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Research report

Left–right functional asymmetry of ventral hippocampus depends on aversiveness of situations

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HIGHLIGHTS

- The ventral hippocampus possesses a left–right functional asymmetry in rats.
- The contribution of each hemisphere depends on the level of aversiveness.
- Both the left and right VH are activated during weaker anxiety.
- Only the right VH is activated during stronger anxiety.

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ABSTRACT

Many studies suggest that animals exhibit lateralized behaviors during aversive situations, and almost all animals exhibit right hemisphere-dominant behaviors associated with fear or anxiety. However, which brain structure in each hemisphere underlies such lateralized function is unclear. In this study, we focused on the hippocampus and investigated the effects of bilateral and unilateral lesions of the ventral hippocampus (VH) on anxiety-like behavior using the successive alleys test. We also examined the expression of c-fos in the VH, which was induced by an aversive situation.

Results revealed that consistent right VH dominance trended with the anxiety level. Weaker anxiety induced both right and left VH functions, whereas stronger anxiety induced right VH function. From these results, we conclude that animals are able to adaptively regulate their behaviors to avoid aversive stimuli by changing the functional dominance of their left and right VH.

1. Introduction

Functional asymmetry between left and right brain hemisphere is well-known. For example, the language area is left-sided in the human brain. However, brain asymmetry is not human-specific. It has been revealed that there are many species that perform some actions asymmetrically, and one of the most well-studied phenomenon is lateralized behaviors during aversive situations. For example, toads can direct their tongues to strike at conspecifics more quickly in the left hemifield than in the right one [1]. In addition, toads exhibit faster avoidance responses at the presentation of a snake model in the left visual field than in the right one [2]. Because information from the left hemifield is sent to the right brain hemisphere through the optic chiasm, these results suggest dominance of the right hemisphere in controlling the fight-or-flight responses. This left eye/right hemisphere preference during aversive situations has been reported in many animals, such as lizards [3], chicks [4], teleost fishes [5],dunnarts [6], dogs [7], cattle [8], horses [9], and baboons [10]. Such evidence in many animal studies indicates the possibility that the existence of right hemispheric dominance in emotional responses is common to almost all animals that have brain hemispheres. However, which brain structure in each hemisphere underlies functional asymmetry remains unclear.

Several studies have reported that some brain structures related to fear/anxiety and stress responses possess functional lateralization. By injecting ibotanic acid solution, Sullivan and Gratton [11] showed that lesions of the right, but not the left, medial prefrontal cortex (mPFC) lead to lower plasma corticosterone levels and smaller ulcers after chronic restraint stress in rats. Coleman-Mesches and Mcgaugh [12] reported that the inactivation of the right amygdala (AMG) with muscimol decreases inhibitory avoidance memory. Guangche and Volker [13] revealed that the right AMG (CeLC) is more preferentially involved in the process of the

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pain sensation. These findings strongly indicate the existence of left–right functional asymmetry in some brain structures, which lead to lateralized behaviors and stress responses during aversive stimuli. AMG and mPFC are well-known structures associated with fear/anxiety and stress responses. Many studies have identified that the ventral hippocampus (VH) is involved in the same functions as AMG and mPFC, particularly anxiety-like behavior [14,15], fear conditioning [16,17], and autonomic responses [18]. The dorsal hippocampus (DH) is involved in learning and memory [e.g.,19,20] and has been shown to have left–right hemisphere differences in memory processing [21,22] and spatial learning [23,24]. The right and left DH have different numbers of cells [25], types of genes [23,26] and proteins [27], and types and densities of synaptic receptors [28,29], and they also generate different gamma oscillations [30,31]. These findings clearly imply the functional asymmetry in DH. However, to date, there has been no study regarding the functional asymmetry in VH. Thus, we investigated whether VH exhibits functional asymmetry during aversive situations. In several previous studies, the elevated plus maze (EPM) [14], successive alleys test (SAT) [32], and light–dark box test [16] were used to measure anxiety-like behaviors of VH-injured animals. In this study, we used SAT, a modified version of EPM, that was developed by Deacon [33]. In this test, the width of successive alleys is gradually narrowed and the anxiety levels of the animal gradually change from the first wide alley to the last narrow one. Furthermore, we used structural lesion and c-fos immunohistochemistry to investigate the functional asymmetry of VH during different anxiety levels in rats. Risk assessment behavior has been related to anxiety and VH function in laboratory animals, and the detailed neural mechanisms of VH for such behavior remain to be revealed. Functional asymmetry of the VH may be a part of the neural mechanisms and discussing the potential advantages VH functional asymmetry has, in any, will be necessary. The present study could be one of the first steps to substantiate the occurrence of VH functional asymmetry in risk assessment behaviors in animals.

2. Materials and methods

2.1. Animals

Experimental subjects were male Wistar albino rats (Shimizu Laboratory Supplies, Kyoto, Japan) that weighed 210–250 g at the time of the surgery. The rats were individually housed in cages with free access to food and water under a light–dark cycle, with the light period between 08:00 and 21:00 h. Behaviors were tested between 10:00 and 12:00 h. All experiments were performedin accordance with the Guidelines for Animal Experiments at Doshisha University and with the approval of the Animal Research Committee of DoshishaUniversity.

2.2. Surgery

One week before the experiment, the rats were anesthetized with sodium pentobarbital (40 mg/kg, i.p.). Lesions were made by passing anodal direct current (2 mA, 30s) using the Lesion Making Device (53500, UGO BASILE SRL, Gemonio, VA, Italy) and a stainless bipolar electrode (150 μm, UB-9007, UNIQUE MEDICAL Co., LTD., Tokyo, Japan). The electrode was inserted into bilateral, right, or left VH ((1) AP, −4.5 mm from bregma; ML, ±5.0 mm from bregma; DV, −6.0 mm from dura; (2) AP, −5.5 mm; ML, ±5.2 mm; and DV, −6.5 mm). For sham lesions, the electrode was lowered to the same coordinates, but no current was passed. All groups consisted of 12 rats. In addition, in order to confirm that the insertion of the electrode did not affect the activity of the VH, three other rats were used for the same surgery with no insertion of the electrode. All rats were allowed to recover for 7 days and were handled for 5 min each day.

