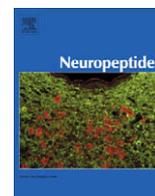




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## Correlations between angiotensinase activity asymmetries in the brain and paw preference in rats

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## ABSTRACT

The function of angiotensin peptides is dependent upon the action of several aminopeptidases (APs) termed angiotensinases. Soluble (SOL) and membrane (MEM)-bound alanyl-AP (AlaAP) and cystinyl-AP (CysAP) are involved in the metabolism of angiotensins and related to the modulation of behavior and memory. To study the interactions between angiotensinase activity in the hippocampus and behavioral lateralization, Wistar rats were selected on the basis of their performance in the paw preference test (left-handed, ambidextrous and right-handed) and the activities of SOL-AlaAP/CysAP and MEM-AlaAP/CysAP were measured in the both hippocampuses. We observed that: (1) the left hippocampus had higher activities of SOL-AlaAP/CysAP and MEM-AlaAP/CysAP than the right hippocampus; (2) rats showed significant differences in the activities of SOL-AlaAP/CysAP and MEM-AlaAP/CysAP in the hippocampus depending on the behavioral lateralization detecting by paw preference; (3) in three groups of rats, hemispheric dominance – %R/T [%R/T = right hemisphere/(right hemisphere + left hemisphere) × 100] activities of MEM-AlaAP, SOL-CysAP and MEM-CysAP was significantly different whereby %RT was lower in left-handed, higher in ambidextrous and intermediate in right-handed rats; (4) individual %R/T activities of SOL-CysAP and MEM-CysAP in the hippocampus were positively correlated with paw preference scores. Finally, we used the passive avoidance behavior test to demonstrate the differences of long-term memory among the three groups. These results suggested that the asymmetric activity of angiotensinase in the rat hippocampus may be associated with both the direction and the intensity of behavioral lateralization as expressed by paw preference.

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### 1. Introduction

Brain asymmetry is thought to be due to functional, anatomical and neurochemical differences. Brain lateralization may be a universal phenomenon that contributes to different functions among members of species in terms of various behaviors (Corballis, 1986). It has been reported that peptides such as angiotensins are related to the modulation of brain functions (Wright and Harding, 1997). Recent investigations proposed that the functional status of these peptides was dependent upon the action of several aminopeptidases (APs) called angiotensinases. Angiotensin IV (ANG IV), which is metabolized from ANG III by alanyl-AP (AlaAP),

has an important role in regulating local blood flow and memory (Wright and Harding, 1997). The brain angiotensin receptor subtype AT<sub>4</sub> has been characterized as a cystinyl-AP (CysAP). It is an insulin-regulated membrane AP (Albiston et al., 2001) that can bind ANG IV with high specificity and affinity to regulate memory and cognitive processing (Albiston et al., 2003).

The hippocampus is an important part of the limbic system. It is considered to be an important region of the brain for learning and memory functioning. A study on the bilateral distribution of the activities of soluble alanyl-AP and cystinyl-AP (SOL-AlaAP/CysAP) and membrane-bound alanyl-AP and cystinyl-AP (MEM-AlaAP/CysAP) showed that there was a left predominance for the activities of MEM-AlaAP, SOL-CysAP and MEM-CysAP in the hippocampus of rats (Banegas et al., 2005). Other studies suggest that strongly right (SR)-handedness is associated with poorer memory performance than non-strongly right (nSR)-handedness (Lyle et al., 2008).

Attempts have been made to correlate behavioral and cerebral asymmetries in animals. Most of these studies focused on the

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relationship between neurochemical asymmetries, in particular dopamine and behavioral lateralization as expressed by spontaneous or amphetamine-induced circling behavior (Cabib et al., 1995). We wanted to investigate the functional asymmetric activation of AlaAP and CysAP in the hippocampus and their potential correlations with behavioral lateralization expressed by paw preference. In rats, postural/motor asymmetries have been described using various tests, including paw preference in a food-reaching or lever-pressing task, side bias in an open field and side preference in a T maze (Papaioannou, 1972; Signore et al., 1991). Paw preference is a food-reaching task model and widely used because of its reliability (Collins, 1968).

Taken together, the experiments described below were designed to investigate the distribution of the activities of AlaAP and CysAP in the left and right hippocampus in left-handed (LH), right-handed (RH), and ambidextrous (L/R) rats. The relationship between the distributions of these APs with behavioral lateralization in rats was investigated.

