行政院國家科學委員會補助專題研究計畫成果報告

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行政院國家科學委員會專題研究計畫成果報告

嫌惡經驗之習得及保留—終紋及其聯絡區之角色

The role of stria terminalis in learning and memory of aversive responses

計畫編號：NSC89-2413-H-002-003

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主持人：梁庚辰 國立臺灣大學心理學系

一、 中文摘要

本研究探討終紋床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紋床核內的正腎上腺素甲型受體以及腎上腺皮質刺激素釋放因子會影響記憶。這些結果顯示終紋床核在記憶形成過程中扮演重要角色。

關鍵詞：終紋床核、正腎上腺素、腎上腺皮質刺激素釋放因子、情緒記憶

二、 英文摘要

The present study examined the role of the bed nucleus of the stria terminalis (BNST) in formation and retrieval of affective memory. Male Wistar rats with cannulae bilaterally implanted into the BNST were trained on a one-trial step-through passive avoidance task. Shortly after training they received bilateral infusion into the BNST of lidocaine, various noradrenergic drugs, or corticotropin releasing factor (CRF). Results showed that posttraining intra-BNST infusion of lidocaine impaired retention. Posttraining intra-BNST infusion of norepinephrine or the α1 antagonist prazosin induced, respectively, a dose- and time-dependent retention enhancement or deficit. The enhancing effect of norepinephrine was attenuated by concurrent infusion of prazosin at a non-impairing dose. Posttraining intra-BNST infusion of the α2 agonist idazoxan or the β antagonist propranolol failed to affect retention. Posttraining intra-BNST infusion of CRF enhanced retention in a dose-dependent manner. Drugs infused shortly before testing did not influence locomotor activity and had no significant effect on retention. The findings suggest that the BNST is involved in memory formation processing of affective experience and NE working through α1 receptors plays a critical role in this function.

Key words: memory, fear conditioning, BNST, lidocaine, prazosin, rats

三、目的與緣起

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 those learning tasks highlighted with emotion arousal. Existing evidence suggests that memory functions of the amygdala may rely on its various afferent-efferent pathways including the stria terminalis (ST). An early study has shown that pretraining lesions of the fornix/ST attenuated the retention enhancing effect of vasopressin injected peripherally. Later studies showed that in an passive avoidance task, pretraining lesions of the ST,

while by themselves caused a negligible or mild retention deficit, attenuated the memory impairing effect caused by posttraining subseizure electrical stimulation of the amygdala. These findings suggest that the ST may play a critical role in mediating memory modulatory influences of the amygdala to elsewhere in the brain.

The ST is also involved in the memory modulatory effect of other treatments: Posttraining injections of epinephrine into the periphery facilitated memory in the passive avoidance task, and this enhancing effect was mediated by release of norepinephrine (NE) in the amygdala. Pretraining lesions of the ST attenuated not only the memory enhancing effect of peripherally injected epinephrine but also that of NE infused into the amygdala. Consistently, ST lesions blocked the memory enhancing effect of systemic injection of clenbuterol, which exerts its action through amygdaloid β receptors. In addition, ST lesions abolished the effects on memory of naloxone or β-endorphin, cholinergic drugs, CCK-8, or glucocorticoid given systemically as well as intra-caudate infusion of oxotremorine. Thus, integrity of the ST appears to be essential for various treatments to exert their influences on memory processes, either through the amygdala or elsewhere in the brain.

Amygdaloid efferents in the ST innervate various target regions including the bed nucleus of the ST (BNST) located at the level of the anterior commissure. This structure has been proposed as part of the extended amygdala in the basal forebrain due to similar patterns of its input-output connections and physiological functions to those of the amygdala. All above findings taken together would expect a role of the BNST in memory processing of affective experience. However, this prediction by far has met with contradictory evidence. On the basis of the evidence that ST fibers contains met-enkephalin which modulated BNST neuronal activity, an early study showed that posttraining intra-BNST infusion of levorphanol, an opioid agonist, caused a memory deficit, and intra-BNST infusion of naloxone attenuated this deficit as well as that caused by subseizure electrical stimulation of the amygdala. These results suggested a role for the endogenous opioid of the BNST in modulating of memory formation.

On the other hand, Davis and his colleagues showed that lesions of the BNST abolished potentiation of acoustic startle caused by corticotropin releasing factor (CRF) or intense light but had no effect on the potentiation caused by an otherwise neutral stimulus that has been associated with electric shocks. These data, consistent with those showing that conditioned freezing and heart rate responses did not rely on the ST and its projection areas, were viewed as evidence that the BNST was not involved in formation and/or expression of conditioned fear responses.

