行政院國家科學委員會專題研究計畫成果報告

安非他命濫用：以動物模型研究其生理與心理的機制—安非他命敏感化之形成與解除的行為及神經機制(III)

Behavioral and Neural Mechanisms underlying Formation and Elimination of Amphetamine Sensitization (III)

計畫類別：整合型計畫
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一、 中文摘要

本三年期計畫利用場地偏好作業與驚跳反應作業探討安非他命致敏與消除之神經機制。此第三年的計畫得到下列的成果：於場地偏好作業訓練後或測試前壓制海馬回功能會分別阻斷偏好記憶的穩固或提取。在訓練時或記憶測試時壓制依核會分別阻斷偏好記憶的習得或提取，周邊安非他命注射可逆轉壓制依核所產生的記憶提取效果，故此一區域顯飛場地記憶儲存之所在。在記憶測試時壓抑前額皮質內側會組斷偏好記憶的提取，但學習前或學習後壓制此一區域對偏好記憶的習得與穩固沒有影響。進一步的研究顯示，杏仁核參與了安非他命酬賞之配對，而依核與腹側海馬回則參與了無酬賞配對與記憶穩固歷程。這些結果顯示：表現偏好記憶時，神經系統會分別經由杏仁核與負測海馬回提取酬賞與無酬賞之相關記憶，然後經過前額皮質內側的綜合而展現對安非他命配對箱的嗜好。另外，我們也發現安非他命在驚跳反應的致敏表現上，受到甚上腺皮質刺激素釋放因子(CRF)的調節：在長期接受安非他命注射後，某些動物表現致敏效果，此效果會被 CRF 拮抗劑所壓抑；有些動物在長期注射安非他命後並未表現致敏，然而這些動物在接受 CRF 拮抗劑後便表現出致敏現象。

關鍵詞：安非他命、致敏作用、二丁卡因、海馬回、依核、前額皮質內側、藥癮、驚跳反應增益、腎上腺皮質刺激素釋放因子

二、 英文摘要

This three-year project aims to explore the neural mechanisms underlying induction and extinction of behavioral changes induced by amphetamine. The results for the third year of this project showed that the amygdala is involved in forming association between reward and cues, the nucleus accumbens is involved in forming

association of nonreward and cues, while the ventral hippocampus is involved in consolidate such association. The medial prefrontal cortex is involved in coordinating these two aspects of association during testing to express preference associated with amphetamine. This study also found that CRF may regulate the expression of sensitization of acoustic startle response induced by amphetamine.

Key words: amphetamine, sensitization, conditioned place preference, amygdala, ventral hippocampus, nucleus accumbens, medial prefrontal cortex, association of reward and nonreward, lidocaine, CRF

三、緣由與目的

The goal for this integrated project is to study behavioral plasticity underlying amphetamine addiction. We focus on the long-term behavioral changes induced by repeated administration of amphetamine, mainly sensitization, and the neural bases underlying such changes. This project based on an assumption that amphetamine addiction involves reinforcement-related learning and memory resulting in indelible brain changes. Thus, intense drug craving, which causes frequent relapses long after detoxification, can only be understood by disclosing the neural circuitry underlying acquisition, retention and extinction of amphetamine reinforced behavior. This subproject specifically aims to investigate the neural mechanisms underlying the acquisition and extinction of conditioned place preference (CPP), which is the most utilized animal model of drug addiction. In this task, a rat was placed into one chamber of a two-chamber apparatus and injected with amphetamine on alternated days; it was placed into the other chamber and injected with saline on the other days. After 4 or more pairings for saline and amphetamine, the rat was tested under no drugs by giving free access to either chamber; the time spent in the amphetamine-pairing chamber relative to the saline-pairing chamber was used as an index of preference. In the 1st year, we developed the paradigm and successfully determined the critical factors involved in retention and extinction of this response. In the 2nd year, we showed that the amygdala was involved in acquisition and expression, but not memory consolidation, of this habit. In the 3rd year, we showed in the following report that this learning actually involved widespread area of the brain.

In addition, this project also investigated the behavioral and neural mechanism underlying sensitization induced by chronic injections of amphetamine. In the first year, we developed a novel paradigm by using acoustic startle to assess the sensitization effect of amphetamine. We found that the development of sensitization relied more on the behavioral context than the environment context, in other words, rats developed sensitization only when amphetamine was induced in the startle session. In the second year, we demonstrated that in contrast to other findings, adrenal hormones were not critical for induction and/or expression of sensitization induced by amphetamine. This led us to investigate the role of the corticotropin releasing factor (CRF).