## 2. Materials and methods

### 2.1. Animals

We have used adequate measures to minimize pain or discomfort of the rats. The research was conducted in accordance with the guidelines published in the NIH Guide for the Care and Use of Laboratory Animals and the principles presented in the “Guidelines for the Use of Animals in Neuroscience Research” by the Society for Neuroscience. All experimental protocols were approved by the Review Committee for the Use of Human or Animal subjects of the Fourth Military Medical University.

Sixty adult male Wistar rats (200–250 g) were purchased from the Animal Center of the Fourth Military Medical University. Rats were housed five per standard polypropylene cage. They were maintained on a 12 h light/12 h dark cycle (07.00–19.00 h) with food and water available *ad libitum*. Behavioral testing and killing were carried out in the second half of the light cycle.

### 2.2. Paw preference test

The paw preference test was modified according to the method of Collins (1985). The paw preference test was carried out about one week after the rats were initially housed in cages. Rats were deprived of food for 18 h. They were then placed in a testing cubicle (width, 9 cm; depth 12 cm) which contained a food pellet in a feeding tube. Rats could get a pellet of food only through the use of one of their paws (left or right). The number of left and right paw reaches for the food pellets was scored. Fifty paw reaches were observed for each rat in each testing session. Rats were tested four times on different days over a two-week period. The first session was considered a training session. Group classification was based on the mean score of the other three sessions. Rats were classified as being right-handed (RH) if the score of the number of right-paw entries (RPE) was  $\geq 29$ , left-handed (LH) if the score was  $\leq 21$ , and ambidextrous (L/R) if the score was between 21 and 29. (Betancur et al., 1991; Fu et al., 2003).

The methods used in the following study were according to those designed by Banegas et al. (2005).

### 2.3. Collection of tissue samples

Tissue samples were collected one week after the end of the paw preference test. Rats were acclimatized to the environment where killing was to be carried out. Rat brains were perfused with saline in the light through the left cardiac ventricle under equi-

thensin anesthesia (2 mL/kg body weight). The equithensin formulation contained: 42.5 g/L chloral hydrate dissolved in 19.76 mL ethanol, 9.72 g/L nembital, 0.396 L/L propylene glycol and 21.3 g/L magnesium sulfate in distilled water. The left and right hippocampus was located according to the stereotaxic atlas of Paxinos and Watson (Paxinos et al., 1980). They were dissected between 7.12 and 5.40 mm anterior to the interaural line in dry ice and then quickly removed (<60 s). To obtain the soluble fraction, tissue samples were homogenized in 10 volumes of 10 mM HCl-Tris buffer (pH = 7.4). They then underwent ultracentrifugation at (100,000g for 30 min at 4 °C). The resulting supernatants were used to measure soluble enzymatic activity and protein content after assaying in triplicate. To solubilize membrane proteins, pellets were re-homogenized in HCl-Tris buffer (pH = 7.4) plus 1% Triton X-100. After centrifugation at 100,000g for 30 min at 4 °C, supernatants were also used to measure membrane-bound activity and proteins. To ensure the complete recovery of activity, adsorbent polymeric Biobeads SM-2 (100 mg/mL) was added to the samples and shaken for 2 h at 4 °C to remove detergent from the medium (Banegas et al., 2005).

### 2.4. Procedures for enzymatic assays

Levels of AlaAP and CysAP were determined in triplicate by fluorometric analysis using (aminoacyl- $\beta$ -naphthylamides: AlaN-Nap and CysNNap) as the substrates (Alba et al., 1986; Greenberg, 1962). Each supernatant (10  $\mu$ L) was incubated for 30 min at 25 °C, with the substrate solution (1 mL): 2.14 mg/100 mL AlaNNap or 5.63 mg/100 mL CysNNap, 10 mg/100 mL bovine serum albumin (BSA), and 10 mg/100 mL dithiothreitol (DTT) in 50 mM of phosphate buffer (AlaAP at pH 7.4) and 50 mM HCl-Tris buffer (CysAP at pH 6.0).

Acetate buffer (1 mL, 0.1 mol/L, pH 4.2) was added to stop all the reactions.  $\beta$ -naphthylamine was released because of the enzymatic activity. It was measured by fluorometric analysis at 412 nm (emission wavelength) and 345 nm (excitation wavelength). Proteins were quantified in triplicate using BSA as a standard (Bradford, 1976). Depending on the substrate and tissue studied, specific soluble and membrane-bound AP activities were expressed as nmol of AlaNNap or CysNNap hydrolyzed per min per mg of protein. Fluorogenic assays were linear with respect to time of hydrolysis and protein content. (Banegas et al., 2005).

