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行政院國家科學委員會專題研究計畫進度報告

情緒記憶古典條件學習與工具條件學習之神經機制探討 (3/3)

Neural Mechanisms underlying Classical Conditioning and Operant Conditioning in Affective Memory

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一、 中文摘要

本研究探討依核在抑制行逃避學習中所扮演的角色。研究結果發現學習後操弄終依核內的麩胺酸受體會影響記憶。結果顯示依核與杏仁核及海馬的互動在記憶形成過程中扮演重要角色。

關鍵詞：依核、杏仁核、海馬、麩胺酸、情緒記憶、大白鼠

二、 英文摘要

This study examined the role of the amygdala, hippocampus, nucleus accumbens (NAc) in formation and expression of aversive memory. Rats were trainined on an inhibitory avoidance task that contains classical and operant conditioning components with the latter playing a more significantly role. In a two-phase training paradigm of an inhibitory avoidance task, CNQX impaired retention if infused into the dorsal hippocampus during the context training phase, into the amygdala during the shock training phase or into the NAc during either phase. Simultaneous infusion of 0.1 μg CNQX into the amygdala plus NAc or into the hippocampus plus NAc caused no addition of effects. The retention deficit caused by 1.0 μg CNQX infused into the hippocampus or amygdala was attenuated by glutamate infused into the NAc. Infusion of NE into the hippocampus or amygdala enhanced retention, which was blocked by CNQX infused into the NAc. These findings suggest that the NAc is involved in mediating influences from the amygdala and dorsal hippocampus on memory processing in the inhibitory avoidance task.

三、緒論

Extensive evidence implicates the amygdala in memory processing of affective information: Manipulation of amygdaloid functions shortly after training caused a time-dependent effect on retention in various emotionally laden learning tasks. However, whether the amygdala subserves storage of affective memory is controversial. Existing evidence suggests that amygdala memory functions may rely on its afferent-efferent pathways including the stria terminalis (ST) and the ventral amygdalofugal path.

Studies showed that in an inhibitory avoidance task, pretraining lesions of the ST, while caused a negligible or mild retention deficit of its own, attenuated the impairing effect on memory caused by posttraining subseizure electrical stimulation of the amygdala or the enhancing effect caused by intra-amygdala infusion of norepinephrine (NE). These findings have led to a proposal that at least in the inhibitory avoidance task, the amygdala may affect retention through the ST with its modulatory influences on memory trace formed elsewhere in the brain. This proposal implies that neural substrates underlying memory storage processing may reside at sites receiving direct or indirect influences of the ST outputs. Amygdaloid efferents in the ST project to various targets including the nucleus accumbens (NAc). Pervasive evidence has shown the involvement of the NAc in processing reward or learning appetitive tasks: Lesions of and pharmacological agents applied to the NAc have been shown to affect acquisition and/or expression of responses in instrumental conditioning, as well as association in classical conditioning reinforced by natural rewards, electrical brain stimulation or addictive drugs. However, in view of profuse innervations of the NAc from both the amygdala and hippocampus that are implicated in aversive learning, one would predict that the NAc should also be involved in formation and/or storage of aversive experience.

Recent findings from this and other laboratories start to shed lights on this issue: Altering the NAc function by lesions or pharmacological agents during training or testing could affect performance in a water maze task, conditioned hypoalgesia as well as inhibitory or active avoidance tasks. Our study showed that infusion of lidocaine into the NAc shortly prior to each training trial of a conditioned place preference (CPP) task blocked acquisition of the CPP response, which appeared to support the reward-processing role conventionally ascribed to the NAc. A further pursue unexpectedly found that lidocaine effectively blocked formation of CPP only when it was infused into the NAc prior to saline-pairing trials, but had no effect if infused prior to amphetamine-pairing trial. Such data suggest that in a CPP task typically view as an appetitive learning task, the NAc may be critical in processing the inherent contrast of missing the expected reward and hence more involved in learning the aversive events.