四、結果

I. <u>Differential involvement of the left and right amygdala in expression, but not acquisition, of CPP</u>

Previous evidence had demonstrated some sort of lateralization of emotional functions in limbic structures. To address

this issue further, rats were trained on the CPP task. They received unilateral infusion of vehicle (Veh) or lidocaine (Lid) just before training and testing of CPP; the infusion was given into the left or/and right amygdala. The results indicated that rats with suppressed left or right amygdala during training still showed robust CPP ($t(5) = 3.12$ or 2.69, $p < 0.05$), therefore, no lateralization of the amygdala function was found in acquisition of the CPP task. However, during testing, suppressing the left amygdala did not blocked CPP, rats stayed longer in the drug chamber ($t(7) = 1.91$, $p < 0.05$). On the other hand, suppressing the right amygdala blocked expression of CPP, rats such treated showed no difference in the time spent in two chambers ($t(7) = 0.91$, $p > 0.10$).

II. Suppressing the dorsal hippocampus failed to affect CPP.

Existing evidence suggests possible involvement of the hippocampus in CPP learning. Rats received bilateral infusion of Veh or Lid into the dorsal hippocampus shortly before or after each training session or shortly after the testing session. Results indicated that Lid infusion before training or testing failed to blocked CPP acquisition or expression, rats in all groups showed significantly longer time staying in the drug chamber ($t(7) = 2.44$ for controls, $t(8) = 2.39$ and $t(5) = 2.50$ for the pretraining and pretesting groups, all $ps < 0.05$). Posttraining Lid infusion into the dorsal hippocampus failed to block formation of CPP memory, all groups of rats spent significantly longer time in the drug chamber ($t(7) = 3.525$ for controls, $t(7) = 3.281$ for the posttraining group, $p < 0.05$).

III. Suppressing the ventral hippocampus impaired CPP memory formation and expression.

Certain evidence suggests that the ventral hippocampus may be more critical in learning involved emotional events than the dorsal hippocampus, which was engaged in learning of complex spatial cues, an attribute, which contributes not much to the mastering of the CPP task. Rats received bilateral infusion of Veh or Lid into the ventral hippocampus shortly before or after each training session or shortly before the testing session. Results indicated that Lid infusion before training failed to block CPP acquisition: Veh- or Lid-treated rats had significantly longer time staying in the drug chamber ($t(6) = 5.18$ for controls, $t(8) = 2.99$ for the pretraining group, all $ps < 0.05$), however, pretesting infusion blocked CPP expression, rats showed no difference in the time spent in either the drug or saline chamber ($t(6) = -0.812$, $p > 0.05$). Posttraining Lid infusion did block CPP memory formation, the Veh group showed preference for the drug chamber ($t(6) = 4.41$, $ps < 0.05$), rats receiving Lid immediately after the training session showed no preference for the drug chamber ($t(10) = 0.831$, $p > 0.5$), yet rats receiving Lid 4 hrs after training showed strong preference for the drug chamber ($t(7) = 4.48$, $p < 0.01$).

IV. Suppressing the nucleus accumbens impaired CPP acquisition and expression

Rats received bilateral infusion of Veh or Lid into the nucleus accumbens shortly before or after each training session or shortly before the testing session. Results indicated that pretraining or pretest Lid infusion blocked CPP acquisition or expression, respectively: Veh -treated rats had significantly longer time staying in the drug chamber ($t(9) = 2.58$, $p < 0.05$), yet rats had pretraining Lid infused into the nucleus accumbens failed to show any preference ($t(7) = 1.32$, $p > 0.5$), neither

did rats receiving pretest Lid infusion ($t(7) = 0.47$, $p > 0.5$). Posttraining Lid infusion did not block CPP memory formation, the Veh group showed preference for the drug chamber ($t(6) = 4.41$, $ps < 0.05$), rats receiving Lid immediately or 4 hrs after the training session also showed preference for the drug chamber ($t(7) = 2.41$ and 3.25, respectively; $p < 0.5$).

V. Suppressing the medial prefrontal cortex impaired expression but not acquisition of CPP.

Rats received bilateral infusion of Veh or Lid into the medial prefrontal cortex shortly before or after each training session or shortly before the testing session. Results indicated that pretraining Lid infusion had no effect on CPP acquisition, but pretest Lid infusion blocked CPP expression. Veh-treated rats had significantly longer time staying in the drug chamber ($t(7) = 3.30$, $p < 0.05$), so did rats had pretraining Lid infused into the medial prefrontal cortex ($t(7) = 2.67$, $p < 0.05$), yet rats receiving pretest Lid infusion showed no preference for the drug or saline chamber ($t(8) = 0.91$, $p > 0.1$). Posttraining Lid infusion did not block CPP memory formation: Rats receiving Lid immediately after the training session showed preference for the drug chamber ($t(4) = 5.99$, $p < 0.5$).

