行政院國家科學委員會專題研究計畫　期中進度報告

不同記憶系統互動之行為與神經機制的探索(2/3)

計畫類別：個別型計畫
計畫編號：NSC93-2413-H-002-004-
執行期間：93 年 08 月 01 日至 94 年 07 月 31 日
執行單位：國立臺灣大學心理學系暨研究所

計畫主持人：梁庚辰

報告類型：精簡報告

處理方式：本計畫可公開查詢

中　華　民　國 94 年 5 月 30 日

Epinephrine Facilitates Latent Learning in an Inhibitory Avoidance Task: Involvement of Amygdaloid Influence on the Hippocampus

K. C. Liang

Department of Psychology,

National Taiwan University

Abstract

The present study investigated the memory-enhancing effect of epinephrine in a latent learning paradigm and roles of the amygdala and hippocampus in this effect. Male albino rats were exposed to the apparatus for inhibitory avoidance training without receiving any footshock on the first day. They were subjected to the normal training procedure with 0.5 mA/0.5 s footshock on the second day and tested for retention on the third day. Rats with long experience of pre-exposure had better retention than those with short or no experience of pre-exposure. The latent learning effect in a long pre-exposure was impaired by 4% lidocaine infused into the dorsal hippocampus immediately after the pre-exposure experience, while lidocaine infused into the amygdala had no such effect. Epinephrine injected systemically after a short pre-exposure experience caused a dose- and time-dependent facilitation of the latent memory. This effect was attenuated by infusion of 1.0 μg propranolol into the amygdala, but the same treatment administered to the dorsal hippocampus caused no attenuation. Intra-amygdala infusion of norepinephrine immediately after the short pre-exposure experience also caused a dose-dependent facilitation of latent memory. Further, intra-hippocampal infusion of lidocaine significantly attenuated enhancing effects of epinephrine given peripherally or norepinephrine infused into the amygdala shortly after a short pre-exposure experience. The present findings, taken together, suggest that in an inhibitory avoidance task, peripheral epinephrine may work through activating the amygdaloid noradrenergic functions in modulating latent learning of contextual information relying on the dorsal hippocampus.

Key words: epinephrine, norepinephrine, β-adrenergic receptors, latent learning, contextual memory

Introduction

In the first year of this project, we demonstrated that pre-exposure to the inhibitory avoidance apparatus resulted in a latent learning effect, that is, rats receiving the pre-exposure experience did not differ from rats without the pre-exposure experience when they were re-exposed to the apparatus, but the former group of rats showed better retention of avoidance behavior than the latter group of rats if a foot shock was administered to both them during the re-exposure trial. This latent learning effect was experience dependent because a long pre-exposure experience resulted in better retention of the avoidance behavior than a short pre-exposure experience if a foot shock was given subsequently. A subcutaneous injection of epinephrine shortly after the short pre-exposure experience enhanced this latent memory in a dose- and time-dependent manner. Further, this memory enhanced by epinephrine lasted more than 3 weeks because the better retention prevailed when the footshock was administered 21 days after the pre-exposure trial. These data established a robust latent learning effect and demonstrate that this effect can be modulated by epinephrine.

In this year, we pursued the neural substrate of this latent learning and epinephrine effect. In this task, latent learning involved familiarization of the context such that it could be associated with an aversive event more readily in a later occasion. The hippocampus has been implicated in formation of a context representation by binding together individual cues existent in the context. Thus we investigated the role of the hippocampus by examining the effect of intra-hippocampal infusion of lidocaine immediately after the pre-exposure trial on retention of avoidance behavior subsequent to footshock training. Previous evidence has shown that in a conventional inhibitory avoidance paradigm, the effect of epinephrine injected peripherally on memory was mediated through releasing norepinephrine from the amygdala. To test whether the memory enhancing effect of epinephrine on latent learning was also dependent upon the same mechanism, this study investigated whether the effect of epinephrine given peripherally could be

blocked by propranolol and mimicked by norepinephrine infused directly into the amygdala shortly after the pre-exposure trial. If the latent learning turned out to rely on the hippocampus, while the enhancement of memory acquired in latent learning by epinephrine turned out to rely on the noradrenergic activation of the amygdala, then it would be interesting to determine whether the memory enhancing effect of epinephrine in latent learning involved modulation of the hippocampal function by influence ensuing from the amygdala.

Methods

Subjects

Male Sprague-Dawley rats weighing 400 grams were used in this study. They were purchased from the National Animal Breeding Center (Nankang, Taiwan) and housed individually with food and water ad lib in the vivarium of Department of Psychology, National Taiwan University with ambient temperature at a 25°Cand relative humidity at 50%. Throughout the study period, a 12/12 light/dark cycle was kept with lights on at 7:00 am. Experiments were carried out from 1:00 pm to 6:00 pm.