Recent evidence suggests that functions of the NAc on aversive memory may depend upon its connection with the limbic structure. For example, Setlow, Roozendaal & McGaugh (2000) have shown that in an inhibitory avoidance task, either bilateral lesions of the NAc or unilateral lesion of the NAc accompanied with contralateral lesion of the basolateral amygdala significantly attenuated the memory enhancing effect of dexamethasone given systemically. While the authors implicated the involvement of projections from the amygdala to NAc in this effect, in view of the reciprocal connections between the amygdala and the NAc, a cross lesion paradigm could not really delineate the direction of influence flow between the two involved structures.

A previous study showed that some afferents projecting from the amygdala to the NAc contain glutamate. In view of that neuroplasticity in various brain regions including the NAc involves glutamate

transmission, this study thus examined the effect of infusion of glutamate agonists or antagonists into the NAc on formation and expression of memory an inhibitory avoidance task as well as whether the NAc glutamatergic transmission mediated the memory modulatory influence from the amygdala.

四、結果

I. Operant conditioning plays a significant role in inhibitory avoidance learning

Four groups of rats were used to assess the independent contribution of classical or operant conditioning to inhibitory avoidance memory. Rats in the Op-Cl group were trained with the typical inhibitory avoidance procedure containing both classical and operant conditioning components. Rats in the Op-only group were put into an alley with two lit chambers and received a shock as they walked from one side to the other. Rats in the Cl-only group were put directly into a lit alley chamber and received a shock as the light turned off. Rats in the control group were put into a lit chamber of an alley and received a shock. The shock intensity was 075 mA/0.75 s. One day after the training, all groups were placed into the lit side of an alley and the latency of stepping into the dark side was measured. The four groups differed in the retention scores: the OP/Cl group was best, the OP-only group was second, the Cl-only group was the third, and the control group was poorest. A Kruskal-Wallis one-way ANOVA indicated a significant difference among the groups ($H'(3) = 27.5$, $p < .0001$). Further paired comparisons indicated that the OP/Cl group had significantly better performance than the Op-only, Cl-only or the control groups ($U = 66$, 30 or 12, $p < 0.05$, 0.0005 or 0.0001). The OP-only group had significantly better retention than the Cl-only or the control groups ($U = 60$ & 22.5, $p < .05$ or .0002). The Cl-only group had significantly better retention than the control group ($U= 65$, $p < .05$). The findings suggest that the traditional inhibitory avoidance task may contain three components, operant conditioning that plays a major role, classical conditioning that plays a minor role and context conditioning that plays a negligible role.

To further evaluate the importance of operant behavior in forming the inhibitory avoidance response, three groups of rats were training on the inhibitory avoidance task. Rats in the Shock-only group were placed directly into the dark chamber and received a shock. Rats in the Inescapable group received the typical inhibitory avoidance training. Rats in the Escapable group received a shock until it ran back into the lit and safe chamber. The alleys were connected in such a way that shock would be initiated in each box at the same time and terminated so as the escapable rat ran back to the safe chamber which opened the circuit. This device guaranteed the three rats run simultaneously received the same amount and same duration of shock. The shock level was set at 0.5 mA, the duration may varied from rat to rat dependent upon when the escapable rat flied back into the safe chamber. Retention was tested 24 hrs later. The results indicated that the three groups differ in retention: The Inescapable group had the best retention, and Escapble group next, and the Shock-only group the poorest. A Kruskal-Wallis one-way ANOVA indicated a significant difference among the groups ($H'(2) = 8.6$, $p < .05$). Further paired comparisons indicated that the Shock-only group had shorter retention latencies than the other groups

(U = 42 & 58.5, p < .05 & .01), but the Inescapable and Escapable group did not differ from each other (U = 58, p > .2). These findings suggest that shock contingent upon behavior create better inhibitory avoidance memory than shock contingent upon darkness only, but whether the shock was escapable or not did not make a difference. Such findings provides further support for the critical involvement of operant conditioning in the inhibitory avoidance memory.

