields if the goal is moved within a cue controlled environment. *Eur. J. Neurosci.* 2, 544 **(1990)**.

35. K. Steele and Rawlins, The effect of hippocampectomy on performance by rats of a running recognition task using long lists of non-spatial items. *Behav. Brain Res.* 54, 1 **(1993)**.

36. L. Tommasi, Mechanisms and functions of brain and behavioural asymmetries. *Phil. Trans. R. Soc. B.* 364, 855 **(2009)**.

37. G. Vallortigara, C. Chiandetti, and A. V. Sovrano, Brain asymmetry (animal) *WIREs Cogn. Sci.* pp. 1–12, doi: 10.1002/wcs.100 **(2010)**.

38. G. Vallortigara, P. Pagni, and V. A. Sovrano, Separate geometric and non-geometric modules for spatial reorientation: Evidence from a lopsided animal brain. *J. Cogn. Neurosci.* 16, 390 **(2004)**.

39. E. Versace, M. Morgante, G. Pulina, and G. Vallortigara, Behavioural lateralization in sheep (Ovis aries). *Behav. Brain Res.* 184, 72 **(2007)**.

Received: 8 February 2013. Accepted: 30 May 2013.

RESEARCH ARTICLE