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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.

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