II. Lesions of the ST impaired retention in the inhibitory avoidance task.

To pursue whether the ST was involved in memory function, four groups of rats received radio frequency lesions or sham operation 2 days before training and were tested 1 or 21 days after 1 mA/1 s footshock trained. The results indicated that sham operated rats showed good memory when tested 1 or 21 days after training, the ST lesioned rats while showed retention not different from the correspondent sham group, had poorer 21-day retention than its sham control group (U = 23, p < .005). The results suggested while rats bearing ST lesions maintained apparent normal retention of a recent event, performance deteriorated significantly as the retention interval lengthened.

To replicate the effect, four groups of rats were trained on the task and received posttraining lesions of the ST at 2, 14 or 21 days after training on 1 mA/1 s footshock, the retention was tested at 31 days after training. The results indicated that posttraining ST lesions induced a great memory deficit when given at any time after training (H'(3) = 12.43, p < .002). Rats with the ST inflicted 2, 14 or 21 days after training had significantly lower retention than the sham control groups (U = 3, 11, 14; p < .001, .001, .05). Such findings suggest that ST lesions indeed impaired retention of a remote event

III. Infusion of CNQX into the NAc during context or shock training impaired memory

In an attempt to delineate the role of the NAc in comparison with those of the amygdala or dorsal hippocampus in inhibitory avoidance learning, rats bearing indwelling cannulae in these three regions were trained on the task with a modified procedure as described in the Method section. The training foot shock was set at 1.0 mA/1.0 s at which differential influences of treatments on context and shock processing have been demonstrated. Four groups of rats with cannulae implanted into one of the three target regions received one of the following treatments administered to the NAc: Veh in each training phase or 1.0 μg CNQX in context training, shock training or both. They were designated, respectively, as the Veh/Veh, CNQX/Veh, Veh/CNQX, CNQX/CNQX groups to denote the treatment received at the context/shock training phase. The infusion was given immediately after each training phase. The results indicate that the three control group receiving Veh showed substantial avoidance to the dark side and CNQX impaired retention if infused into the dorsal hippocampus after context training, into the amygdala after shock training or into the NAc after either phase of training. The data were analyzed by three separate Kruskal-Wallis one-way ANOVAs.

Analyses revealed a significant difference among the hippocampal infusion groups (H'(3) = 14.7, p = 0.01). Further paired comparisons indicated that the Veh/Veh group had significant better retention than the CNQX/Veh and CNQX/CNQX groups (U = 29, 44.5, p <

0.01 & 0.05; respectively). The Veh/CNQX group, which did not differ from the Veh/Veh group, also showed better retention than the CNQX/Veh and CNQX/CNQX groups ($U = 16$ & 26, $p < 0.01$ & 0.001; respectively).

Overall significant difference was found among the amygdala infusion groups ($H'(3) = 24.1$, $p = 0.0001$). Further paired comparisons indicated that the Veh/Veh group had significant better retention than the Veh/CNQX and CNQX/CNQX groups ($U = 29, 18$; respectively, $p < 0.001$). The CNQX/Veh group, which did not differ from the Veh/Veh group, also showed better retention than the Veh/CNQX and CNQX/CNQX groups ($U = 21$ & 10, respectively; $p < 0.001$).

Significant overall difference was also detected among the NAc infusion groups ($H'(3) = 37$, $p < 0.003$). Further paired comparisons showed that the Veh/Veh group had significant better retention scores than the CNQX/Veh, Veh/CNQX and CNQX/CNQX groups ($U = 21, 11,$ & 5, respectively; $p < 0.05, 0.01,$ & 0.001). The CNQX/CNQX group showed poorer retention scores than the CNQX/Veh group ($U = 9$, $p < 0.05$), but did not differed from the Veh/CNQX group.