2.3. Apparatus

The experimental apparatus (Fig. 2A) was SAT, as devised by Deacon [33], and we followed its experimental procedure. In brief, the apparatus is composed of four 30-cm-long alleys. The widths and side walls of the alleys gradually narrowand lower as the number of alleys increases (Alley 1, 9-cm width/30-cm height; Alley 2, 9-cm width/2.5-cm height; Alley 3, 6.7-cm width/0.5-cm height; and Alley 4, 3.5-cm width/0.3-cm height). Alleys 1–4 were painted black, gray, white, and white, respectively. The apparatus was placed 50 cm above the floor under 200 lx illumination. Behaviors were recorded using a camera (BSW32KM03SV, BUFFALO INC., Aichi, Japan) that was mounted directly above the apparatus.

2.4. Successive alleys test

First, the rats were placed in Alley 2 that faced the direction of Alleys 3 and 4. The animals were then allowed to explore the apparatus for 600 s. A trial consisting of this procedure was performed once a day for 7 days (Days 1–7) continuously. After each trial, the surfaces of all alleys were cleaned. From the recorded videos of the animals, the time spent in each alley and the number of entries into each alley were calculated by using a system for automated analysis (ANY-maze software, Stoelting Co., IL, USA). An entry was scored if the animals moved into the next alley with 80% or more of their bodies (this criterion was considered to be comparable to the invasion of all four of the animal's paws in this software). The ratio of Alley 4/Alley 3 entries (number of entries into Alley 4 compared with those into Alley 3) is an indicator of how often the rats entered Alley 4 after entering Alley 3. A value of 0 would mean that the rats never entered Alley 4, and a value of 0.5 would mean that the rats always entered Alley 4 (if the rats always entered Alley 4 through Alley 3, the ratio of Alley 3 and Alley 4 entries would be 2:1). After all trials were completed (Day 8), the rats of the Sham lesion group and three non-injected rats were blinded for 4 h in their cages. They were then placed in Alley 4 isolated from Alley 3 with a 12-cm wide/30-cm tall board for 30 min to allow time for expression of the c-fos proteins (Fig. 2B). The rats were then returned to their cages. One hour thereafter, they were moved to the histology process described below. These series of procedures were not performed on the other three groups (Bilateral lesion, Right lesion, and Left lesion).

2.5. Histology

On Day 8, the rats in all groups were deeply anesthetized with an overdose of sodium pentobarbital (220 mg/kg) and were transcendentally perfused with 0.01 M PBS and 4% paraformaldehyde (PFA). The brains were then removed and stored in PFA overnight, before transferring them to 30% sucrose. We obtained coronal brain sections (50 μm) using a cryostat and mounted them on slides. Cresyl violet solution was used as a background stain to detect the lesion area. Brain regions were identified according to the Rat Brain Atlas [34]. The lesion sizes were calculated using a software program (ImageJ software, National Institutes of Health, MD, USA).

2.6. Immunohistochemistry

Immunohistochemical staining was performed using a rabbit-specific HRP/DAB detection kit (ab64261, Abcam, Cambridge, MA, USA), according to the manufacturer's protocol. In brief, the sections (AP = −4.80 mm) were incubated with protein block solution for 10 min to eliminate nonspecific background staining.