Surgery

One month after arrival, some rats underwent stereotaxic surgery to implant cannulae into the amygdala and/or dorsal hippocampus. After atropine sulfate (Sigma, St. Louis, MO, USA) pretreatment (0.4 mg/kg, i.p.) to prevent respiratory congestion, rats were anesthetized with an injection of sodium pentobarbital (45 mg/kg, i.p., MTC Pharmaceuticals, Cambridge, Ontario, Canada) and mounted on a stereotaxic instrument (David Kopf Instruments, DKI-900, Tujunga, CA, USA). The coordinates of bilateral cannulae implantation for the amygdala and the dorsal hippocampus were AP. - 3.0 mm, ML. $\pm$ 4.8 mm and DV. - 6.5 mm and AP. - 3.5 mm, ML. $\pm$ 2.0 mm and DV. - 2.0 mm. The incisor bar was set at - 3.3 mm according to the rat brain atlas by Paxinos and Watson (1997). Cannulae made of 23-G stainless steel tubing with 0.33 mm inner diameter and 0.63 mm outer diameter were implanted bilaterally into the target area. Cannulae for the amygdala and dorsal hippocampus

were at a length of 15 and 10 mm, respectively. Two jewelry screws were implanted over the right frontal and the left posterior cortices serving as anchors. The whole assembly was affixed on the skull with dental cement. Rats were kept warm until resurrection from anesthesia. They recuperated for at least two weeks before subjected to behavioral experiments.

Behavioral Tasks

Animals of the present study were subjected to the step-through inhibitory avoidance task. The apparatus was a trough-shape alley divided by a sliding door into a safe compartment and a shock compartment. The safe compartment was lit by a 20 W light bulb and the shock compartment was dark. In order to assess latent learning, the procedure was modified from the typical one-trial inhibitory avoidance learning and has been previously described (Liang, 2001). Briefly, the rat was placed into the lit site facing away from the door. On the first day of training, the rat was placed into the lit chamber. As the rat turned around, the door was opened. The rat was allowed to step into the dark side without receiving any shock. In the low pre-exposure condition, the rat was taken out 10 s after entering the dark site, and this procedure was repeated for 3 times. In a high pre-exposure condition, the rat was placed into the lit side and allowed for free exploration of the alley for 3 min after stepping into the dark site. On the second day of training, the rat was placed again into the lit side and the door was open, as the rat stepped through, the door was closed and an inescapable footshock (0.5 mA/0.5 s) was administered through a constant current shocker controlled by a timer (Lafayette Instruments, Model 80240 and Model 58010, Lafayette, IN, USA). The shock intensity was calculated as the root mean square of the sinusoidal alternating currents. After the shock, the rat was removed from the alley to receive the assigned posttraing treatment and then returned to its home cage.

Retention was tested 24 hrs after shock administration. The rat was put into the alley again and its latency to step into the dark side was taken as a retention score. If the rat did not step through in 10 min, the test trial was ended and a ceiling score of

600 (seconds) was assigned.

Drugs and Drug Administration

Epinephrine was obtained from a local drug company in a 1 mg/ml solution and dl-norepinephrine was obtained from RBI (Natick, MA, USA). Epinephrine was diluted with saline (Sal) to the appropriate concentration and injected subcutaneously. Norepinephrine was dissolved into a specific brain buffer which in 100 ml contained 0.9 g of NaCl, 4.5 ml of 0.2 M Na_2HPO_4, and 0.95 ml of $NaH_2PO_4 \cdot 2H_2O$. All concentrations were calculated as the salt weight. Vehicle (Veh) for the central infusion was specific brain buffer. The intra-cranial infusion device was constructed as follows: A piece of 0.5 m polyethylene tubing (Intramedic PE-20, Sparks, MD, USA) was connected to a 10 μl microsyringe (Hamilton 701-N, Reno, NV, USA) on one end and cemented to a 30 G dental needle on the other. The syringe and the tubing were first filled with distilled water. Drug solutions were then introduced from the injection needle and separated by a tiny air bubble from the distilled water. Drug infusion was administered to a conscious rat shortly before or after training. The rat was gently held and the injection needles were inserted into the cannulae after removing the stylet. Caution was taken not to stress the animal. Bilateral intra-crainial infusion was administered at a rate of 0.5 μl per min through a syringe pump (Carnegie Medicin, CMA/100, Stockholm, Sweden). The infusion volume on each side was 0.5 μl. To facilitate drug diffusion, the needle remained in the cannula after infusion for an additional min before withdrawn. The stylet was then replaced immediately to prevent back flow.