IV. Concomitant infusion of CNQX into the NAc, amygdala or hippocampus induced no additive effects

To evaluate whether possible interaction between the NAc and amygdala or NAc and hippocampus, groups of rats were trained on the task with a 1.2 mA/1.2 s footshock. Immediately after training, Veh or 0.1 µg of CNQX was infused into the NAc, amygdala, hippocampus, NAc plus amygdala or NAc plus hippocampus to assess whether effects induced by affecting individual sites would be additive. Because rats receiving Veh infusion into the NAc plus amygdala or NAc plus hippocampus showed comparable retention scores, they were collapsed into a combined control group. The results indicated that 0.1 µg CNQX infused locally into the NAc, amygdala or hippocampus caused a mild deficit, infusing CNQX simultaneously into the NAc plus amygdala or hippocampus caused no additional effect. While a Kruskal-Wallis one-way ANOVA revealed no overall significant difference among various groups ($H'(5) = 8.8$, $p > 0.1$), paired comparisons indicated that the control group had significantly better retention scores than all CNQX-treated groups ($U = 62, 81, 111, 79,$ & 70; $p < 0.05$). The group receiving CNQX in the NAc plus amygdala or hippocampus did not show retention poorer than the group receiving CNQX in the NAc, amygdala or hippocampus alone.

V. Intra-amygdala or hippocampal infusion of CNQX did not attenuate the memory enhancing effect of glutamate infused into the NAc.

To study further possible interaction between the amygdala or hippocampus and NAc on retention, groups of rats bearing cannulae in both the NAc and amygdala or hippocampus were trained on the task with a 0.7 mA/0.7 s footshock, which would generate intermediate control performance optimal for demonstration of both enhancing and impairing effects on memory. Immediately after training, rats received one of the following amygdala or hippocampus/NAc treatments: Veh/Veh, Veh/0.1 µg glutamate, 1.0 µg CNQX/Veh, 1.0 µg CNQX/0.1 µg glutamate and 1.0 µg CNQX/0.001 µg glutamate. Because

rats receiving Veh infusion into the amygdala or hippocampus did not differ, so they were collapsed together. A Kruskal-Wallis one-way ANOVA revealed significant differences among the groups (H' (7) = 41.5, $p < 0.0001$). In comparison with the moderate retention performance of the control group, infusion of 0.1 μg glutamate caused an enhancing effect, rats given 0.1 μg glutamate into the NAc showed significantly better retention than controls ($U = 56$, $p < 0.01$). Infusion of 1.0 μg CNQX into the amygdala or hippocampus caused an impairing effect: Rats with CNQX infused into one of these two regions showed significantly poorer retention than the controls ($U = 45$ & 30 for amygdala and hippocampus; respectively, $p < 0.001$). When both amygdala/hippocampus and NAc treatments were applied simultaneously, the enhancing effect of intra-NAc infusion of glutamate dominated: Rats having CNQX into the amygdala or hippocampus plus 0.1 μg glutamate into the NAc showed better retention than both the controls and the corresponding group infused only with CNQX ($U = 50.5$ & 25, $p < 0.01$ & 0.05, respectively for the amygdala treated groups; $U = 61.5$ & 3, $p < 0.05$ & 0.001, respectively for the hippocampus treated groups). The amnestic effects of CNQX were also attenuated by a non-enhancing dose of glutamate: Rats having CNQX into the amygdala or hippocampus plus 0.001 μg glutamate into the NAc showed better retention than the corresponding group infused only with CNQX ($U = 26.5$ & 17, $p < 0.05$), but did not differ from the control group.

VI. Intra-NAc infusion of CNQX blocked the memory enhancing effect of NE infused into the amygdala or hippocampus after training

To further test that the amygdala or hippocampus could affect memory through its influence on the NAc, six groups of rats with indwelling cannulae in the amygdala or hippocampus as well as NAc were trained on the task with a 0.5 mA/0.5 s footshock. Immediately after training, a memory-enhancing agent—NE (0.2 μg)—was infused into the amygdala or hippocampus. At the mean time CNQX (0.1 μg or 0.01 μg) or Veh was infused into the NAc. Retention performance in the 1-day test is shown in Figure 6. Retention scores did not differ between rats with Veh infused into the NAc plus amygdala and those with Veh infused into the NAc plus hippocampus, therefore they were collapsed into a combined control group. NE infused into the amygdala or hippocampus immediately after training caused a memory enhancing effect that was completely abolished by 0.1 or 0.01 μg CNQX infused concurrently into the NAc. A Kruskal-Wallis one-way ANOVA revealed a significant overall difference among the groups (H'(6) = 36.8, $p < 0.0001$). For rats having amygdala/NAc infusion, the NE/Veh group had significantly better retention scores than the control, NE/0.01 μg CNQX or NE/0.1 μg CNQX group ($U = 26$, 25, & 17, $p < 0.01$, 0.05 & 0.01; respectively), while the latter three did not differ among themselves. For rats having hippocampus/NAc infusion, the NE/Veh group also had significantly better retention scores than the control, NE/0.01 μg CNQX or NE/0.1 μg CNQX group ($U = 16$, 1, & 2, $p < 0.0001$, 0.0005 & 0.0001; respectively), the latter three did not differ among themselves either.