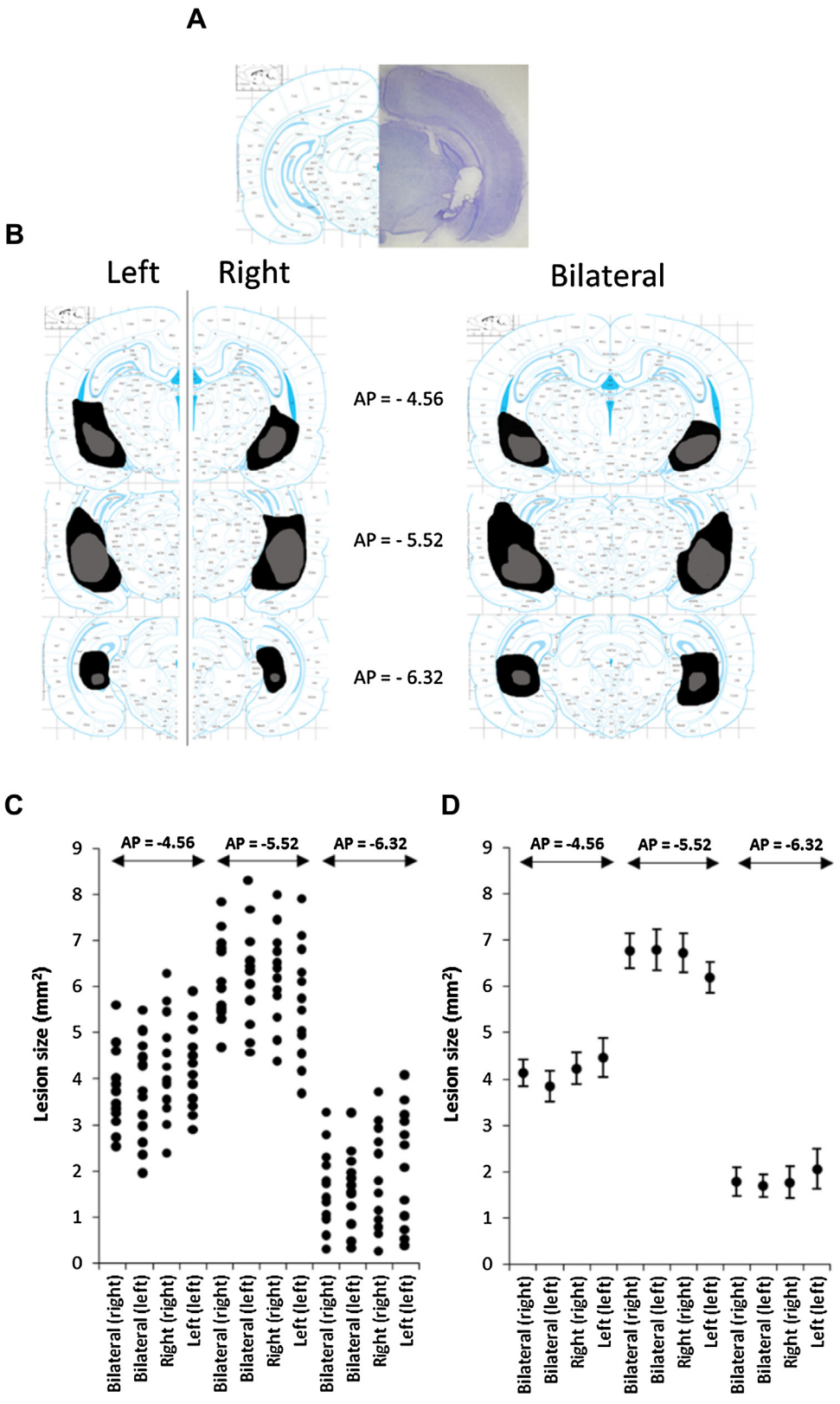

Fig. 1. Locations of the lesioned areas. (A) A raw sample of an electrical lesion of the ventral hippocampus. This section was stained with cresyl violet to identify brain regions more clearly. The brain maps (AP = −4.56, −5.52, and −6.32 mm) derived from Paxinos and Watson [33] represent (B) left, right, and bilateral lesion areas. The gray area indicates the minimum extent of tissue damage and the black area indicates the maximum. (C) Distribution of the lesioned areas of all rats for Bilateral lesion (right hemisphere), Bilateral lesion (left hemisphere), Right lesion (right hemisphere), and Left lesion (left hemisphere) groups in AP = −4.56, −5.52 and −6.32 mm. (D) Means ± SEM of the lesioned areasof each group. No significant differences were detected among the groups. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

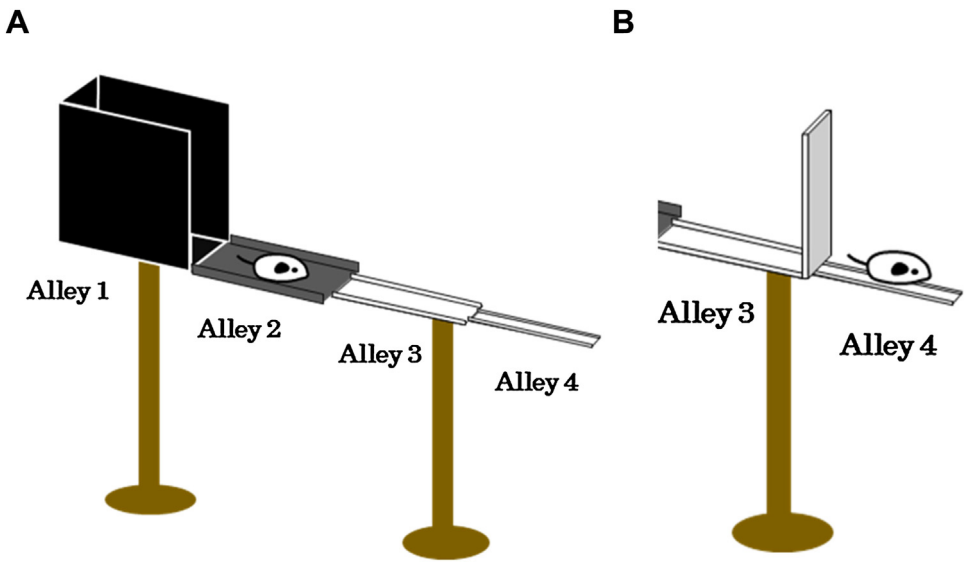

Fig. 2. (A) An image of the successive alleys test. Each alley is labeled Alley 1, 2, 3, and 4. (B) An animal isolated in Alley 4 by a high wall board for the measurement of c-fos expression.

After washing in a buffer, sections were incubated with rabbit anti-c-fos antibody (1:500, sc-52, Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 2 h at room temperature. Then, they were washed and incubated with biotinylated goat anti-rabbit immunoglobulin G for 1 h at room temperature followed by 30 min of incubation in streptavidin–biotin complex/HRP and 10 min in DAB solution. Finally, the stained slices were dehydrated using ethanol solutions and xylenes and coverslipped with the mounting reagent. Each section (DV = −7.0 to −9.0 mm) was scanned at 20 × magnification using a light microscope (Axioplan 2 Imaging, Carl Zeiss Microscopy, LLC, NY, USA) equipped with a camera (DFC300 FX, Leica Microsystems Inc., IL, USA). The number of c-fos-positive cells in VH subregions (CA1 and CA3) was counted with the ImageJ software. First, section images were digitized in gray scale with eight bits and then noise smoothing was performed for all images. Second, the background noise of each image was eliminated, and the obtained images were thresholded to convert them into binary ones. The threshold used for each image was set to 0–180 points (in this software, this value of the threshold was optimal for screening the object to be measured in the present sections). Last, the circular bodies—that is, the c-fos positive cells—were automatically counted.

2.7. Data analysis

Experimental data are shown as the means ± SEM. A mixed-design analysis of variance (ANOVA) with Condition (Sham lesion, Bilateral lesion, Right lesion, Left lesion) as the between-subject factor and Day as the within-subject factor was used to analyze these results; the time spent in each alley and the number of entries into each alley on successive 6 days (Days 2–7) and the results of the sum of entries and the ratio of Alley 4/Alley 3 entries during all 7 days (Days 1–7) among the Sham, Bilateral, Right, and Left lesion groups. Comparison of the time spent in each alley and the number of entries into each alley on Day 1, the sum of entries on Day 1 v.s. on Day 7 and the immunohistochemical results were performe dusing the Student's *t*-test. The regression-based TOST equivalence test (Package 'equivalence' of the R software (https://cran.r-project.org/)) was used to analyze the equivalence of the lesion extents.

