Research report

Excitotoxic basolateral amygdala lesions potentiate the memory impairment effect of muscimol injected into the medial septal area

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Abstract

In rats, the septo-hippocampal system is important for memory encoding. Previous reports indicate that muscimol, a specific GABAergic agonist induces learning and memory deficits when infused into the medial septal area. The basolateral nucleus of the amygdala (BLA) modulates memory encoding in other brain areas, including the hippocampus. To explore the interactions between the septo-hippocampal system and amygdala in memory, we studied the effects of intra-medial septal infusions of muscimol in rats with BLA lesions. Animals received sham surgery or excitotoxic BLA lesions and were given infusions of either vehicle or muscimol (5 nmol) into the medial septal area 5 min prior to training sessions in inhibitory avoidance and water maze tasks. In the inhibitory avoidance task, muscimol-induced memory impairment was potentiated by BLA amygdala lesions. Additionally, in the water maze task, BLA-lesioned rats given muscimol infusions into the medial septal also showed memory impairment. These findings indicate that the MSA interacts with the BLA in the processing of memory storage. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

Extensive studies with rats have shown that the hippocampus plays a key role in the acquisition and retention of inhibitory avoidance, as well as water and radial maze learning tasks. The medial septal area (MSA) projects to the hippocampus through cholinergic fibers that run in the fornix and enter the hippocampus through the fimbria. Hippocampal theta activity, which according to some reports is strongly associated with learning, is controlled by MSA neurons through this pathway [11,20,21,27,30]. Spatial learning and spatial mapping by hippocampal cells [18,19] are impaired by MSA and fimbria–fornix lesions [4,9]. Muscimol and benzodiazepine agonists administered into the MSA disrupt several forms of learning, including one-trial inhibitory avoidance and water maze learning [3,4,16,30,32,33].

It is well established that the amygdala plays an important role in modulating the storage of affectively influenced memory [1,2,5,7,8,10,22,26,36,46]. In particular, the basolateral nucleus of the amygdala (BLA) is implicated in this function. Although in inhibitory avoidance tasks, excitotoxic lesions of the BLA alone do not induce significant learning deficits, they do prevent many modulatory influences on memory storage [25,26,37–41,47]. Recent electrophysiological studies reporting functional interactions between the BLA and the hippocampus [10,14,15,24] suggest that the hippocampus and the amygdala may interact in memory formation.

Anatomical findings have shown extensive interconnections between septal and amygdaloid nuclei and the hippocampus involving the entorhinal cortex [6,13,49]. Previous findings suggest that the MSA and the BLA may interact in modulating memory processing involving the hippocampus [9,17,49]. Here we further explore this issue by studying the effect of three different treatments, (1) BLA lesions, (2) micro-infusion of muscimol into the MSA prior to training, and (3) a combination of both

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treatments in two different memory tasks: inhibitory avoidance and water maze spatial learning.

2. Materials and methods

2.1. Subjects

Male Sprague–Dawley rats (n = 70; 300–325 g at time of surgery) from the Charles River Laboratories (Wilmington, MA) were used. They were individually housed in a temperature and light controlled vivarium (22°C; 12:12-h light:dark cycle; lights on at 0700 h), with food and water available ad libitum.

2.2. Surgical procedures

The rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.). Additional doses of pentobarbital were administered as needed. Bilateral lesions of the BLA were made with an N-methyl-D-aspartate (NMDA) solution (Sigma Chemical; 1 mg/100 ul phosphate buffer, pH 7.4) using a stereotaxic apparatus (Kopf, Tujunga, CA) and the following coordinates according to the atlas of Paxinos and Watson [34]: AP, −3.0 mm; ML, ±5.0 mm; DV, −8.4 mm. The NMDA solution was back-filled into a 30-gauge needle that was attached by a polyethylene tube to a 10μl Hamilton syringe driven by a minipump Sage Instruments, Boston, MA. NMDA infusions were made over a period of 30 s total. Injections were made using a 30-gauge needle connected to a 10-μl Hamilton syringe by polyethylene tubing. The top of the needle was bent in such a way that the distal end protruded to 2 mm beyond the tip of the cannula. The styloids were removed from the cannula and the needle was inserted into the cannula. The injection solution was delivered (0.5 ul/38 s) by an automated syringe pump (Sage Instruments). After the injection was completed, the needles were kept in the cannulae for an additional 30 s to maximize drug diffusion. The styloids were replaced as soon as the needles were removed. Following injections, the animals were returned to their home cages and trained 5 min later.