### 2.5. Passive avoidance behavior test

Twenty-four rats were chosen at random from three groups for the passive avoidance behavior test. It was carried out in a shuttle-box consisting of a white illuminated compartment and a dark compartment separated by a sliding door in the lower middle part. The test was conducted for two consecutive days including one training trial (day-1). Rats were placed into the white illuminated compartment facing away from the dark compartment, and left for 5 min to acclimatize to the apparatus. After the adaptation period, the shutter between the two compartments was removed. This allowed access to the dark compartment, which was equipped with a stainless steel grid floor for electrical stimulation. As soon as the rat moved into the dark compartment with all four paws, an electrical current (0.3 mA, 30 V) was applied through the floor grid. The maximal duration of the stimulus was 2 s. In the entire period, rats had a free opportunity to escape the negative stimulation by moving into the light compartment. In the testing trial on day-2, the basic procedure was the same as on day-1 and lasted a maximum of 300 s. The latency of the first movement from the light compartment into the dark compartment was measured and defined as “memory retention”, and the time taken by the rat to enter the dark compartment was recorded as the “time of entrance”. If rats

did not leave the dark compartment within 10 s of electrical stimulation, they were removed from the chamber. If rats failed to enter a dark compartment within 5 min, a latency of 300 s was assigned.

## 2.6. Statistical analyses

Rats were classified by RPE scores for each behavioral lateralization (LH, L/R, RH). Two measurements were considered for statistical analyses: the activities of SOL-AlaAP/CysAP and MEM-AlaAP/CysAP (nmol/min/mg prot) for each hemisphere and the percentage of right dominance were calculated for each rat according to the following formula:

$$\%R/T = \frac{\text{right hemisphere}}{\text{right hemisphere} + \text{left hemisphere}} \times 100$$

The activities of SOL-AlaAP/CysAP and MEM-AlaAP/CysAP (nmol/min/mg prot) in each side of the hippocampus in each group were analyzed. Statistical analyses of RPE scores were performed by one-way analysis of variance. Statistical analyses of the activities of SOL- and MEM-AlaAP/CysAP in each hippocampus were performed by two-way analysis of variance (ANOVA) with behavioral lateralization as between factor (three levels: LH, L/R, RH) and hemispheres as within factor (two levels: L, R). Simple effect analyses were tested by one-way ANOVA for each hippocampus (three levels: LH, L/R, RH) followed by *post hoc* tests (Duncan test). A paired *t*-test was performed for the differences between the activities of SOL-AlaAP/CysAP and MEM-AlaAP/CysAP in the right and left hippocampus of each group.

Differences among the %R/T activities of SOL-AlaAP/CysAP and MEM-AlaAP/CysAP characterized by different behavioral lateralization were tested by one-way ANOVAs for each brain area (three levels: LH, L/R, RH) followed by *post hoc* tests (Duncan). Pearson's correlations were also calculated between %R/T activities of SOL-AlaAP/CysAP and MEM-AlaAP/CysAP and individual RPE scores. The results of the passive avoidance behavior test in rats characterized by different behavioral lateralization were also tested by one-way ANOVAs followed by *post hoc* tests (Duncan).  $P < 0.05$  was considered significant.

## 3. Results

### 3.1. Behavioral results of the paw preference test

Sixty Adult male Wistar rats were obtained from the Animal Center of the Fourth Military Medical University. After the behavior test, we observed a right paw preference of 39 RH rats (65%); a left paw preference of 12 LH rats (20%); and ambidextrous paw preferences for 9 L/R rats (15%). 6 rats were randomly chosen to be used in the next experiment separately from each paw preference group.

Table 1 shows the mean RPE scores of the three experimental groups (LH, L/R, RH) used for enzymatic assays. The number of rats per group was 6 LH, 6 L/R and 6 RH. One-way ANOVA revealed a

**Table 1**

Right paw entry scores of left-handed, ambidextrous and right-handed rats selected for these experiments ( $n = 18$ ).

	LH	Ambidextrous	RH
RPE scores (Mean $\pm$ SD)	12.2 $\pm$ 1.2	25.2 $\pm$ 0.8	35.8 $\pm$ 1.5

*Post hoc* comparisons (Duncan) revealed significant differences ( $p < 0.01$ ) among all groups. LH (left-handed), RH (right-handed).

significant main effect of behavioral lateralization [ $F(2,17) = 616.748, p < 0.05$ ].

