# 安非它命及古柯鹼引發制約性場地偏好行為的神經行為機制之研究（三）

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中文摘要

制約性場地偏好的行爲模式以往被廣泛的使用於研究心理性興奮劑藥物所具有刺激滿足慾的特性，該類藥物中的安非它命及古柯鹼已被證明可以很明顯的引發個體對特定場地制約而形成偏好的行爲反應。本研究計劃第三年的實驗繼續探討安非它命及古柯鹼引發大白鼠制約性場地偏好的神經行爲機制。就行爲層面而言，三個實驗分別利用不同的壓力源去檢測其對由安非它命或古柯鹼引發制約性場地偏好之行爲的影響。實驗一利用夾尾形成的壓力源發現其週邊注射安非它命引發制約性場地偏好行爲的習得階段無干擾效果，但會對習得後的表現造成減抑效果。另外，這種夾尾壓力源對週邊注射古柯鹼形成制約性場地偏好行爲的習得與習後階段皆有干擾效果。實驗二結果顯示由團體居住環境所形成的壓力源明顯會干擾週邊注射安非它命引發之制約性場地偏好行爲，但實驗三利用不定時供給食物的壓力源卻強化了週邊注射安非它命引發之制約性場地偏好行爲。有關神經機制的探討，基於本實驗及他人已發現安非它命直接作用在度巴胺系統的依核可以有效形成制約性場地偏好行爲，實驗四利用中樞微量注射這兩種興奮劑進入依核以外的腦區域進行測試。古柯鹼直接注入內側前額葉皮質可以引發制約性場地偏好行爲，但該藥物注入杏仁核或海馬體則無此效果。另外，安非它命若採較高劑量注入內側前額葉皮質的次級區域亦可引發制約性場地偏好行爲。綜合以上本研究第三年的實驗結果除了確認心理性興奮劑引發制約性場地偏好如何受壓力源的影響外，並結合前二年的結果提供安非它命及古柯鹼的酬賞性藥效在這種行爲作業過程係基於不同的神經機制。

**關鍵詞**：制約性場地偏好，安非它命，古柯鹼，壓力，中樞微量注射，
度巴胺系統，邊緣系統，大白鼠

## Abstract

Conditioned place preference (CPP) paradigm has been utilized with great deal to study the appetitive properties of psychostimulants. Both amphetamine (AMP) and cocaine (COC) have been found to reliably induce CPP effects. For the 3rd year, this study continuously investigates the neurobehavioral mechanisms of CPP induced by AMP and COC in the rat. From behavioral perspective, the effects of mild stressors on the CPP induced by AMP and COC were examined in three experiments. Experiment 1 using tail-pinch showed that this mild stressor significantly disrupted the acquisition, rather than the expression, of CPP induced by systemic injection of AMP. Tail-pinch applied in both stages of acquisition and expression significantly suppressed the CPP of systemic COC. The results of Experiment 2 demonstrated that the stressful experience from group housing significantly impaired the formation of CPP by systemic AMP. The results of Experiment 3 demonstrated that the stressful experience from inconsistent feeding enhanced the magnitude of CPP of systemic AMP or COC. In terms of neural mechanisms, Experiment 4 was designed to examine whether the CPP can be induced by central microinjection of AMP or COC into the DA related brain areas other than the nucleus accumbens. Microinjection of COC into the medial prefrontal cortex (MPFC), but not the amygdala or hippocampus, produced a marked CPP. In addition, CPP was solely observed when a higher dose of AMP locally infused into the infra-limbic area of MPFC. Together, these results verify how the stressful experience affects drug-induced CPP and continuously highlight important differences between the neural substrates for the reward effects of AMP and COC in the CPP task.