五、討論

Combining the results of the three years, we can reach the following conclusions:

1. Inhibitory avoidance learning contains the following components: context conditioning, classical conditioning and operant conditioning. Among the three, operant conditioning contributes most to the formation of the avoidance response.
2. Pretraining or posttraining lesions of the ST induced a more fragile memory trace, rats with lesions as such showed poorer retention than the sham rats in the inhibitory avoidance task.
3. In an inhibitory avoidance task, posttraining infusion of CNQX into the NAc, a target of the ST projection, induced a retention deficit that is dose-dependent and time-dependent, it also has receptor and site specificity. Posttraining infusion of glutamate into the NAc improved memory of the response. Pre- or posttraining intra-NAc infusion of CNQX caused similar deficits in the active avoidance and Morris water maze tasks. These data suggest that AMPA receptors in the NAc are involved in memory formation processing of aversive events.
4. In a two-phase inhibitory avoidance paradigm, suppressing the amygdala with CNQX impaired retention when the drug was given at the shock training phase. Suppressing the hippocampus with CNQX impaired retention when the drug was given at the context training phase. Suppressing the NAc impaired retention when the drug was given at either the shock or context training phase.
5. Concurrent infusion of CNQX at a sub-threshold dose into the amygdala and NAc showed any additive effect; neither did concurrent infusion of CNQX into the hippocampus and the NAc. Yet concurrent infusion of CNQX into the amygdala and hippocampus showed additive effect, suggesting that these two sites may converge their influences to the NAc.
6. Intra-amygdala or-hippocampal infusion of CNQX did not abolish the memory enhancing effect of intra-NAc infusion of glutamate. In contrast, intra-NAc infusion of CNQX attenuated the enhancing effect of amygdaloid or hippocampal NE infusion, and intra-NAc infusion of glutamate attenuated the amnesic effect of amygdala or hippocampal CNQX infusion. These findings suggest a flow of memory modulatory influences from the amygdala to the NAc instead of in a reversed direction.
7. Pretest-intra-NAc infusion of CNQX impaired expression of inhibitory avoidance tasks acqiored 1-day or 21-days ago. The same treatment also impaired expression of 1-day active avoidance memory, but had no effect on memory expression in the Morris water maze task. Thus, the NAc and /or its projected targets may be involved in operating long-term inhibitory avoidance memory.

Previous studies have shown that pretraining infusion of DNQX into the NAc would block acquisition/retention in a CPP task. Such changes in acquisition or retention performance could be due to altered sensory/motor or motivational factors instead of learning or memory per se. Intra-NAc infusion of CNQX indeed decreased shock sensitivity and exploration according to the present results. To rule out such confounding, the present study adopted a one-trial learning

paradigm and a posttraining infusion regimen that could not have affected performance during acquisition.

Our laboratory has observed that pretraining intra-NAc infusion of CNQX impaired acquisition/retention in the Morris water maze task. Thus, these results extend the previous findings by showing not only that the NAc AMPA receptors, in addition to their involvement in appetitive learning, are also involved in aversive learning, but also that these receptors are indeed engaged by memory formation processing. This notion is consistent with the results that AMPA receptors were involved in glutamate transmission in the NAc as well as certain forms of neuronal plasticity in the NAc.