### 3.2. Activities of AlaAP and CysAP in the absence of behavioral lateralization

Table 2 shows the activities of AlaAP and CysAP in the left and right hippocampus of all rats without consideration of behavioral lateralization. The activities of SOL-AlaAP/CysAP and MEM-AlaAP/CysAP were higher in the left hippocampus than in the right hippocampus ( $p < 0.05$ ). This indicated that there were significant differences in the activities of AlaAP and CysAP between the left and right hippocampus in normal rats.

Table 3 shows the activities of AlaAP and CysAP in the bilateral hippocampuses of LH, L/R and RH rats. The activities of SOL-CysAP and MEM-AlaAP/CysAP were higher in the left hippocampus than in the right hippocampus in the three behavioral rats ( $p < 0.05$ ), except that there was no significant difference in the activities of SOL-AlaAP between the bilateral hippocampuses of three groups ( $p > 0.05$ ).

### 3.3. Activities of AlaAP and CysAP in the presence of behavioral lateralization

A significant main effect of behavioral lateralization was found in the activities of SOL-AlaAP [ $F(2,30) = 14.498, p < 0.05$ ]; MEM-AlaAP [ $F(2,30) = 19.304, p < 0.05$ ]; SOL-CysAP [ $F(2,30) = 17.928, p < 0.05$ ], and MEM-CysAP [ $F(2,30) = 22.626, p < 0.05$ ] (Fig. 1). This indicated major differences among bilateral activities of the hippocampus in the different experimental groups. A significant interaction between behavioral lateralization and hemispheric activity was found only in SOL-CysAP [ $F(2,30) = 46.606, p < 0.05$ ] and MEM-CysAP [ $F(2,30) = 3.762, p < 0.05$ ].

Individual between-groups comparisons (Duncan test) revealed that the activity of SOL-AlaAP in the left hippocampus was higher in ambidextrous rats than that in RH rats and LH rats ( $p < 0.05$ ). The activity of MEM-AlaAP in the left hippocampus was higher in L/R rats than in RH rats ( $p < 0.05$ ). The activity of SOL-CysAP and MEM-CysAP in the left hippocampus was higher in LH rats than that in L/R rats and RH rats ( $p < 0.05$ ).

The right hippocampus had higher activities of SOL-AlaAP/CysAP and MEM-AlaAP/CysAP in L/R rats than in LH rats and RH rats ( $p < 0.05$ ) (Fig. 2).

### 3.4. %R/T activities of AlaAP and CysAP in the hippocampus

No significant effect of behavioral lateralization was found in the hippocampus in the %R/T activity of SOL-AlaAP [ $F(2,17) = 0.105, p > 0.05$ ] (Fig. 3). A significant effect of behavioral lateralization was demonstrated in the %R/T activity of MEM-AlaAP [ $F(2,17) = 9.756, p < 0.05$ ]; %R/T activity of SOL-CysAP [ $F(2,17) = 26.676, p < 0.05$ ]; %R/T activity of MEM-CysAP [ $F(2,17) = 26.676, p < 0.05$ ]; %R/T activity of MEM-CysAP [ $F(2,17) = 26.676, p < 0.05$ ].

**Table 2**

Activities of SOL- and MEM-AlaAP/CysAP (nmol/min/mg prot, Mean  $\pm$  SD) in the left and right hippocampus in rats ( $n = 18$ ).

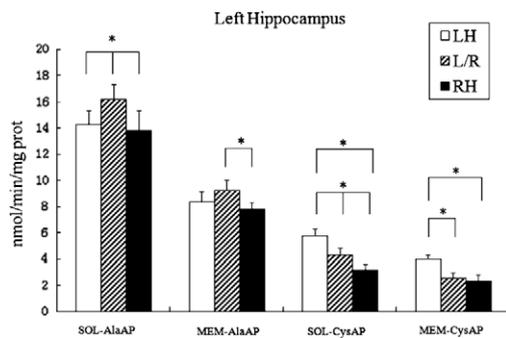
	Mean $\pm$ SD		<i>t</i>	<i>df</i>	<i>p</i>
	Left hippocampus	Right hippocampus			
SOL-AlaAP	14.77 $\pm$ 1.48	13.77 $\pm$ 1.68	2.614	17	<0.05
MEM-AlaAP	8.46 $\pm$ 0.89	4.89 $\pm$ 1.20	19.718	17	<0.05
SOL-CysAP	4.38 $\pm$ 1.20	2.00 $\pm$ 0.59	6.868	17	<0.05
MEM-CysAP	2.95 $\pm$ 0.85	1.06 $\pm$ 0.32	7.491	17	<0.05

Statistically significant differences between right and left hippocampus in each group (paired-samples *t*-test).