3. Results

3.1. Histology

We observed that the stereotaxic passing of an anodal direct current destroyed most VH structures. Fig. 1A shows a basic sample of the electrical lesion, and Fig. 1B indicates the lesion areas of the Bilateral, Right, and Left lesion groups (n = 12 in each group). The extent of the lesion is shown with reference to the horizontal sections found in the Rat Brain Atlas [34]. Two rats whose lesions were over-destroyed – that is, whose lesion areas at AP = −5.52 mm included more than one third of the DH structure – were excluded from analysis. Minimum lesion areas (gray color) were observed in the ventral dentate gyrus, CA1 and CA3, but maximum lesion areas (black color) were not observed in the structures outside of the hippocampus. The lesions in the right and left hemispheres were highly symmetrical. Fig. 1C represents the lesion sizes of individual rats (n = 12) in each group (Bilateral lesion, Right lesion, and Left lesion groups). Fig. 1D shows the same data with means ± SEM. There was no significant difference among these four sites in all three sections (AP = −4.56, −5.52 and −6.32 mm). The Sham lesion group had little-to-no damage in these areas.

3.2. Behavioral test

SAT was used to measure anxiety-like behaviors that depend on the anxiety levels. The apparatus is shown in Fig. 2A. No rats fell from the apparatus during the experiments. The results of the mixed-design ANOVA are summarized in Tables 1 and 2. On Day 1, compared with the Sham lesion group, the Bilateral lesion group spent significantly less time in Alley 1 ($P < 0.01$) and long times in Alleys 2 ($P < 0.01$), 3 ($P = 0.024$), and 4 ($P < 0.01$); unilateral right and left lesions had no influence on the behavior, and there was no significance between the two groups (Fig. 3A). The ANOVA revealed the following differences from Day 2–7, which are listed in increasing amount of time spent: Bilateral group (B) < Right lesion group (R) < Left lesion group (L) < Sham lesion group (S) in Alley 1; S < L < R < B in Alley 2; S < L < R = B in Alley 3; and S = L < R < B in Alley 4 ("<" indicates a significance and " = " indicates no significance) (Fig. 3B). No interaction in the time spent was detected between any of the groups. Analysis of the number of entries on Day 1 revealed that

Table 1
Effects of bilateral and unilateral lesions of the ventral hippocampus on the time spent in each alley.

Level	Factor	Alley 1	Alley 2	Alley 3	Alley 4
Sham–Bilateral	Condition	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$
		$F(1, 12) = 189.59$	$F(1, 12) = 48.69$	$F(1, 12) = 65.11$	$F(1, 12) = 251.09$
	Day	n.s.	n.s.	n.s.	$P < 0.05$
		$F(5, 12) = 1.76$	$F(5, 12) = 0.92$	$F(5, 12) = 1.97$	$F(5, 12) = 2.73$
Sham–Right	Condition	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$
		$F(1, 12) = 63.80$	$F(1, 12) = 25.49$	$F(1, 12) = 21.06$	$F(1, 12) = 14.70$
	Day	n.s.	n.s.	n.s.	n.s.
		$F(5, 12) = 1.36$	$F(5, 12) = 0.54$	$F(5, 12) = 0.15$	$F(5, 12) = 0.61$
Sham–Left	Condition	$P < 0.01$	$P < 0.05$	$P < 0.05$	n.s.
		$F(1, 12) = 8.63$	$F(1, 12) = 4.80$	$F(1, 12) = 7.82$	$F(1, 12) = 3.41$
	Day	n.s.	n.s.	n.s.	$P < 0.05$
		$F(5, 12) = 0.72$	$F(5, 12) = 1.08$	$F(5, 12) = 0.42$	$F(5, 12) = 2.38$
Bilateral–Right	Condition	$P < 0.01$	$P < 0.05$	n.s.	$P < 0.01$
		$F(1, 12) = 8.80$	$F(1, 12) = 5.52$	$F(1, 12) = 0.87$	$F(1, 12) = 30.69$
	Day	$P < 0.01$	n.s.	$P < 0.05$	$P < 0.01$
		$F(5, 12) = 4.41$	$F(5, 12) = 0.83$	$F(5, 12) = 2.77$	$F(5, 12) = 3.51$
Bilateral–Left	Condition	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$
		$F(1, 12) = 81.71$	$F(1, 12) = 24.39$	$F(1, 12) = 11.19$	$F(1, 12) = 216.02$
	Day	$P < 0.05$	n.s.	n.s.	$P < 0.05$
		$F(5, 12) = 2.91$	$F(5, 12) = 1.66$	$F(5, 12) = 0.75$	$F(5, 12) = 2.23$
Right–Left	Condition	$P < 0.01$	$P < 0.01$	$P < 0.05$	$P < 0.01$
		$F(1, 12) = 20.94$	$F(1, 12) = 11.20$	$F(1, 12) = 5.54$	$F(1, 12) = 41.73$
	Day	$P < 0.05$	n.s.	n.s.	n.s.
		$F(5, 12) = 2.90$	$F(5, 12) = 0.96$	$F(5, 12) = 1.83$	$F(5, 12) = 1.87$

n.s., non-significant. No interaction was detected between all groups.

Table 2
Effects of bilateral and unilateral lesions of the ventral hippocampus on the number of entries into each alley.