Previous findings from this and other laboratories have shown that various neurochemicals processes in the amygdala including activation of α or β noradrenergic receptors or glutamatergic receptors play a critical role in memory processing of aversive experience. The profuse projections from the amygdala to the NAc, which presumably contain glutamatergic fibers, raises a possibility that glutamate inputs to the NAc may mediate modulatory influences from the amygdala on memory storage processes. This conjecture gains support from the present results that intra-NAc infusion of CNQX attenuated the memory enhancing effect of NE infused into the amygdala immediately after training. These findings lend further support for a recent suggestion that integrity of the amygdala-NAc pathway is necessary for the memory modulation effect of dexamethasone. However, the present study extends the previous one by showing that blocking the postsynaptic sites of glutamatergic inputs, which could arise among other sources from the amygdala, indeed abolished the memory enhancing effect of stimulating the amygdala noradrenergic α receptors. Because the attenuating dose of CNQX (0.1 μg) by itself possessed a weak memory-impairing effect, it could be argued that the amygdala noradrengergic system and the NAc glutamatergic system have provided independent but opposite influences that summate at a third structure. In this case, the observed attenuation would bear no relevance on mechanism. However, such an interpretation appears to be contradictory with the findings that the sub-maximal effects caused by CNQX infused into each structure showed no addition when this drug was simultaneously infused into both structures. The occlusion of summation observed after concurrent infusion of CNQX to both the NAc and amygdala suggests that the two structures may be in series with regard to affecting memory processes. Thus, any influence from an upstream site would be overshadowed by manipulation of its downstream target. Our data provide definite evidence supporting the notion that the amygdala modulates memory through its influences on elsewhere in the brain. However, the present study did not explore whether the projection from the NAc to the amygdala also plays a role in memory formation, thus could not rule out a possibility that the two sites may be contained in a reverberating neural loop involved memory formation processing as that has been proposed for the amygdala and medial prefrontal cortex.

If the amygdala modulates inhibitory avoidance memory through its influences on the NAc, it would be interesting to

inquire whether the NAc would be a site of memory storage for the inhibitory avoidance response. Impairment of retention performance in the inhibitory avoidance task caused by lesions or temporary suppression of the NAc at any time after training would be consistent, although not necessarily prove, such a notion. Experiment VI yielded results consistent with this conjecture: 1.0 μg CNQX infused into the NAc shortly before the testing session impaired retention performance in both the 1-day and 21-day retention tests. The poor performance could not be due to heightened exploratory activity because intra-NAc infusion of CNQX actually depressed rather than elevating activity levels. These results suggest that to retrieve or express either recent or remote emotional memory normally relies on integrity of the NAc.

However, it should be noted that blocking AMPA receptors during testing only impaired, but did not demolished, retention in a recent or a remote test. Such results suggest that other neurochemical processes in the NAc may also be involved in memory expression of aversive experience. For example, 6-OHDA lesions of the NAc could attenuate the enhancing effect of angiotensin II on expression of inhibitory avoidance memory. The role of DA and other related systems in the NAc in expression of aversive memory should be pursued in the future. Alternatively, structures other than the NAc may also be involved in expressing or retrieving recent or remote affective memory. In addition to the NAc, the amygdala also projects profusely to the BNST via the ST. A recent study from this laboratory has shown that manipulating functions of the BNST shortly after training indeed enhanced or impaired retention in the inhibitory avoidance task, however, various treatments applied to this area shortly before tests with various retention intervals had no effect on memory. These data thus exclude a role of this structure in memory storage.

The ST also connects the amygdala with the septal area and substantia innominata, cholinergic fibers from these two regions project, respectively, to the hippocampus and widespread cortical areas. It has been proposed that the amygdala may also exert its modulatory influences on the hippocampus or cerebral cortex. Our previous results have shown that lidocaine-induced suppression of the hippocampus with CNQX or lidocaine impaired retention performance in the 1-day test but not in the 21-day test. In contrast, suppressing the medial prefrontal cortex or insular cortex with similar agents impaired retention in the 21-day test but not in the 1-day test. This profile of data suggests that expression of either recent or remote emotional memory appears to rely on a network of structures. The NAc appears to collaborate with other structures to mediate expression of both recent and remote inhibitory avoidance memory.

These findings suggest that the NAc may receive through the ST or other fiber tracts convergent inputs from the amygdala and hippocampus, where association was forged by context conditioning and classical conditioning, and play an important role in the operant component of the inhibitory avoidance response.