The inconsistent findings and lack of a clear explanation for them demand more research on the role of the BNST in learning and memory. The BNST receives dense innervation of noradrenergic fibers from the A1, A2 regions and locus coeruleus of the brain stem. The nuclei contain the highest concentration of dopamine-b-hydroxylase immunoreactivity in the brain as well as abundance of α1a, α1b, α2 and β receptors. Stress of immobilization caused in vivo release of NE in the BNST. In view of the importance of NE in memory formation, it is worthwhile to investigate the role of NE, as well as various subtypes of its receptor within the BNST in memory. The BNST also contains high densities of CRF immuno-

reactive cell bodies and neural processes. This neuropeptide is implicated in modulating emotional experience: Avoidance of water stress activated expression of c-fos in the BNST, which was mediated through CRF. In view of the previous findings that infusion of CRF into the amygdala or hippocampus enhanced retention of an πασσιωε αωοιδανχε task, this study also examined the effect of posttraining infusion of CRF into the BNST on memory.

四、結果

I. Effects of intra-BNST infusion of lidocaine on memory

Four groups were used in this experiment. They were trained on the task with a 1 mA/1 s footshock. Two groups received intra-BNST infusion of Veh or 2% (w/v) lidocaine immediately after training, while the other two groups received intra-BNST infusion of Veh or lidocaine 3 min before the 1-day retention test. Results showed that suppression of the BNST immediately after training caused a retention deficit: The median retention score of the Veh group was 600 s (Q1/Q3: 600/600, n=15), while that of the lidocaine group was 133.4 s (Q1/Q3: 18.9/377.1, n=13), the difference was statistically significant ($U=40$, $p<0.01$). Conversely, suppression the BNST during testing failed to affect retention performance: The median retention scores of the Veh and lidocaine groups were, respectively, 593.2 s (Q1/Q3: 284.5/600, n=10) and 600 s (Q1/Q3: 600/600, n=9), this difference was not statistically significant ($U=34.5$, $p>0.10$).

II. Effects of intra-BNST infusion of prazonsin on memory

Five groups of rats were trained on the task with a 1 mA/1 s footshock. They received intra-BNST infusion of Veh or 0.3, 1.0 or 3.0 μg prazosin immediately after training or 1.0 mg prazosin 4 hrs after training. The 1-day retention performance analyzed by a Kruskal-Wallis one-way ANOVA revealed a significant difference among various groups ($H(4)=10.95$, $p<0.05$). The effect was mainly due to significantly poorer retention scores in rats receiving immediately after training 1.0 μg prazosin than in the Veh controls ($U=64$, $p<0.05$). On the other hand, rats receiving the drug treatment 4 hrs after training had retention scores not significantly different from those of the Veh controls'($U=51.5$, $p>0.1$). While the difference between the groups having 1.0 μg prazosin either immediately or 4 hrs after training failed to reach statistical significance ($U=45$, $p>0.10$), a median test showed that the difference in the two score distributions approached statistical significance ($\chi^2=3.0$, $0.05<p<0.10$), suggesting that the delay infusion group appeared to have fewer rats than the immediate infusion group (2 vs. 10) showing scores lower than the grand median of the two groups pooled together.

This experiment additionally explored the effect of intra-BNST infusion of the β antagonist propranolol on retention and found that 5.0 μg of propranolol administered into the BNST immediately after training yielded a median retention score of 373.6 (Q1/Q3: 2.3/ 600, n=9) and that was comparable to the median retention score of the control group (363.7, Q1/Q3: 102.4/ 600, n=10) ($U=49$, $p>0.1$).

Two additional groups of rats were trained on the task without receiving any treatment before or shortly after training. Intra-BNST infusion of Veh or 1.0 μg prazosin was administered 3 min before the 1-day retention test. The medians for the Veh and prazosin groups, respectively, were 600 (Q1/Q3: 475.2/ 600, n=7) and 334.8 (Q1/Q3: 155.4/ 600, n=7). While the prazosin group appeared to have

poorer retention performance, the difference failed to reach statistical significance ($U=13.5$, $p>0.10$).