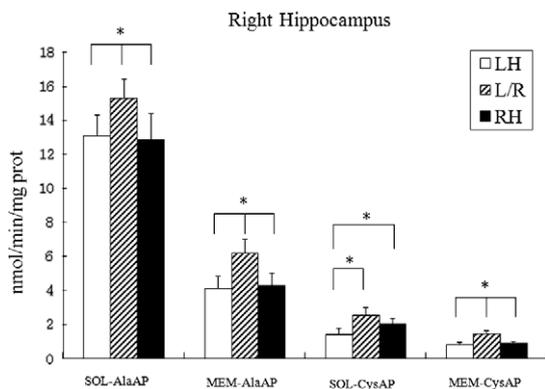
**Table 3**  
Activities of SOL- and MEM-AlaAP/CysAP (nmol/min/mg prot, Mean  $\pm$  SD) in the right and left hemispheres of rats selected for their different right paw entry scores.( $n = 18$ ).

Behav, lateral Hippocampus	LH		Ambidextrous		RH	
	L	R	L	R	L	R
SOL-AlaAP	14.29 $\pm$ 1.00 ( $t = 1.667$ , $df = 5$ , $p > 0.05$ )	13.11 $\pm$ 1.21	16.21 $\pm$ 1.05 ( $t = 1.140$ , $df = 5$ , $p > 0.05$ )	15.34 $\pm$ 1.11	13.81 $\pm$ 1.48 ( $t = 1.513$ , $df = 5$ , $p > 0.05$ )	12.85 $\pm$ 1.54
MEM-AlaAP	8.36 $\pm$ 0.75 ( $t = 21.418$ , $df = 5$ , $p < 0.05$ )	4.13 $\pm$ 0.70	9.23 $\pm$ 0.78 ( $t = 10.962$ , $df = 5$ , $p < 0.05$ )	6.21 $\pm$ 0.82	7.78 $\pm$ 0.50 ( $t = 13.000$ , $df = 5$ , $p < 0.05$ )	4.33 $\pm$ 0.72
SOL-CysAP	5.74 $\pm$ 0.51 ( $t = 32.383$ , $df = 5$ , $p < 0.05$ )	1.41 $\pm$ 0.35	4.28 $\pm$ 0.54 ( $t = 9.529$ , $df = 5$ , $p < 0.05$ )	2.55 $\pm$ 0.44	3.13 $\pm$ 0.42 ( $t = 20.108$ , $df = 5$ , $p < 0.05$ )	2.04 $\pm$ 0.30
MEM-CysAP	3.99 $\pm$ 0.30 ( $t = 18.751$ , $df = 5$ , $p < 0.05$ )	0.83 $\pm$ 0.13	2.53 $\pm$ 0.39 ( $t = 4.675$ , $df = 5$ , $p < 0.05$ )	1.44 $\pm$ 0.21	2.32 $\pm$ 0.45 ( $t = 6.384$ , $df = 5$ , $p < 0.05$ )	0.91 $\pm$ 0.10

Statistically significant differences between right and left hippocampus in each group (paired-samples  $t$ -test). LH (left-handed), RH (right-handed).



**Fig. 1.** The activities of SOL- and MEM-AlaAP/CysAP (nmol/min/mg prot) in the left hippocampus of left-handed (LH), ambidextrous (L/R) and right-handed (RH) rats ( $n = 18$ ). The vertical lines above the bars indicate standard deviation. \* $p < 0.05$  (Duncan test).

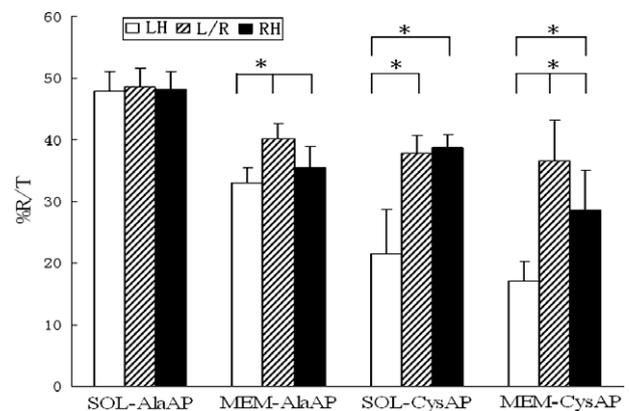


**Fig. 2.** The activities of SOL- and MEM-AlaAP/CysAP (nmol/min/mg prot) in the right hippocampus of left-handed (LH), ambidextrous (L/R) and right-handed (RH) rats ( $n = 18$ ). The vertical lines above the bars indicate standard deviation. \* $p < 0.05$  (Duncan test).