2.3. Drug preparation and infusion procedures

Muscimol (Sigma) was dissolved in saline solution and injected at a dosage of 5.0 nmol into the MSA 5 min prior to training. Injections of vehicle or drugs into the MSA were made using a 30-gauge needle connected to a 10-μl Hamilton syringe by polyethylene tubing. The top of the needle was bent in such a way that the distal end protruded to 2 mm beyond the tip of the cannula. The styloids were removed from the cannula and the needle was inserted into the cannula. The injection solution was delivered (0.5 ul/38 s) by an automated syringe pump (Sage Instruments). After the injection was completed, the needles were kept in the cannulae for an additional 30 s to maximize drug diffusion. The styloids were replaced as soon as the needles were removed. Following injections, the animals were returned to their home cages and trained 5 min later.

2.4. Inhibitory avoidance apparatus and procedures

The animals were randomly assigned treatment schedules and then given intraseptal injections of 5 nmol muscimol or buffer solution 5 min prior to training in an inhibitory avoidance task.

The inhibitory avoidance apparatus consisted of a trough-shaped alley (91 cm long, 15 cm deep, 20 cm wide at the top, 6.4 cm wide at the bottom) separated into two compartments (30 and 60 cm long) by a stainless-steel door that opened by receding into the floor. The 30 cm starting compartment was illuminated by a Tensor lamp (25 W). The floor of the starting light-compartment was constructed of plastic. The floor of the dark compartment was constructed of stainless-steel plates through which footshock could be delivered. Training and testing were conducted in a sound and light attenuated room. At the time of training, the rat was placed in the lighted start compartment facing away from the door. When the rat turned around the door was opened and a timer was started. After the rat entered the dark compartment, the door was closed, a footshock (0.5 mA, 1.0 s constant current) was administered and the rat was immediately removed and returned to its cage. The latency of the rats to enter the dark compartment was recorded. On the retention test, performed 48 h later, the animals were placed in the starting compartment using the same procedure as in the training session. The timer recording the retention latency was started immediately after the animal turned to face the
darkened compartment or after 10 s if the animal did not turn around within that interval. No footshock was administered on the retention test. The latency to enter the darkened compartment (maximum of 600 s) was used as an index of retention. The training and test were conducted by a researcher who was not aware of the treatment given to the animals.

2.5. Water maze apparatus and procedures

One week after completion of inhibitory avoidance testing, the rats were quasi-randomly assigned to new treatment groups and then trained in a spatial task in the Morris water maze. The maze was a circular galvanized steel tank, 183 cm in diameter and 58 cm in height, and filled with water (25°C) to a depth of 20 cm. It was located in a room containing several extramaze cues. Four starting positions were equally spaced around the perimeter of the pool. A perspex platform (20 × 25 cm) was placed 25 cm away from the edge of the pool in a fixed location. The top of the platform was submerged 2.5 cm below the surface of the water.

Five minutes prior to the start of the water maze training, the rats received infusions of 5 nmol muscimol or buffer solution into the MSA. Before the first training trial the rats were placed on the submerged platform for 30 s. On each of the training trials (i.e., swims), the rat was placed into the tank, facing the wall of the tank, at one of four designated starting positions and then allowed to swim to and escape onto the submerged, non-visible,
platform. The latency to find the platform was recorded. If an animal failed to escape within 60 s it was manually guided to the platform. After mounting the platform, the animal was allowed to remain there for 20 s and then placed into a holding cage for 30 s until the start of the next trial. All animals received six training trials. On the retention test 48 h after training three retention test trials were given with the platform in the same location as during the training trials. A maximum time of 60 s per test trial was allowed for the rat to locate the platform. If it did not find the platform within 60 s it was removed without being guided to the platform, returned to a holding cage for 20 s, and then placed again into the tank at one of the starting positions. Each of the escape latencies, as well as the average of the three latencies, were used as measures of retention.

2.6. Statistics

Inhibitory avoidance training and test results are expressed in medians (interquartile range) and were analyzed using the Kruskal–Wallis $H$-test. The differences between two groups were analyzed using two tailed Mann–Whitney $U$-tests. Non-parametric statistics were used for inhibitory avoidance data because of indeterminate scores resulting from the use of a maximum latency of 600 s.