III. Effects of intra-BNST infusion of NE on memory

Five groups of rats were trained on the task with a 0.6 mA/0.6 s footshock. They received intra-BNST infusion of Veh, 0.02, 0.2, 1.0 μg of NE or 0.2 mg NE plus 0.3 mg prazosin. An additional group trained simultaneously received intra-BNST infusion of 0.2 mg NE 4 hrs after training. Immediate posttraining intra-BNST infusion of NE caused a dose- and time-dependent retention enhancement, which was attenuated by prazosin. A Kruskal-Wallis one-way ANOVA revealed a significant difference among the various groups ($H(5)=20.76$, $p<0.001$). Further paired comparisons showed that rats having 0.2 or 1.0 μg NE immediately after training showed significant better retention scores than the Veh controls ($U=92$, $p<0.05$, $U=23.5$, $p<0.001$). In contrast, rats receiving 0.2 μg NE 4 hrs after training failed to show retention scores significantly different from those of the Veh controls ($U=62.5$, $p>0.10$). While the difference between the two groups having 0.2 μg of NE either immediately or 4 hrs after training failed to reach statistical significance ($U=85$, $p>0.10$), a median test indicated that the difference in the two score distributions approached statistical significance ($\chi 2=3.36$, $0.05<p<0.10$), suggesting that the immediate infusion group appeared to have more rats than the delay infusion group (14 vs. 3) showing scores higher than the grand median of the two groups pooled together.

Further, rats receiving 0.2 μg NE plus 0.3 mg prazosin showed retention scores not different from the Veh group ($U=80$, $p>0.10$) but significantly lower than those receiving only 0.2 μg NE ($U=39$, $p<0.01$), indicating that the $\alpha 1$ antagonist attenuated the retention enhancing effect of NE. Consistent with this notion, posttraining intra-BNST infusion of the $\alpha 2$ agonist idazoxan (1.0 μg) failed to produce a significant retention enhancement: The idazoxan group had a median score of 204.4 (Q1/Q3: 93.0/394.8, n=13), while the Veh controls had a median score of 117.0 (Q1/Q3: 79.4/242.4, n=10), no significant difference between the two groups was detected ($U=51.5$, $p>0.10$). To examine the effect of NE on memory retrieval, two additional groups of rats were trained as described above without being treated around training. They had intra-BNST infusion of Veh or 1.0 mg NE 3 min prior to the 1-day retention test. The results showed that pretest intra-BNST infusion of NE did not affect retention performance. The median scores for the Veh and the NE groups were 6.6 (Q1/Q3: 4.7/155.5, n=7) and 85.7 (Q1/Q3: 53.6/455.6, n=7), respectively. Although the NE group appeared to have higher scores, the difference was not statistically significant ($U=14$, $p>0.10$).

IV. Effects of intra-BNST infusion of CRF on memory

Four groups of rats were trained on the task with a 0.7 mA/1 s footshock, and received intra-BNST infusion of Veh or 0.03, 0.1 or 0.3 μg CRF immediate after training. The 1-day retention performance analyzed by a Kruskal-Wallis one-way ANOVA showed that difference among various groups only approached statistical significance ($H'(3)=7.5$, $p<0.06$). However, paired comparisons showed that the 0.1 mg group had significantly higher retention scores than the Veh control or 0.03 μg group ($U=7$, $p<0.05$ for both comparisons). Difference in other pairs of comparisons failed to reach a statistical significance level.

V. Effects of intra-BNST infusion of lidocaine or prazosin on locomotion

Four groups were used in this experiment: Two of them received intra-BNST infusion of 2% lidocaine or its vehicle—the specific brain buffer, while the other two received intra-BNST infusion of 1.0 mg prazosin or its vehicle—10% propylene glycol. The results revealed a clear trend of habituation in locomotion over the testing period with a gradually descending trend of the travel distance (in centimeters). Neither lidocaine nor prazosin infused into the BNST seemed to have any effect. These data were analyzed separately for effects of lidocaine and prazosin by two 2×5 repeated measure design two-way ANOVAs with Drug as the between subject variable and Block as the within subject variable. In both analyses, the Block main effect was significant ($F(4, 92)=16.34$, $p<0.001$ for the lidocaine experiment, $F(4, 48)=4.74$, $p<0.01$ for the prazosin experiment), yet neither the Drug main effect nor the Drug×Block interaction effect was statistically significant (all $Fs<1$).

五、討論

This study reported the following findings on a passive avoidance task: First, suppression of the BNST with lidocaine immediately after training impaired later retention. Second, immediate posttraining intra-BNST infusion of NE or a $\alpha 1$ antagonist prazosin caused, respectively, dose-dependent enhancement or impairment of retention. Application of drugs activating or blocking other types of adrenergic receptors appeared to have little effect. Third, immediate posttraining intra-BNST infusion of CRF enhanced retention. Fourth, various drug treatments applied to the BNST during testing failed to alter expression of the passive avoidance response.