Level	Factor	Alley 1	Alley 2	Alley 3	Alley 4	Sum	Alley 4/Alley 3
Sham–Bilateral	Condition	n.s.	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$
		$F(1, 12) = 0.94$	$F(1, 12) = 18.95$	$F(1, 12) = 34.56$	$F(1, 12) = 44.24$	$F(1, 12) = 28.09$	$F(1, 12) = 16.11$
	Day	$P < 0.05$	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$	n.s.
		$F(5, 12) = 2.45$	$F(1, 12) = 3.84$	$F(5, 12) = 4.83$	$F(5, 12) = 4.30$	$F(5, 12) = 5.31$	$F(5, 12) = 0.88$
Sham–Right	Condition	n.s.	$P < 0.05$	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.05$
		$F(1, 12) = 1.08$	$F(1, 12) = 7.38$	$F(1, 12) = 15.62$	$F(1, 12) = 24.25$	$F(1, 12) = 13.96$	$F(1, 12) = 4.62$
	Day	$P < 0.05$	$P < 0.01$	n.s.	n.s.	$P < 0.01$	n.s.
		$F(5, 12) = 2.64$	$F(5, 12) = 4.34$	$F(5, 12) = 2.28$	$F(5, 12) = 1.71$	$F(5, 12) = 6.11$	$F(5, 12) = 0.49$
Sham–Left	Condition	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
		$F(1, 12) = 0.15$	$F(1, 12) = 0.49$	$F(1, 12) = 0.22$	$F(1, 12) = 0.00$	$F(1, 12) = 0.28$	$F(1, 12) = 0.50$
	Day	n.s.	n.s.	$P < 0.01$	$P < 0.01$	$P < 0.05$	$P < 0.01$
		$F(5, 12) = 0.82$	$F(5, 12) = 1.56$	$F(5, 12) = 3.49$	$F(5, 12) = 4.82$	$F(5, 12) = 2.62$	$F(5, 12) = 5.15$
Bilateral–Right	Condition	n.s.	$P < 0.01$	$P < 0.05$	$P < 0.05$	$P < 0.01$	n.s.
		$F(1, 12) = 0.08$	$F(1, 12) = 9.52$	$F(1, 12) = 4.60$	$F(1, 12) = 4.80$	$F(1, 12) = 8.64$	$F(1, 12) = 0.40$
	Day	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.05$	$P < 0.01$	$P < 0.01$
		$F(5, 12) = 4.83$	$F(5, 12) = 6.04$	$F(5, 12) = 5.26$	$F(5, 12) = 3.32$	$F(5, 12) = 5.67$	$F(5, 12) = 6.26$
Bilateral–Left	Condition	n.s.	$P < 0.05$	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$
		$F(1, 12) = 0.08$	$F(1, 12) = 6.70$	$F(1, 12) = 29.27$	$F(1, 12) = 51.72$	$F(1, 12) = 17.44$	$F(1, 12) = 31.57$
	Day	$P < 0.05$	n.s.	$P < 0.01$	n.s.	$P < 0.01$	n.s.
		$F(5, 12) = 2.41$	$F(5, 12) = 1.65$	$F(5, 12) = 5.57$	$F(5, 12) = 1.90$	$F(5, 12) = 3.53$	$F(5, 12) = 0.94$
Right–Left	Condition	n.s.	$P < 0.05$	$P < 0.01$	$P < 0.01$	$P < 0.05$	$P < 0.01$
		$F(1, 12) = 0.20$	$F(1, 12) = 4.63$	$F(1, 12) = 12.19$	$F(1, 12) = 29.25$	$F(1, 12) = 7.73$	$F(1, 12) = 30.34$
	Day	$P < 0.05$	$P < 0.05$	$P < 0.05$	n.s.	$P < 0.01$	n.s.
		$F(5, 12) = 3.01$	$F(5, 12) = 2.84$	$F(5, 12) = 2.64$	$F(5, 12) = 1.99$	$F(5, 12) = 3.78$	$F(5, 12) = 0.57$

n.s., non-significant. No interaction was detected between all groups.

the Bilateral lesion group had more entries into Alleys 2 ($P < 0.01$), 3 ($P < 0.01$), and 4 ($P < 0.01$) than the Sham lesion group. Similar to the results of the time spent in each alley, unilateral lesions had no effect on the number of entries, and there was no difference between the Right and Left lesion groups (Fig. 4A). The mixed-design ANOVA revealed the following differences from Day 2–7, listed in increasing number of entries: S = L = R = B in Alley 1; S = L < R < B in Alleys 2, 3, and 4 (Fig. 4B). The sum of entries to all four alleys can be described as S = L < R < B, and comparison between Days 1 and 7 showed significant differences in all groups (Sham, $P < 0.01$; Bilateral, $P < 0.01$; Right, $P = 0.034$; Left, $P = 0.010$; Fig. 4C). The ratio of Alley 4/Alley 3 entries was S = L < R = B. The values of the Bilateral and Right lesion groups were approximately 0.5 on all 7 days, whereas those of the Sham and Left lesion groups decreased from approximately 0.45–0.3 in the first 3 days and then increased to approximately 0.4 in the last 4 days (Fig. 4E). No interaction in the number of entries, sum of entries and ratio of Alley 4/Alley 3 entries was detected between all groups.

3.3. C-fos expression

To determine the amount of neural activity in VH during aversive situations, the animals in the Sham lesion group were restricted to Alley 4, the most anxiogenic among the four alleys, and c-fos