**Key Words:** conditioned place preference, amphetamine, cocaine, stress, microinjection, dopamine systems, limbic systems, rat

## Background

Conditioned place preference (CPP) paradigm has been utilized with great deal to study the appetitive properties of psychostimulants (Carr et. al., 1989). Systemic administration of amphetamine (AMP) or cocaine (COC) has been found to reliably induce CPP effects (Hoffman, 1989; Tzschentke, 1998). Albeit both AMP and COC have been recognized as relatively similar effects in several pharmacological aspects, some studies applying CPP task indicate that different effects potentially exist between these two agents. The study continuously investigated the neurobehavioral mechanisms of CPP induced by AMP and COC in the rat. From behavioral perspective, factors such as stressful experience can potentially be affecting AMP- or COC- induced CPP's which have not been fully attended in the past. The mesolimbic dopamine (DA) systems previously recognized to mediate the general reward effects (Koob & Bloom, 1988; Le Moal & Simon, 1991; Wise & Rompre, 1989). In terms of nural mechanisms, this project has been using microinjection technique to evaluate the specific brain areas involved in the formation of CPP induced by AMP and COC. Extended from previous finding of differential locations for AMP and COC activating in the subareas of the nucleus accumbens(NAC) (Liao et. al., 2000), several other areas were further examined as their potentialities to induced CPP by either agents in the last 12 months. Accordingly, four experiments were designed and executed in the 3rd year of this project. Experiment 1 manipulated mild stress induced by tail-pinch to examine how this stressor would affect in the acquisition and expression of CPP of AMP and COC. The development of CPP of AMP under different ways of housing (isolated vs. grouped) and feeding (regular vs. randomized) as stressor were verified in Experiment 2 and Experiment 3, respectively. Experiment 4 was designed to examine whether the CPP can be induced by central microinjection of AMP or COC into several areas other than the NAC. The data collected from this project were expected to further elucidate the neurobehavioral mechanisms for the effects of AMP and COC on CPP than that currently known.

## General Methods

***Subjects:*** The subjects were naive male Wistar rats weighing 200 $\pm$ 25 g at the start of the experiments. They were purchased from the Center of Experimental Animal of the National Taiwan University Hospital, Taipei, Taiwan. Except those subjects in Experiment 2, each rat was housed individually in a vivarium with a 12/12 hr light dark cycle. All the experimental sessions were conducted in the light cycle. The temperature of the animal colony was maintained at 23 $\pm$ 1 °C throughout the experiment. Except an irregular schedule of feeding in Experiment 3, rats were provided with Purina lab chow (5001) and tap water ad libitum.

***Drugs:*** D-amphetamine HCl and cocaine HCl (Sigma Chemical Co, St. Louis, MO, USA.) were each dissolved in saline (0.9% NaCl w/v). Vehicle injections were 0.9 % physiological saline. Drug solutions were freshly prepared just before administration at the specified dosages expressed as the salt.

***Apparatus:*** The CPP apparatus was made of Plexiglas and consisted of 3 different compartments. The central compartment (20 L x 10 W x 12 H cm) was connected to two equal-sized chambers (45 L x 45 W x 45 H cm). A chamber was painted gray on each wall and had the wire-meshed floor with wooden bedding below in one side, while the other was painted with black and white vertical stripes (4 cm each) and had a grid floor made by stainless steel rods running in parallel. In addition to these contextual differences, a tiny amount of vinegar was smeared along the top edge of the black and white striped wall during the CPP procedure. The entrance of each

side chamber was partitioned by a Plexiglas plate during the conditioning sessions, but left open for free access during pre-conditioning exploration and post-conditioning test sessions. The CPP apparatus was located in an isolated room with a dim light.
***CPP Procedure:*** Each rat was handled 10 min daily for two weeks of acclimation before experimentation. The CPP procedure required 15 daily sessions divided into three phases of pre-conditioning exploration, conditioning, and a post-conditioning test. During the first two daily sessions, designated the pre-conditioning phase, each subject was allowed to move freely in all the three compartments of the apparatus for 10 min. Rats showed no consistent preference to either compartment ($p>0.05$). Subsequently on each of twelve days in the conditioning phase, subjects received an injection of, alternately, a psychostimulant drug or vehicle and were immediately confined to one of the side chambers for 30 min. The order of injection and the chamber associated with drug were counterbalanced within groups.