(2,17) = 18.151,  $p < 0.05$ ]. Individual comparisons between groups (Duncan test) revealed that the %R/T activity of MEM-AlaAP was significantly higher in L/R rats than that in LH rats and RH rats ( $p < 0.05$ ); %R/T activity of SOL-CysAP was significantly higher in L/R rats than in LH rats and higher in RH rats than in LH rats ( $p < 0.05$ ); %R/T activity of MEM-CysAP was significantly higher in L/R rats than that in LH rats and in RH rats ( $p < 0.05$ ).

### 3.5. Correlations between individual %R/T activities of AlaAP and CysAP and RPE scores

We further analyzed the correlations between the between RPE scores and the %R/T activities in the hippocampus. The correlation



**Fig. 3.** %R/T activities of SOL- and MEM-AlaAP/CysAP in hippocampus from left-handed (LH), ambidextrous (L/R) and right-handed (RH) rats ( $n = 18$ ). The vertical lines above the bars indicate standard deviation. \* $p < 0.05$  (Duncan test).

between the %R/T activity of SOL-AlaAP and MEM-AlaAP and RPE failed to reach statistical significance: %R/T activity of SOL-AlaAP ( $R = 0.041$ ,  $n = 18$ ,  $p > 0.05$ ); %R/T activity of MEM-AlaAP ( $R = 0.305$ ,  $n = 18$ ,  $p > 0.05$ ) (Fig. 4). A positive correlation between RPE scores and %R/T activities of SOL-CysAP ( $R = 0.904$ ,  $n = 18$ ,  $p < 0.05$ ) and MEM-CysAP ( $R = 0.500$ ,  $n = 18$ ,  $p < 0.05$ ) were observed (Fig. 5). These data indicated a strong relationship between the intensity of behavioral lateralization and hemispheric asymmetry in the activity of CysAP in the rat hippocampus.

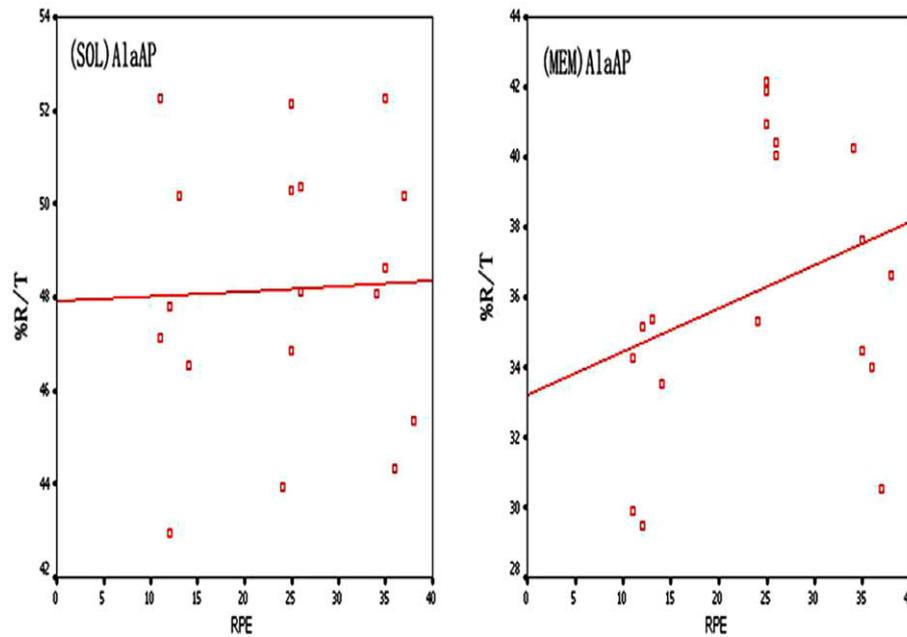
### 3.6. Results of the passive avoidance behavior test

The results of the passive avoidance behavior test are shown in Fig. 6. On day-2, there was no significant main effect of groups on latency to enter the dark compartment [ $F(2,23) = 3.045$ ,  $p > 0.05$ ] and the time of entrance [ $F(2,23) = 0.950$ ,  $p > 0.05$ ]. But Individual between-groups comparisons (Duncan test) revealed that the latency to enter the dark compartment was higher in LH rats than in RH rats ( $p < 0.05$ ).