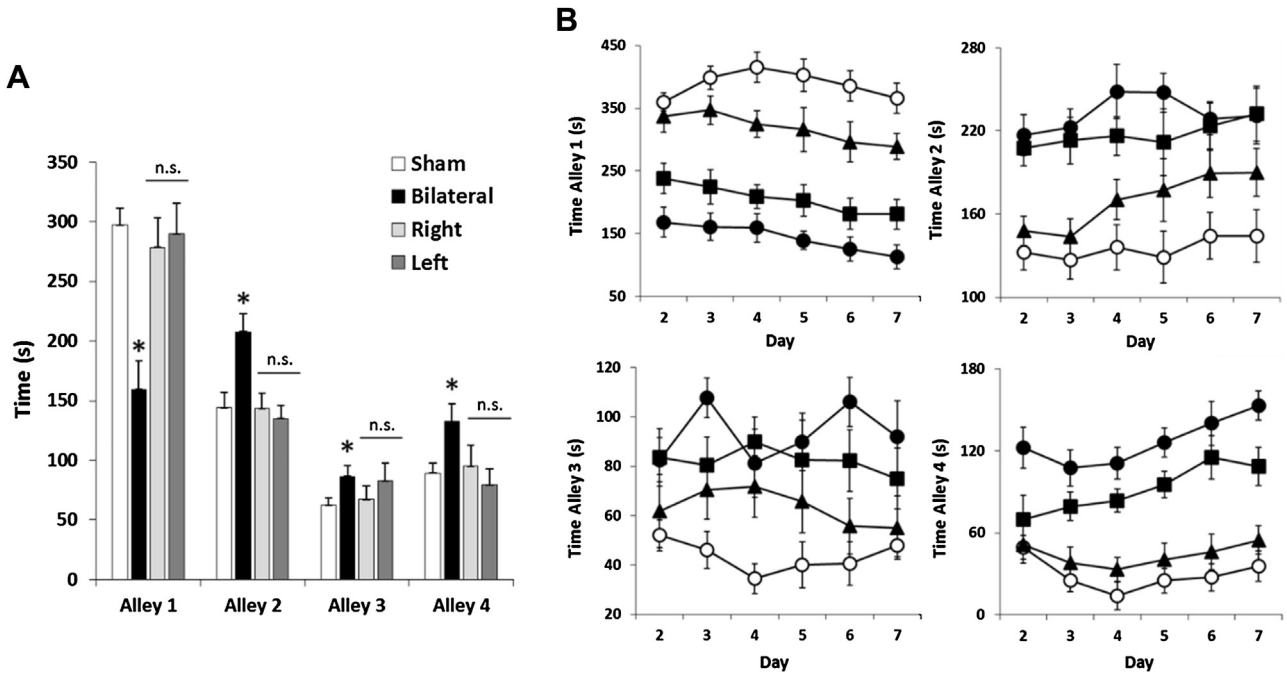

Fig. 3. Results of the time spent in each alley. All the rats in the Sham (white bar or ○), Bilateral (black bar or ●), Right (light gray bar or ■), and Left (dark gray bar or ▲) lesion groups (n = 12 in each group) explored the alleys for 10 min. Time spent in each alley are shown for Day 1 (A) and for Days 2–7 (B). All data are shown as means ± SEM. *P < 0.05 compared with the Sham lesion group. "n.s." means non-significant.

expression was measured. A basic picture of a VH section is shown in Fig. 5A. No electrode stabbing was observed in any of the animals. There was no significant difference in the amount of c-fos expression between the animals who had not had the electrode inserted (n = 3) and the Sham lesion group (who had electrodes inserted). An exposure of 30 min to Alley 4 induced different amounts of c-fos expression between the right and left ventral CA1 ($P < 0.01$) and CA3 ($P = 0.012$) of Sham lesion group. The right hemisphere had a significantly higher number of c-fos-positive cells than the left hemisphere in both subregions (Fig. 5B).

4. Discussion

This study aimed to assess whether the rat VH possesses functional asymmetry in response to an aversive situation and to reveal, if any, what external factors make the asymmetry exhibited. From behavioral and immunohistochemistrical experiments, we found functional asymmetry associated with the anxiety in VH. Moreover, we revealed that the emergence of asymmetry depended on the anxiety level. Therefore, we concluded that the rat VH has some lateralized functions that enable adaptive behavior according to different aversive situations.

4.1. Behavioral test

Functional dissociation along the hippocampal long-axis has already been described, and the rodent VH mainly regulates fear/anxiety and stress responses [19]. It has been revealed that this region is functionally associated with AMG, mPFC, and hypothalamus [35]. Therefore, we investigated how the left and right VH performed during anxiogenic situations by measuring the time spent in and the number of entries into each alley of SAT for 7 days. On Day 1, the Bilateral lesion group spent less time in Alley 1 and more time in Alleys 2, 3, and 4 than the Sham lesion group. The number of entries into Alley 2, 3, and 4 were also more in the Bilateral lesion group than in the Sham lesion group. These results suggest that bilateral VH lesions lead to decreased anxiety-like behavior, in agreement with Mchugh et al. [32]. The results of that study [31] differed slightly from our results, in that they observed a longer time spent only in Alley 2. This might be explained by the differences in experimental conditions, such as the brightness of the light or the time of each trial. In contrast to the Bilateral lesion group, lesions in the left and right VH did not affect anxiety-like behaviors, showing that there was no laterality in these two groups. Other brain areas related to anxiety or fear, such as AMG and mPFC, might work complementarily instead of the unilateral VH. In contrast, analysis of the time spent in each anxiogenic alley (Alleys 2, 3, and 4) from Days 2–7 resulted in a pattern (listed in increasing amount of time spent) of S < L < R < B in Alley 2, S < L < R = B in Alley 3, and S = L < R < B in Alley 4. These results suggested that either the right VH was consistently superior to the left one in mediating anxiety-like behavior or the left and right VH worked together against relatively weak anxiety (such as Alleys 2 and 3, with wider widths); the right VH exclusively worked against strong anxiety (such as Alley 4, with a narrower width). The fact that there was a significant difference between the Sham and Right lesion groups and no significant difference between the Sham and Left lesion groups may have confirmed these possibilities. The presence or absence of the main effects by Day among each group differ for each alley, but this could be attributed to such left/right differences depend on the strength of the anxiety.