VI. Differential involvement of various structures in the reward and nonreward association of the CPP task.

In a CPP task a rat had to combine two pieces of information obtained on alternating days: Certain cues were associated with amphetamine reward, while others were associated with saline nonreward. During testing, the rat made a preference choice on the basis of the two associations. It is unclear whether those structures critical for acquisition or memory formation of CPP were involved in processing both associations or simply involved in one of them. To address this issue, we infused Lid into the above structures specifically during the amphetamine-pairing trials but save the saline-pairing trials or vice versa. The results indicated that when infused into the amygdala, Lid blocked CPP if given before the amphetamine-pairing trials: Rats such treated showed little preference for the drug chamber ($t(7) = 0.435$, $p < 0.676$). However, the same treatment applied during the saline-pairing trials had no effect: Rats such treated showed clear preference for the drug chamber ($t(7) = 2.683$, $p < 0.05$). The opposite was found when Lid was infused into the nucleus accumbens: Lid given prior to the amphetamine-pairing trials had no effect on acquisition of CPP, rats treated as such showed clear preference for the amphetamine chamber ($t(7) = 3.05$, $p < 0.05$), on the other hand, Lid infused prior to the saline-pairing trials blocked acquisition of CPP, rats treated as such showed no preference for the drug chamber ($t(14) = 1.537$, $p = 0.147$). Likewise Lid infused into the ventral hippocampus blocked memory formation of CPP when given immediately after the saline trials: Rats treated as such showed no preference for the drug chamber ($t(9) = 1.479$, $p > 0.5$). On the other hand, Lid given immediately after the amphetamine-pairing trials did not block CPP memory formation: Rats treated as such showed clear preference for the drug chamber ($t(6) = 6.39$, $p < 0.01$).

VII. Differential effects of peripheral injections of amphetamine as reminding cue for expression of CPP memory under suppression of various brain structures.

The above findings indicated that the

expression of CPP is most sensitive to disruption of brain functions, it was readily blocked by suppression of the amygdala, ventral hippocampus, nucleus accumbens and medial prefrontal cortex. In a CPP task a rat was tested for memory under a drug-free state. It is interesting to know whether additional retrieval cues presented during testing would overcome the deficits caused by lesioning various brain regions. Previous studies has shown that retrieval of operant behavior in obtaining drug rewards, such bar pressing for self-administration of opiates, is facilitated by giving the subject a priming injections prior to the testing session. Accordingly, this study investigated whether a pretest injection of amphetamine (1.5 mg/kg) could serve as a reminding cue for rats with suppression of various brain regions. Various groups of rats bearing cannulae in different brain structures were trained on the CPP task as previously described. They received Lid infusion into the target regions just prior to testing, however, a peripheral injection of amphetamine was given simultaneously. The results indicated that rats received Lid infused into the amygdala, ventral hippocampus and medial prefrontal cortex showed no preference for the drug chamber ($t(6) = -0.681$, $t(7) = -0.547$, $t(6) = 1.642$, $p > 0.5$). On the other hand, rats with Lid infused into the nucleus accumbens showed clear preference for the drug chamber after receiving a peripheral injection of amphetamine ($t(5) = 3.675$, $p < 0.05$). These data suggest that amphetamine can serve as an effective reminding cue to overcome the deleterious effect of nucleus accumbens suppression.