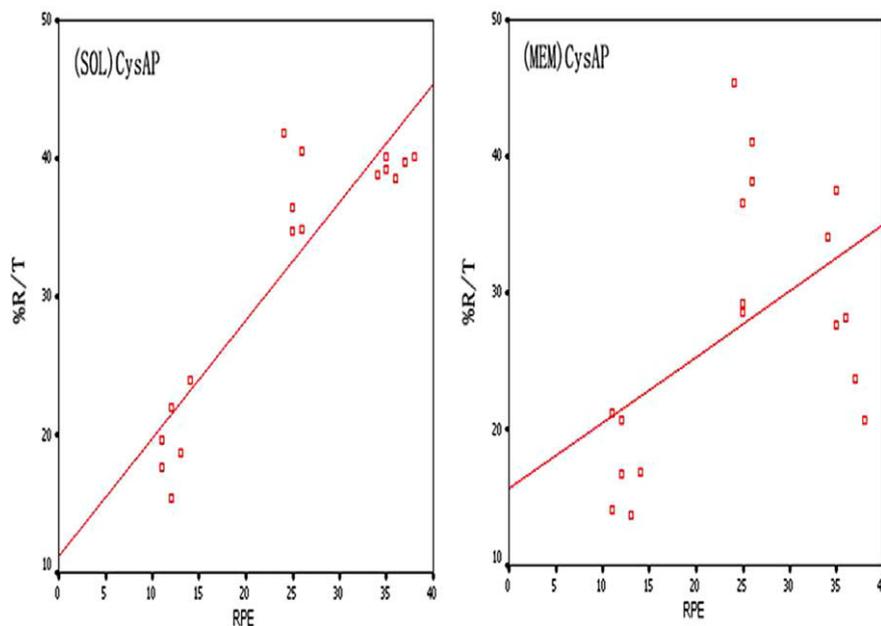
## 4. Discussion

The present study aimed to demonstrate a correlation between behavioral lateralization (described as paw preference and the asymmetric activities of brain angiotensinase (AlaAP and CysAP)) and the interhemispheric differences in AP activities. Our data suggested a positive correlation between hemispheric asymmetries in AP activities and individual paw preference scores.

ANG IV was thought to be an inactive product of the degradation of ANG II, but it was subsequently discovered that the hexapeptide markedly enhanced learning and memory



**Fig. 4.** Correlations between individual %R/T activities of SOL- and MEM-AlaAP in the hippocampus and RPE scores ( $n = 18$ ).



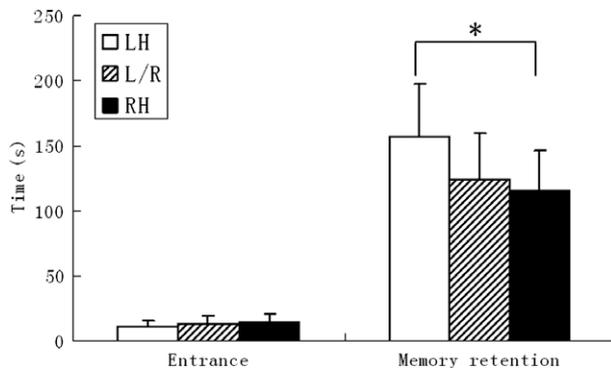
**Fig. 5.** Correlations between individual %R/T activities of SOL- and MEM-AlaAP in the hippocampus and RPE scores. ( $n = 18$ ).

(Alescio-Lautier and Soumireu-Mourat, 1998). It could also reverse memory deficits in animal models of amnesia.  $AT_4$  was recently discovered and characterized to bind ANG IV preferentially (Wright and Harding, 1995). This ANG IV/ $AT_4$  system was believed to be prominent in brain structures associated with cognitive processes and memory. First, one study demonstrated high concentrations of the  $AT_4$  receptor site in the hippocampus of the rat, guinea pig and monkey, which has a close correlation with memory (Wright et al., 1993). Second, it was reported that intracerebroventricular infusion of ANG IV could influence the hippocampus, because it could selectively activate c-Fos immunoreactivity in the pyramidal and granular cells of the hippocampus (Roberts et al., 1995). Third, administration of  $AT_4$  receptor antagonists could disrupt the retention of associative and spatial memory in rats, whereas, ANG IV could facilitate these process (Wright et al.,

1995, 1999). Taken together, these data strongly suggested that the angiotensins (AlaAP and CysAP) in the hippocampus have important roles in regulating learning and memory.