The number of entries into each anxiogenic alley resulted in a pattern (listed in increasing number of entries) of S = L < R < B for all alleys. This suggests that the right VH is more active on entry into anxiogenic areas. Moreover, the ratio of Alley 4/Alley 3 entries was also significantly different between the Right and Bilateral lesion groups and the Sham and Left lesion groups. The Right and Bilateral lesion groups had a consistent value of approximately 0.5 on all 7 trial days. In contrast, the Sham and Left lesion groups showed more widely variable values. On Days 1–3, the values gradually decreased from approximately 0.5–0.3, indicating that the animals in the Sham and Left lesion groups experienced stronger anxiety in Alley 4 during this period. In contrast, on Days 5–7, the values gradually increased to nearly 0.5, indicating that the animals in these two groups were habituated to Alley 4 during the latter periods of

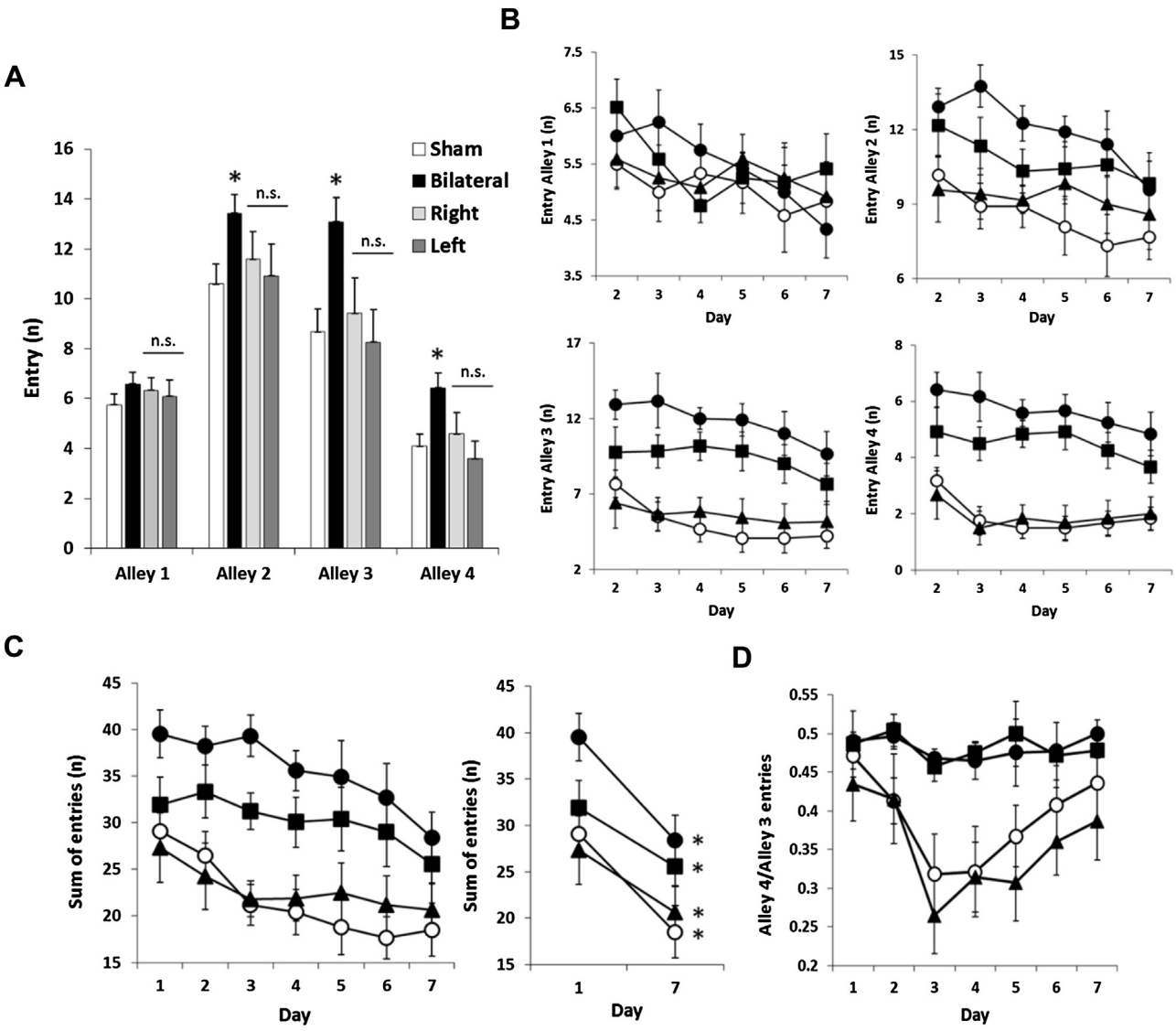

Fig. 4. Results of the number of entries into each alley. All the rats in the Sham (white bar or ○), Bilateral (black bar or ●), Right (light gray bar or ■), and Left (dark gray bar or ▲) lesion groups (n = 12 in each group) explored the alleys for 10 min. The number of entries into each alley are shown for Day 1 (A), and Days 2–7 (B). *P < 0.05 compared with the Sham lesion group. The sum of entries on all 7 days (C-left), the sum of entries on the first and last days (C-right) (*P < 0.05 compared with Day 1), and ratio of Alley 4/Alley 3 entries (D) are shown. All data are shown as means ± SEM. "n.s." means non-significant.

the test. These results suggested that the right VH worked more strongly to exhibit behaviors associated with anxiety than the left VH, in agreement with other studies of lateralized behaviors [1–10] and brain functions [11–13,36–40]. In addition, the total number of entries into the alleys decreased in all groups from Day 1–7, indicating that both unilateral and bilateral VH lesions, gradually formed over this time period, had no influence on the spatial memory.

The above results suggest the existence of functional differences in the left and the right VH, which affect behaviors. Although the equivalence of the extent of damage in the left and right hemispheres is one of the most crucial factors in revealing brain functional asymmetries, there was no significant difference between the left and the right VH in the degree of the damage between all groups, as shown in Fig. 1C and D. Therefore, it could not be considered that the interhemispheric differences in the present study could be attributed to different degrees of damage between the hemispheres. However, as the lesion method in this study was electrical damage, the present research cannot exclude the possibility of functional compensation by other brain regions. Thus, further verification of the functional lateralization with reversible functional inhibition methods will be necessary. Moreover, *in vivo* electrophysiological methods should also be used in future work to investigate left/right asymmetrical activation in greater detail.