Histology Verification

After the experiments, animals with implants into various brain regions were sacrificed with an overdose of sodium pentobarbital (50 mg per rat, i.p.) and perfused through the heart with physiological saline followed by 10 % formalin. The brain was removed, stored in formalin for at least 48 hours. The brains were then sectioned into 40-μm slices and stained with cresyl violet. The cannula placements were examined by projecting the stained slides onto brain atlas charts (Paxinos and

Watson, 1997). Only animals with both cannulae within the target structures were accepted for final data analyses.

Statistical Analysis

Because the distribution of retention scores was truncated at 600, medians and interquartile ranges were used to represent the central and dispersal tendencies of group. The difference among the groups was first analyzed by non-parametric one-way Kruskal-Wallis one-way ANOVA and followed by two-tailed Mann-Whitney U-tests to detect difference between pairs of groups.

Results

Experiment I: Suppression of the hippocampus, but not the amygdala, impaired latent learning

Rats with cannulae implanted in the amygdala or hippocampus were subjected to training of the long pre-exposure condition. Immediately after the pre-exposure experience, they received infusion of vehicle or 4% lidocaine into the amygdala or hippocampus and on the second day all groups were subjected to footshock training. Retention performance on the third day is shown in Figure 1. All treatments applied after the pre-exposure session had no effect on the entrance latency during training, yet infusion of lidocaine into the hippocampus immediately after the pre-exposure experience impaired retention performance, whereas the same treatment given to the amygdala had no discernable effect. Because rats receiving intra-amygdala or intra-hippocampal infusion of vehicle had comparable retention scores, they were pooled into a single combined control group to increase the power of statistics. A Kruskal-Wallis test revealed no significant difference among groups in the entrance scores on the training day ($H'(2) = 2.5$, $p > .2$), but significant difference in entrance scores among groups on the testing day ($H'(2) = 10.6$, $p < 0.01$). Further paired comparisons indicated that the group receiving intra-hippocampal infusion of lidocaine had significantly poorer retention scores than the combined control group or the group receiving intra-amygdala infusion of lidocaine ($U = 5$ & $22.$, $p < 0.001$ &

0.05; respectively).

Insert Figure 1 about here

Experiment II. Infusion of propranolol into the amygdala had no effect but attenuated the facilitation effect of epinephrine on latent learning

This experiment tested whether the facilitation effect of epinephrine on latent learning involves the amygdala or hippocampus. In the first part of the experiment, rats bearing cannulae in the amygdala were subjected to the low pre-exposure training procedure. Immediately after the pre-exposure experience, rats received infusion of Veh or propranolol at a dose of 0.2, 1.0 or 5.0 μg into the-amygdala. All rats were trained and tested identically on the second and third days. As shown in Figure 2, intra-amygdala infusion of propranolol immediately after the pre-exposure experience did not affect latent learning: Kruskal-Wallis one-way ANOVAs failed to detect significant difference in the entrance latencies on either the shock-training day ($H'(3) = 1.7$, $p > 0.10$) or the testing day ($H'(3) = 4.5$, $p > 0.10$). While rats having 5.0 μg of propranolol tended to have lower retention scores than the control group, the difference failed to reach statistical significance ($U = 20$, $.05 < p < .07$).

In the second part of the experiment, rats bearing cannulae in the amygdala or hippocampus were trained with the low pre-exposure procedure. Immediately after the pre-exposure experience, rats received peripheral injection of saline (Sal) or 0.01 mg/kg epinephrine (E) and infusion of vehicle (Veh) or 1.0 μg propranolol (Prop) into the amygdala or hippocampus. All rats were trained identically on the second day. Figure 3 shows the retention scores of these groups on the third day. Because rats infused with Veh into the amygdala or hippcampus did not differ significantly in retention scores, these animals were collapsed into two groups according to their peripheral treatments and designated as Veh/Sal and Veh/E groups, while the other two groups were designated as Prop(amyg)/E and Prop(hipp)/E groups. Epinephrine injected immediately after the exposure experience facilitated retention, propranolol infused into the amygdala attenuated this facilitation, but into the hippocampus had no

effect. Kruskal-Wallis tests revealed no significant difference among groups in entrance scores on the training day ($H'(3) = 1$, $p > 0.5$) but significant difference in retention scores on the testing day ($H'(2) = 24.9$, $p = 0.0001$). Paired comparisons indicated that the Veh/E group had significantly better retention than the Veh/Sal group ($U = 23$, $p < 0.0001$). Retention scores of the Prop(amyg)/E group while were significantly lower than those of the Veh/E group ($U = 37.5$, $p < 0.05$), but were still significantly better than those of the Veh/Sal group ($U = 56$, $p < 0.05$). On the other hand, the Prop(hipp)/E group did not differ from those of the Veh/E group in retention, but had significantly better retention than the Veh/Sal and Prop(amyg)/E groups ($U = 9$ & 21, $p < 0.05$).