Altered performance in a learning task could be caused by influences on sensory-motor reactivity and/or motivation factors in addition to acquisition and/or retention processes per se. In the present study, all drugs were administered after a single trial of training; therefore, it is unlikely that the treatment affects retention by acting on performance factors. As a matter of fact, when two drugs used in this study were infused into the BNST, no change in locomotion was observed. Further, these drugs failed to affect retention when applied before testing, ruling out any possibility that animals treated as such were incapable of sensory discrimination or impaired in motivational function. Both NE and prazosin induced a prominent effect if applied to the BNST immediately after training but had little effect if applied 4 hrs later. This time-dependent effect argues strongly for a role of the BNST in formation processing of passive avoidance memory.

Extensive evidence has implicated the central noradrenergic activity in memory processing. Consistently, the present study found that posttraining infusion of NE into the BNST, a brain region most densely innervated by NE fibers, improved formation of affective memory in a dose-dependent manner: It caused no effect at 0.02 μg but significant enhancement at 0.2 and 1.0 μg. Infusion of prazosin—a $\alpha 1$ antagonist—into the same region caused an opposite effect and created an inverted U dose-response curve: 1.0 μg impaired retention but neither lower nor higher doses had any effect. The reason that a high dose of prazosin had no effect is not readily clear. However, a previous study did report a similar inverted U-shape dose-response curve for influences of prazosin injected subcutaneously on water maze learning. The possibility

that prazosin at a high dose may bind nonspecifically to other types of receptors and antagonize the effect of α1 transmission blockade should be explored in the future. The enhancing and impairing effects of NE and prazosin acting on the BNST, respectively, paralleled to those caused by infusing these agents into the amygdala. The present study showed that prazosin at a dose of 0.3 μg blocked enhancement of memory induced by 0.2 μg of NE. This dose of prazosin had no impairing effect of its own; the attenuation thus could not be due to a pure summation of two opposite effects bearing no relevance in receptor mechanisms. These results suggest that NE infused into the BNST may act on α1 adrenergic receptors to affect memory formation. These findings are consistent with the reports that α1 agonists enhanced retention when administered peripherally or directly into the brain. The present study also reported that 1.0 μg of idazoxan—an α2 agonist—infused into the BNST failed to cause a significant enhancing effect, which may reduce, but not completely eliminate, the probability of participation of BNST α2 receptors in memory processing. That 1.0 μg of prazosin induced a memory deficit by itself is worth noting. This finding implies that NE is released endogenously in the BNST during training and works as an intrinsic memory modulator under natural conditions. Indeed, there is evidence that emotional events such as immobilization stress caused in vivo NE release in the BNST, which played a crucial role in endocrinal and behavioral responses to a conditioned fear stimulus. In the present context, it is of interests to note that the stress-induced NE release in the BNST and CRF-like immunorecactivity in the hypothalamus were subjected to influences from the central amygdala nuclei.

With regard to noradrenergic modulation of learning-related neural plasticity, early evidence has implicated solely a β-mediated mechanism: Posttraining intra-amygdala infusion of the β blocker propranolol impaired retention and attenuated the memory enhancing effect of NE, the latter effect was mimicked by posttraining intra-amygdala infusion of a β agonist isoproterenol or 8-bromo-cAMP, an analogue of the second messenger coupled to activation of β receptors. However, the present study failed to find an effect of posttraining infusion into the BNST of propranolol at the same dose that had a marked effect when given to the amygdala (5.0 μg). While a wider range of doses should be tested before a definite conclusion could be reached for the role of BNST β receptors in memory, recent findings have nonetheless shown that noradrenergic modulation of memory processes involved complicated interaction among various subtypes of adrenergic receptors. In particular, β receptors appeared to act cooperatively with α1 receptors in modulating memory formation: Pazosin infused into the basolateral amygdala nucleus attenuated the memory enhancing effect of a β agonist clenbuterol, but not that of 8-bromo-cAMP, by shifting the dose-response curve to the right. In addition, concomitant infusion of a β antagonist atenolol blocked an otherwise significant memory enhancing effect of activating α1 receptors in the same region. It was thus suggested that in the basolateral amygdala, the influence of activating α1 receptors on memory might be mediated via modification of cAMP generation caused by activation of β receptors. In view of these findings, one should seriously consider the possibility that perturbing β receptors in the BNST would produce a more evident effect only under a

condition when α1 function there is concomitantly altered.