The post-conditioning test was conducted one day after the last session of the conditioning phase. Each subject was placed into the central compartment and allowed to move freely for 10 min. Note that the subject received no injection prior to the CPP test session. Times spent in each compartment during the pre-conditioning and post-conditioning test sessions were recorded. Subjects were judged to be in a compartment only when all four limbs were in that compartment, which definition represents a more rigorous criterion for representing choice than has previously been employed (e.g. Hemby et. al, 1992). For each subject, two raw scores calculated by the difference in time spent in both the drug- and saline-associated side from pre-conditioning sessions to post-conditioning test were collected for statistical analysis. Changed from the pre-conditioned session to the post-conditioned test, a significant difference in the time spent in the drug-paired versus the saline-paired chambers was considered as successful place conditioning. Normally, CPP is indexed as time increased on the drug-associated side in comparison to time decreased on the saline-associated side.
***Experiment 1:*** Three groups (n=8 each) were assigned to test whether the exposure to the tail-pinch right before the acquisition and expression stages of CPP induced by systemic injection of either AMP or COC. To induce stressful experience, tail-pinch was conducted for 10 min right before the behavioral session (Chang et. al. 2000). For the control groups, the doses for systemic injection (IP) of AMP and COC to reliably induce CPP were 2 and 10 mg/kg, respectively (Liao et. al., 1998).
***Experiment 2:*** Two groups (n=9 each) were assigned to test the CPP effects of systemic AMP following two ways of housing, isolated and grouped. The isolated housing was the regular way to raise experimental subject in the lab (see general methods/subject), whereas the procedure for grouped housing raised three rats in a single cage. In other word, the living space was one third for each subject of the group housing as comparing to that in the isolated housing. These housing manipulation were conducted for 24 days before the protocol of drug induced CPP. In addition to manipulating housing, the ages of subject were compared in weaning and adult.
***Experiment 3:*** Four groups (n=8 each) were designed to compare the CPP induced by systemic AMP and COC under either regular or irregular schedule of feeding. The protocol of regular feeding was just as described in the general methods, whereas the irregular feeding schedule was manipulated by providing the same amount of food in a randomized schedule. In the irregular schedule of feeding, the daily amount of food was in-equalized, and each daily food supply was offered randomizedly in different times during light cycle (09:00, 12:00, and 17:00).

***Experiment 4:*** Several groups were assigned to test if AMP or COC can be directly infused into brain areas other than the NAC to induce CPP. Thus, the drug administration was conducted in microinjection instead of systemic injection for this experiment. A general stereotaxic surgery was conducted to implant the cannulae for microinjecton aimed at the (central) amygdala, the (dorsal) hippocampus, and the medial prefrontal cortex (MPFC). The tips of the guide cannulae terminated 1.5 mm above the acute injection site. Stainless steel stylets were inserted into the guide cannulae to keep the guides patent until the microinjections were conducted. At the end of surgery, penicillin (50000 I.U.) was intramuscularly administered to reduce the likelihood of postoperative infection. Subjects were allowed at least 7 days to recover from surgery. At the time of microinjection conducted in the conditioning phase, the stylets were replaced by 28 gauge injection needles connected with PE20 tubing to the 2 μl Hamilton microsyringes. Drug or vehicle solution was administered in a volume of 0.5 μl over 1 min. The injector needles were left in place for an additional minute to enhance diffusion from the injection site and to reduce the possibility of reflux. After behavioral testing, all subjects were sacrificed for the histological verification regarding the infusion sites. Behavioral data from individual subjects were excluded if the bilateral injections fell beyond the boundary of the target site or not symmetrical.

## Results

***Experiment 1:*** Consistent to previous work, systemic administrations of AMP and COC significantly produced CPP in two control groups. When tail-pinch applied on the CPP test day, this mild stressor significantly disrupted the expression of CPP induced by systemic injection of AMP. Such impairment was not occurred in the group of AMP when tail-pinch applied during the conditioning phase. Tail-pinch applied in both stages of acquisition and expression significantly suppressed the CPP of systemic COC.

***Experiment 2:*** In contrast to the CPP of AMP in the isolated group, stressful experience from group housing significantly impaired the formation of CPP by systemic AMP. These effects were also true for both weaning and adult subjects.

***Experiment 3:*** The results of Experiment 3 demonstrated that the stressful experience from inconsistent feeding enhanced the magnitude of CPP of systemic AMP or COC.