4.2. Immunohistochemistry

C-fos expression represents neural activity [41,42], and its expression is increased in the rodent VH after anxiety-like behavior [43,44]. We counted the number of c-fos-positive cells to confirm whether neurons in the rat VH were activated asymmetrically in an anxiogenic situation. The expression of c-fos in the right VH (CA1 and CA3 subregions) was significantly increased compared with that in the left VH of the rats that were isolated in Alley 4. This result is consistent with that of other studies that showed right hemispheric dominance of c-fos and arc expression in AMG and mPFC [38,40,45,46]. As the protein expression of c-fos reflects neuronal activation, our results suggested that right ventral CA1 and CA3 dominantly worked against anxiety that was induced by the narrow Alley 4. A previous study [24] reported that there are fewer neurons in the right hippocampus than in the left hippocampus, and

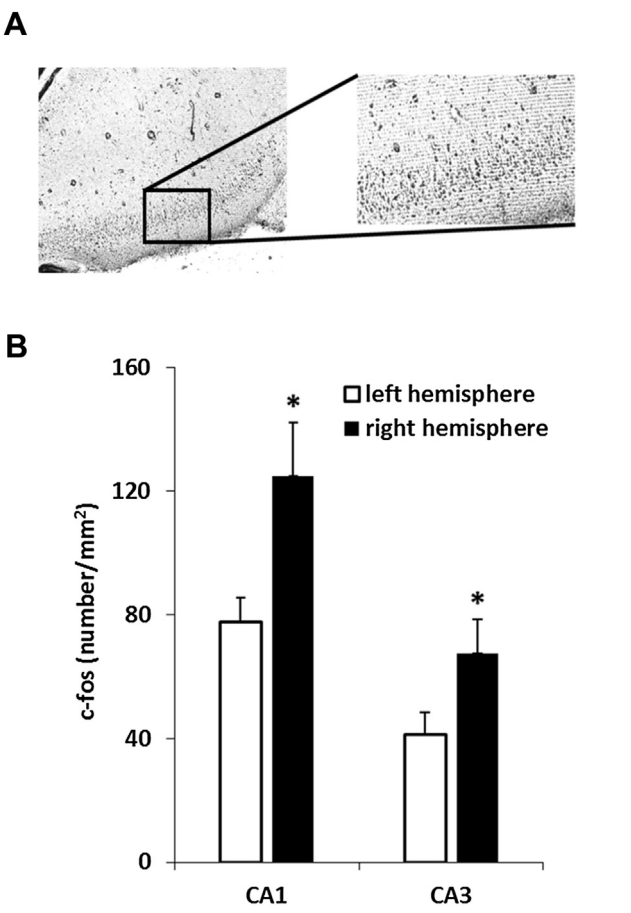

Fig. 5. C-fos expression in VH. (A) A basic sample of a VH section showing c-fos expression. (B) The densities (number/mm^2) of c-fos-positive cells in the right and left ventral CA1 and CA3 of Sham lesion group. All measures are shown as means ± SEM. *P < 0.05 compared with the Sham-Left group.

the number of rat CA1 and CA3 cells is different between the right and left hemispheres, with the right hippocampus containing 6% fewer neurons in the CA3/2 subfield and 21% fewer neurons in the CA1 subfield than the left hippocampus. Thus, it would not be true that being more cells activated in the right VH is derived from being more cells are in the right VH. Furthermore, no stab of the electrode insertion was observed in AP = −4.80 mm sections of any animal, and there were no significance differences in the c-fos expression between non-inserted animals and Sham lesion (inserted) animals. Thus it can be inferred that either the insertion of the electrode did not affect c-fos expression or the effect was extremely small. The dominance of the right VH in our study clearly suggests that more neurons were activated in response to anxiety in the right VH than in the left VH.

4.3. General discussion

The left eye/right hemisphere preference in avoidance behaviors has been repeatedly reported in many animals. Thus, the dominance of the right brain is no doubt involved in adaptive behaviors to cope with aversive situations. Additional research has also suggested that AMG and mPFC also display functional asymmetries associated with fear/anxiety, pain processing, and stress responses [11–13,32–36]. Furthermore, in recent years, many studies have pointed out that VH plays an important role in these same functions [14–18,30]. In this study, we confirmed the relationship between VH andanxiety-related functional asymmetry. VH projects to AMG [47], mPFC [48], and hypothalamus [49], all of which are part of neural circuits that control emotion, indicating that all these regions may exhibit functional asymmetry. In fact, the hippocampus, AMG, and mPFC have left/right asymmetries in the amounts of neurotransmitters secreted in those areas after exposure to stressors and release of corticosterone [11,50], noradrenaline [51], dopamine [52], serotonin [53,54], and angiotensin [55]. A considerable advantage of this functional asymmetry in emotional circuits might be that the activation of a unilateral brain region allows the animal to more quickly perform some urgent behaviors (e.g., fight or flight). In contrast, animals might be able to perform higher behaviors to cope with complex situations or solve difficult problemsby working their two hemispheres interactively.

5. Conclusion

In this study, we investigated functional asymmetry in the rat VH in response to aversive stimuli that cause different anxiety levels. The results of our tests for anxiety-like behavior and the c-fos expression revealed that (1) VH possessed a noticeable functional asymmetry associated with anxiety, (2) the right VH more dominantly worked against anxiety than the left VH, and (3) the extent of the functional asymmetry depended on the anxiety levels, with stronger anxiety enhancing right-hemispheric dominance of VH and weaker anxiety making it less distinct. This is the first study to reveal functional left–right asymmetry of VH and its dependence on the aversiveness of situations. These findings provide new insights on the functional lateralization and its interaction in the brain for adaptive behaviors.

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