Insert Figure 2 & 3 about here

Experiment III. Intra-amygdala infusion of NE facilitated latent learning

Five groups of rats were subjected to the low pre-exposure training procedure. Immediately after the pre-exposure experience, they received intra-amygdala infusion of vehicle, 0.001, 0.01, 0.1 μg NE or 0.1 μg NE plus 1.0 μg propranolol. Retention tested on the third day is shown in Figure 4: Intra-amygdala infusion of NE shortly after pre-exposure facilitated latent learning in a dose-dependent manner, the group receiving 0.01 or 0.1 μg NE showed better retention than the vehicle group. Kruskal-Wallis tests revealed that the difference in entrance scores among the various groups was not significant on the training day ($H'(4) = 7.5$, $p > 0.1$), but was significant on the testing day ($H'(3) = 25.5$, $p = 0.001$). Further paired comparisons indicated that the group receiving 0.01 or 0.1 μg NE had significantly better retention than the controls ($U = 12$ & 32, $p < 0.01$ & 0.05, respectively), while retention scores of the 0.001 μg NE group did not differ from those of the controls. Further, infusion of 1.0 μg propranolol completely blocked the effect of 0.1 μg norepinephrine: The group receiving NE and propranolol currently showed significantly lower retention scores than the group infused with 0.1 μg of NE ($U = 14$m $p < 0.005$), but did not

differ from the controls (U = 49, p > 0.1).

Insert Figure 4 about here

Experiment IV. Intra-hippocampal infusion of lidocaine attenuate the enhancing effect of E or NE on latent memory

To evaluate whether facilitating effects of peripheral E or amygdala NE relied on the dorsal hippocampus, rats were trained and tested as previously described. Three groups had cannulae in the dorsal hippocampus received one of the following intra-hippocampal/peripheral treatments immediately after the pre-exposure experience: vehicle/saline, vehicle/0.01 mg/kg E and 4% lidocaine/0.01 mg/kg E. They were denoted as the Veh/Sal, Veh/E, Lid/E groups, respectively. The other three groups had indwelling cannulae in both the hippocampus and amygdala received one of the following intra-hippocampal/intra-amygdala treatments immediately after the pre-exposure experience: vehicle/vehicle, vehicle/0.01 μg NE and 4% lidocaine/0.01 μg/kg NE. They were denoted as the Veh/Veh, Veh/NE, Lid/NE groups, respectively. The results are shown in Figure 5. In replication of previous results, peripheral injections of E or intra-amygdala infusion of NE induced memory facilitation in the latent learning paradigm, however, both enhancing effects were attenuated by infusion of lidocaine into the dorsal hippocampus. The data were analyzed separately by two Kruskal-Wallis one-way ANOVAs. There is a significant overall difference among the three peripheral injection groups (H'(2) = 9.6, p < 0.01). Paired comparisons indicated that the Veh/E group had significant better retention than the Veh/Sal or Lid/E group (U = 14 & 16, p < 0.01). There is also a significant overall difference among the amygdala infusion group (H'(2) = 15.1, p < 0.001). Further paired comparisons indicated that the Veh/NE group had significant better retention scores than the Veh/Veh or Lid/NE group (U = 6 & 10, p < 0.001 & 0.005, respectively).

Insert Figure 5 about here

Conclusion

Major findings of this study can be recapitulated as follows: 1. Exposure to the apparatus of an inhibitory avoidance task enhanced subsequent learning of the inhibitory avoidance response when the rat was formally trained with footshock. 2. This facilitating effect of pre-exposure was abolished by lidocaine suppression of the hippocampus. 2. Posttraining systemic injections of E enhanced the effect of a short pre-exposure experience, which was otherwise insignificant, in facilitating retention in formal learning. This facilitation effect is time-dependent, dose-dependent, and long lasting. 3. The E-induced facilitation of the pre-exposure effect on subsequent avoidance learning was mimicked by NE and attenuated by propranolol infused into the amygdala immediately after the pre-exposure. 4. Lidocaine suppression of the hippocampus attenuated the facilitation of pre-exposure effect on subsequent avoidance learning caused by E given systemically or NE given into the amygdala. These data demonstrated that the amygdala mediates the influences of epinephrine ensuing from the periphery and modulates the memory formation process going on in the hippocampus during a latent learning paradigm. Research on the exact pathway of how the influences from the amygdala reach the hippocampus is now undergoing.