#### 4.1. Activities of AlaAP and CysAP in the absence of behavioral lateralization

Results in the present study were in accordance with a study showing a significant difference in AlaAP and CysAP activities between the left and right hippocampus in rats (Banegas et al., 2005). The hippocampus exhibited a bilateral pattern of AP activities with left predominance. We also identified increased activities of AlaAP and CysAP in the left hippocampus. CysAP activity was believed to be identical to the  $AT_4$  receptor distributed in the hippocampus (Albiston et al., 2001), which has a high



**Fig. 6.** The results of passive avoidance behavior test in left-handed (LH), ambidextrous (L/R) and right-handed (RH) rats ( $n = 24$ ). The figure displays the time response of the entrance and memory retention(s). The vertical lines above the bars indicate standard deviation. \* $p < 0.05$  (Duncan test).

density and is associated with brain functions of learning and memory (Alescio-Lautier et al., 2000). Additionally,  $AT_4$  receptors occur in high levels in the basal nucleus of Meynert, CA1 to CA3 regions in the hippocampus, and throughout the neocortex, areas important for cognitive processing. Despite the dramatic central effects of Ang IV and abundance of the receptor in the central nervous system, the identity of the  $AT_4$  receptor and the mechanism by which its ligands mediate their actions are unknown (Albiston et al., 2001). Here, we found a higher level of activity of AlaAP and CysAP in the left hippocampus, suggesting their relative important functional asymmetries on this side. Furthermore, asymmetrical brain would have important roles in the modulation of learning and memory in humans. Typical cerebral lateralization is associated with left cerebral dominance for language. In the present study, the left hippocampus with its higher AP activities may have a more important role than the right hippocampus in the facilitation of learning and memory.

#### 4.2. Activities of AlaAP and CysAP in the presence of behavioral lateralization

The present study clearly demonstrated that the activities of AlaAP and CysAP in the hippocampus were associated with lateralization in normal rats. The activity of AlaAP was highest in L/R rats and the activity of CysAP was highest in LH rats, intermediate in L/R rats and lowest in RH rats in the left hippocampus. The right hippocampus had higher AlaAP and CysAP activities in L/R rats than in LH and RH rats. Both AP activities were higher in the left hippocampus than in the right hippocampus. This showed that important components of the AP in the hippocampus were also associated with brain lateralization. Significant asymmetries in the activity of CysAP (but not AlaAP) in rats showed that the ipsilateral relationship between APs and preferred paws observed in the present study and links between different APs and brain lateralization were different. We commonly accept that LH rats had right cerebral dominance, whereas RH rats had left cerebral dominance. The present study showed that the basal activity of CysAP in LH rats was higher than in RH rats in the left hippocampus, but a significant difference was not found in the right hippocampus for LH and RH rats.

#### 4.3. Correlations between individual %R/T activities of AlaAP and CysAP in the hippocampus and RPE scores

We studied lateralization using paw preference because this behavior appears to be more stable; >90% of rats presented a consistent paw preference score with time. The model enables the

quantitative and objective study of lateralization. The relationship between hemispheric dominance of AP activities in the hippocampus and preferred paw at the group level was revealed in the present study. The present study is the first demonstration of a linear correlation between %R/T activities of AP and individual paw preference scores. We found a positive correlation between the activity of CysAP in the hippocampus and the intensity of behavioral lateralization.

#### 4.4. The passive avoidance behavior test

The passive avoidance behavior test is an index of long-term memory. In the present study, LH rats showed longer memory retention than RH rats. Previous studies revealed that strongly right-handedness is associated with poorer memory performance than non-strongly right-handedness (Lyle et al., 2008). Together with the results of the activities of AlaAP and CysAP in the presence of behavioral lateralization, we suggest that the LH rats have a higher activity of AP than RH rats and could perform preferential brain function in memory.

The present work showed that, in general, the left hippocampus had higher activities of AlaAP and CysAP than the right hippocampus. It also suggested that the asymmetric activity of AP (particularly CysAP) has a strong relationship with direction and the intensity of rat behavior. We are only beginning to comprehend the nature of the pathways linking the activities of AP and paw preferences. The possible implications of behavioral lateralization and asymmetrical brain functions need further investigation.

## 5. Conclusion

The present study supports the view that different measurements of postural/motor asymmetries may reflect different brain asymmetries and suggest a strong correlation between behavioral lateralization expressed as paw preference and the asymmetric activity of AP (CysAP) in the rat hippocampus.

#### Conflict of interest statement

None declared.

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