The findings that NE-containing fibers synapse with CRF immunoreactive sites in the BNST suggest possible interaction between these two neurochemicals in certain behavioral functions. Evidence has suggested a role of CRF in modulating formation of memory for affective experience. The present study showed that post-training intra-BNST infusion of CRF caused a dose-dependent enhancement of passive avoidance memory with 0.1 mg being most effective. Similar effect was also reported for infusing the same dose of CRF into the amygdala after training. Thus, the BNST is among the several brain sites on which endogenously released CRF may act to modulate memory. In the dentate gyrus, it has been shown that the memory enhancing effect of CRF may be due to its presynaptic facilitation of NE release from its terminals, because the effect was attenuated by infusion into the same region of either propranolol or N-2-chloroethyl-N-ethyl-2-bromobenzylamine (DSP-4, an NE neurotoxin), the noradrenergic modulation of memory in turn relied upon an N-methyl-D-aspartate (NMDA) mechanism because it can be blocked by MK-801. In view of the anatomical evidence that NE fibers in the BNST terminate on CRF containing dendrites or dendritic spines and the physiological evidence that in the BNST through an NMDA mechanism glutamate enhanced release of NE, which in turn exerted negative feedback on release of glutamate through an α2 mechanism, it is unclear whether CRF, NE and glutamate would interact in a similar cascade in the BNST to modulate memory as that in the dentate gyrus. On the other hand, NE released by glutamate in the BNST could modulate memory by affecting CRF release. The exact mode of interaction among these neurochemicals in the BNST to participate in memory processes would be a subject of great interests for further elucidation.

On basis that BNST lesions blocked light-induced or CRF-induced potentiation of startle but failed to block conditioned fear potentiation of startle, it has been proposed that the BNST mediates unconditioned but not conditioned fear responses, implying that this structure may not be involved in learning and memory. This implication is consistent with the report that changes in Fos- or Fos-like protein induced by intra-ventricular infusion of arginine-vasopressin were detected in the BNST of unconditioned, but not conditioned, rats. On the contrary, the present study found a significant effect on retention of a passive avoidance response by reversibly suppressing this nucleus with lidocaine or perturbing its adrenergic or CRF function shortly after training. Consistently, lesions of the BNST have been shown to attenuate various conditioned endocrine responses. Further, presentation of a conditioned inhibitor readily induced expression of c-fos in the BNST. These results thus provide support for a role of the BNST in acquisition or expression of conditioned association.

The exact cause for the above discrepant findings remains elusive. Conditioned and unconditioned responses might engage different neural substrates in the BNST. For example, in mice, the CRF1 and CRF2 receptors of the lateral septum and dorsal hippocampus were shown to be differentially involved in enhancing acquisition of fear conditioning and inducing anxiety, respectively. In view that both CRF1 and CRF2 receptors are found in the BNST, various studies may just probe into different aspects of the BNST func-

tion due to subtle difference in manipulation regimens and target regions. Alternatively, there has been evidence that certain posttraining treatments were effective only in one-trial learning paradigms such as the πασσιωε αωοιδανχε task used in the present study but not in multiple-trial paradigms such as the conditioned freezing responses. However, we have shown that pre- or posttraining intra-BNST infusion of prazosin or NE affected acquisition/retention in a multi-trial and multi-session learning task—the Morris water maze (unpublished observation). The discrepancy could also be due to that classical conditioning of fear responses is less susceptible to posttraining manipulation of the BNST function than inhibitory or active avoidance learning, in which successful performance requires execution of an instrumental act in addition to association of an otherwise neutral cue with an aversive unconditioned stimulus. As a matter of fact, previous studies have shown that the ventral amygdalofugal pathway, but not the ST, was solely responsible for classical conditioning of fear responses, whereas both of these pathways were critical for a passive avoidance task, although they subserved different roles. Clarification of these issues should be pursued in the future.

The BNST has been proposed as part of the extended amygdala. To a certain extent, this notion is substantiated by the present findings that CRF and NE in the BNST, just as those in the amygdala, were also involved in modulating formation of aversive memory. However, the function of these two structures in learning and memory appeared not to be totally identical. In a previous study, we have shown that suppression of the amygdala with 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) during testing impaired memory expression in the 1-day but not the 21-day test. In contrast, the present study showed that pretest intra-BNST infusion of lidocaine, prazosin or NE had no effect on memory expression in the 1-day test. With caution in mind that negative findings and comparisons between effects of different drugs could be hard to interpret, the findings taken together imply that the role of the BNST may be limited to the consolidation phase and yet that of the amygdala may be extended to early expression of a learned response. Given the intimate reciprocal connections between the two structures, the BNST and amygdala may form, among others, a circuitry allowing reverberation of neural activity to occur, which has long been hypothesized to be crucial for consolidation of memory traces elsewhere in the brain. Alternatively, the BNST may simply serve as a relay station for conveying the amygdala influences to other brain sites during the memory formation period. Further research addressing these two possibilities should be undertaken.

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