VIII. Modulation of amphetamine-induced acoustic startle by α-helical-CRF

The finding that adrenalectomy failed to abolish amphetamine-induced sensitization of acoustic startle led us to explore the role of central CRF in this effect. Rats were subjected to daily acoustic startle sessions for consecutive 7 days and received pre-session injections of CRF (5.0 mg/kg), as described in the last year's report. Two days later, they received 4 challenge tests in the following order: vehicle, amphetamine 3.0 mg/kg, amphetamine 3.0 mg/kg + α-helical-CRF 10 μg and amphetamine 3.0 mg/kg. In the initial analysis of the results, we detected minor but significant effect of sensitization, yet no effect of αhCRF was found. However, careful examination of the data revealed that the subjects could be divided into two groups: One showed conspicuous sensitization in the amphetamine-challenge test, while the other showed little sensitization. Further analyses of the data by treating appearance of sensitization as a factor revealed a significant 3-way interactive effect ($F(3, 24) = 3.30$, $p < 0.05$): for the group which showed prominent sensitization after chronic amphetamine injections, αhCRF depressed the sensitized responses, the effect was significant on the 2nd block of the testing session ($p < 0.05$). In contrast, for rats which failed to show sensitization at the initial amphetamine challenge, icv infusion of αhCRF unmasked an otherwise non-apparent sensitization effect, the αhCRF treated-animals showed pronounced potentiation of the startle response in the amphetamine + αhCRF test ($p < 0.01$). It is even more interesting to note that on the fourth test when αhCRF was withdrawn and only amphetamine was given, the effect of αhCRF still persisted: Rats with sensitization unmasked by αhCRF showed sensitized responses to further amphetamine-only challenge, and rats with sensitization depressed by αhCRF showed no sensitized responses to

further amphetamine-only challenge. The interactive effect between types of treatment and the initial response profile was significant ($F(3, 24) = 4.92$, $p < 0.01$).

五、討論

This study investigated the neural substrates underlying association of neutral cues with rewarding effect of amphetamine through a CPP paradigm. The results showed that pretraining, but not posttraining, infusion of lidocaine, a local anesthetic, into the amygdala or nucleus accumbens impaired acquisition of CPP. Posttraining, but not pretraining, infusion of lidocaine into the hippocampus impaired memory consolidation of CPP. Neither pretraining nor posttraining infusion of lidocaine into the medial prefrontal cortex had any effect on acquisition or memory consolidation of CPP. However, inactivation of all above areas prior to the retention test blocked expression of CPP memory. The above structures appeared to be involved in different aspects of CPP memory. That blocking the amygdala was effective only during the amphetamine trials suggests involvement of this structure in coding association of cues and drug reward. While most findings in the field implicated importance of the nucleus accumbens in reward learning, our findings suggest on the contrary that it is involved in coding the association between cues and non-reward. Manipulation of the ventral hippocampus was effective only when given after training as the rat has returned to the home cage. Given that the hippocampus is involved in context learning, it may be critical for a rat subjected to CPP learning to detect the difference between a nonreward environment in the task context and a nonreward environment in the non-task context. This discrimination is of great importance for a rat to have good performance in the CPP task because different modes of behavior should be initiated in these two situation. In a task context, encounter of cues associated with nonreward may activate movement and search for where the rewarding cues are present. On the other hand, such movement may not be initiated in a non-task situation because any search of this kind will be futile. In a sense, a CPP memory could not be viewed as well formed until when the rat not only can discriminate the drug and the nondrug chambers but also can discriminate the task and non-task conditions.

It is interesting to note that the medial prefrontal cortex, while not involved in acquisition or consolidation of CPP memory, it is critical involved in expression of CPP memory. The medial prefrontal cortex possesses reciprocal connections, either directly or indirectly, with the ventral hippocampus, amygdala and nucleus accumbens. Thus anatomically, this area is in a strategic position to integrate all the information required for performing in a CPP test.

All the aforementioned areas except for the nucleus accumbens are essential for operating CPP memory. The present study showed that the memory retrieval deficit induced by suppressing the nucleus accumbens can be overcome by a dose of amphetamine. Such results suggest that in correct performance of CPP, information concerning the nonreward place is not irreplaceable, a rat would still showed correct preference if the input from the reward association is augmented by a reminding injection of amphetamine.

In the previous year, we showed that adrenalectomy or adrenal medullectomy failed to suppress the induction or expression of amphetamine-induced sensitization of acoustic startle responses. Given

the suggestion that the HPA axis is critically involved, we pursued the role of CRF in expression of sensitization. We found that endogenous CRF may work as a toggle switch in expressing sensitization induced by CRF. Its presence may either turn on or off the sensitization response depending on the present status of the animal. For those animals which showed sensitization in the first place, αhCRF turned off the sensitization response, and the animal remained desensitized in the following amphetamine challenge even without further administration of αhCRF, for those rats which showed no sensitization in the initial challenge, αhCRF turned on the response, and the response remained sensitized. The mechanism underlying this biphasic modulatory effect remains unclear. However, it is likely that there is an optimal level of CRF for the amphetamine sensitized response to appear, too high or to low may conceal expression of the sensitization. In view of the notion that CRF may serve as an endogenous mediator of stress or anxiety, such findings may bear significance on how stress be a critical factor for the specific system sensitized by amphetamine to express the abnormal function.