The water maze training results are presented as mean values with standard errors (S.E.) and analyzed additionally using two-way analysis of variance (ANOVA). Treatments were assessed (four levels) as the between-subjects variable and trial numbers (training, six levels) as the within-subject variable. In the water maze test results the means of the three test trials were analyzed with two way

Fig. 2. Brightfield photomicrographs of thionin stained, coronal sections of rat brain. Photo A shows areas of a non-Muscimol-treated rat. A squared area of the MS is shown magnified in photo B. Photo C shows a muscimol-treated section of rat brain and photo D shows the squared area of the MS magnified with needle trace extending into the MS. Above this is the cannula trace. The bar is 100 $\mu$m in A and C and 200 $\mu$m in photos B and D. Medial septal area (MS), lateral septal area (LS), lateral ventrical (LV).
analysis of variance (ANOVA), with the treatments; lesion (two levels) and drug treatment (two levels) as the between-subjects variable and trial numbers (test 3 levels) as the within-subject variable. The Fisher post hoc test was used to determine the source of the detected significance in the ANOVA. All statistics were evaluated for significance using a criterion of \( p < 0.05 \).

3. Results

3.1. Histology

A representative lesion of a BLA nucleus is shown in Fig. 1. Histological examination revealed considerable decreases in neuronal density and gliosis in the lesioned areas. The anterior–posterior extensions of the BLA lesions were between \(-2.3\) and \(-3.3\) mm caudal from bregma. In the animals included in the analysis of each lesioned group, at least 50% of the BLA was damaged bilaterally. There was mild damage in the cortex adjacent to the BLA, on at least one side, in 35 of the animals. These findings are consistent with previous histological descriptions of NMDA induced lesions[37,39]. Data from 15 animals in the BLA lesioned groups were excluded because the lesions failed to meet the criterion or because extensive damage was observed in adjacent structures. Inspection of cannulae and tip of needle placements showed that 48 of 55 selected animals had acceptable placements in the MSA. Fig. 2 shows the placements of the cannulae above, and the needle trace into the MSA of a muscimol-treated rat.

3.2. Inhibitory avoidance results

The groups did not differ in training session step-through latencies [Kruskal–Wallis analysis of variance (\( H = 3.53; df = 3; p = 0.317 \)], Table 1. The median (interquartile range) training step-through latency was 8 (8.75) s.

Retention test latency data are shown in Fig. 3. Kruskal–Wallis analysis revealed a significant group effect in these animals (\( H = 23.67; df = 3; p < 0.0001 \)). The latencies of the sham lesioned-muscimol group were significantly shorter than those of the sham lesioned-vehicle group (\( U_{12,13} = 36, p < 0.04 \)). The latencies of the BLA lesioned-vehicle group did not differ significantly from those of the sham lesioned-vehicle controls or sham lesioned-muscimol group. The latencies of the BLA lesioned-muscimol group were significantly shorter than those of the sham lesioned-vehicle group (\( U_{12,12} = 6, p < 0.0005 \)), the sham lesioned-muscimol group (\( U_{12,13} = 32, p < 0.01 \)) and the BLA lesioned-vehicle group (\( U_{12,11} = 13, p < 0.001 \)).

3.3. Water maze results

Table 1 shows the mean latency to find the submerged non-visible platform in the training trials for the four groups of animals. In the training session a two-way ANOVA with one repeated measure (latency to the platform) revealed no significant treatment effects (\( F_{1,260} = 2.21 \)). However, significant trial effects were found (\( F_{2,260} = 11.61, p < 0.0005 \)), indicating that all groups learned the water-maze task.
Fig. 4 shows the mean latency to find the submerged non-visible platform on the retention test conducted 48 h after training. A repeated measures ANOVA across the three retention trials revealed a significant effect for treatment \( F_{3,31} = 4.54, p < 0.01 \). Rats with BLA lesions and muscimol infused into the MSA were significantly impaired in their retention of the platform location as compared to all other groups (\( p < 0.01 \)). Between-group comparisons: sham-lesioned vehicle animals (T1 \( p < 0.005 \); T2 \( p < 0.05 \); T3 \( p < 0.02 \)), the sham-lesioned muscimol animals (T1 \( p < 0.02 \); T2 \( p < 0.05 \); T3 \( p < 0.04 \)), and the BLA-lesioned vehicle rats (T1 \( p < 0.002 \); T2 \( p < 0.005 \); T3 \( p < 0.05 \)). There were no significant differences between the other groups.

4. Discussion

The principal finding of this study is that rats with BLA lesions, and prior to training infused with muscimol in the MSA, showed impaired memory for inhibitory avoidance and spatial water maze training control animals. This result is consistent with previous evidence suggesting that the BLA and the septo-hippocampal system are both involved in regulating the learning and memory of inhibitory avoidance and water maze tasks. Importantly, our results support the hypothesis that the amygdala and the septo-hippocampal system work together in learning and memory processes related to these two different avertively motivated tasks.

Extensive evidence points to the BLA as a brain region that plays a central role in modulating the acquisition and/or consolidation of avertively motivated tasks [10,24,26]. Although excitotoxic BLA lesions induced prior to training do not block the acquisition of IA tasks, such lesions block the memory modulatory effects of several classes of neurotransmitters and related drugs [26,31, 37,39,41,47]. Thus, our finding that BLA excitotoxic lesions per se do not cause significant learning deficits in the IA task are consistent with those of previous studies.

Participation of the BLA in water maze tasks is less well established. Although it is clear that the water maze is an aversive task, lesions of the BLA do not impair learning and memory of this task [9,37–39]. However, posttraining infusion of norepinephrine into the BLA improves retention of water-maze learning [12] whereas infusion of an inhibitor of glucocorticoid receptors into the BLA impairs memory in this task [40]. Furthermore, BLA lesions prevent the effect of several drugs on memory consolidation of water maze spatial learning [39,41]. These observations strongly suggest that the amygdala/BLA acts to modulate the learning and memory of water maze tasks in rats.

The hippocampus is clearly involved in memory processes of water maze and inhibitory avoidance tasks [9,16,23,29]. In view of the well-known multiple interconnections among MSA, BLA and the hippocampus [13,42–44], our results may suggest a cooperative or interactive role of the MSA and the BLA in hippocampal memory processing of these two tasks. It is possible that the role of the MSA may be mediated by its strong cholinergic projection to the hippocampus which regulates neuronal activity relevant to memory processing [11,20,21,48].

The present findings agree with those published previously by Decker and colleagues [9], who studied the effect of combined septal and amygdala lesions on memory in the inhibitory avoidance and the water maze spatial task. An important difference is our observation of impaired memory in the rats following intraseptal muscimol infusion in the inhibitory avoidance task, whereas Decker et al. did not observe a consistent effect of septal lesions on memory in this task.
Our results suggest that the BLA and the MSA can be viewed as major modulators of areas that function to facilitate the encoding of memories of emotionally motivated learning. These areas could include the hippocampus, the entorhinal cortex and neocortical areas. Findings from other laboratories are consistent with this general hypothesis. Ikegaya et al. [14,15] demonstrated that the BLA plays a modulatory role for LTP induction (one model for neuronal plasticity) in the hippocampus. Molnar et al. [28] showed that MSA lesions can reduce the amplitude of the maximal population spikes in the hippocampus and can produce an impairment in the increase in population spikes evoked by high frequency stimulation of the perforant path (LTP). Importantly, physostigmine normalized the parameters evaluated. Rashidy-Pour et al. [35] demonstrated that animals submitted to MSA inactivation showed a faster decay of LTP in dentate gyrus of the hippocampus in vivo. They attributed these results to the elimination of the MSA output amplification on synaptic responses that might be mediated by excitatory amino acids in the hippocampus. Stackman et al. [45] showed that benzodiazepine ligands infused into the MSA modulate evoked responses and LTP in the hippocampus. Since the BLA and the MSA also project to other brain areas, treatments affecting these two structures would be expected to influence consolidation of memory in sites under direct or indirect influence of the BLA and the MSA, including many other areas besides the hippocampus, such as the entorhinal cortex and neocortical areas. This view is further supported by the work of Mitchell et al. [27] who demonstrated that lesions of the MSA provoke disruption of the theta rhythm and cholinergic system in the entorhinal cortex.

In summary, our results support the concept that both structures, the amygdala and the septo-hippocampal system, work together to modulate the encoding processes of long term memories of emotional-spacial/contextual related events.

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