***Experiment 4:*** Microinjection of COC into the MPFC, but not the amygdala or hippocampus, produced a marked CPP. In addition, when AMP infused into MPFC subareas, a CPP was solely observed when a higher dose of AMP locally infused into the infra-limbic area of MPFC.

## Discussion

The CPP can be specifically induced by psychostimulant drugs in association with a whole perspective of environmental cues. However, the behavioral mechanism for place conditioning and the neural substrates for psychostimulant drugs to activate their rewarding effects remain obscure. The project aims to investigate the neurobehavioral mechanisms for CPP induced by psychostimulants.

In terms of the rewarding effects of drugs like psychostimulants, it is therefore possible that the development of repeated drug use (or abuse) can be relied on the interaction between the individual and environment (for review see Altman et al, 1996). It is well known that a large number of people administer with drugs for variable periods of time, only some of them develop a real addiction. From the behavioral perspective, a theoretical consideration of this individual-centered vision of

addiction is based on that drug abuse is a preexisting pathological condition exposed by the drug. Addiction would appear only in certain individuals because their biological features will generate a pathological response to drugs. This pathological response would make the appetitive properties of drugs greater in some subjects, thus increasing their propensity to develop a drug addiction. The stress can be one of the most distinguishing factors related to the environmental experience. When the body is exposed to harm or threat, the result is a cluster of physiological changes that is general referred as the stress. Recently, a series of work has been done by Piazza and his associates suggest that the stress play an important role in drug-self administration (reviews by Piazza & Le Moal, 1998 &1996). It is suggested that stress experiences facilitate individual to establish drug self-administration, increase the rate of responding duration retention, and can precipitate reinstatement. As known, both self-administration and CPP are the most widely used animal models of evaluating the reward properties of drugs. The data regarding the three types of stress applied in the present report (Experiment 1-3) clearly show the stressful experience does affect the drug induced CPP. The potential interaction between the CPP and stress may be contributed to the common reaction from the mesolimbic DA systems under stress and drug/place conditioning. However, the directions to alter psychostimulant-induced CPP are dependent upon the types of stressors used and what stage of place conditioning applied. The CPP was disrupted by experimental manipulations of tail-pinch and group housing, while the enhanced degree of place conditioning was observed from the irregular food-supply. In considering the current CPP data and those reported by the use of self-administration, the distinctiveness on behavioral meaning or theoretical background exists between these two animal models.

As simultaneously comparing the effects of AMP and COC in Experiment 1, the finding is in agreement with one of the assumptions in the present project. That is the CPP effects of AMP and COC can not be identical. It seems to be the former one is more solid than the latter one. The CPP of COC was disrupted by tail-pinch applied in either the acquisition or the expression stage, whereas the CPP of AMP was only impaired when tail-pinch applied in the expression stage. These data suggest that the formation of CPP of COC is subtle under the stress.

Based on the previous findings from this lab, microinjection of AMP into the core, but not the shell, area of the NAC produced a marked CPP. Conversely, such CPP was solely observed when COC with higher dose was infused into the shell area (Liao et. al., 2000). Our results complement previous evidence of CPP initiated by microinjection of AMP into the NAC (Carr & White, 1983 & 1986), and further showing this effect can be attributed to the drug action occurred in the core area of NAC. In conjunction with most of the previous work reporting that AMP administered in both systemically and centrally produces a more robust CPP effect than COC (Hoffman, 1989), these findings suggest an important discrepancy between the neural substrates for AMP and COC CPP. Accordingly, the present project continuously investigates the brain sites for inducing these CPP's. In the last year, this project focused on the brain areas other than the NAC. The CPP was induced by COC but not by AMP infused the MPFC. However, when given with a higher dose, AMP infused into a MPFC subarea (infra-limbic) was found to induce CPP. Although these results indicate that the action of COC in MPFC is more sensitive to produce a rewarding stimulus as associated with the environmental cues than that of AMP, it is possible that hereterogeneous functions exist in the MPFC. A growing body of evidence suggests that the PFC (including MPFC) should not be treated as a

homogeneous brain area (Fuster, 1997). Moreover, the differences of MPFC microinjections of COC and AMP on the CPP were not compatible to that both drugs could be self-administered into the MPFC (Koob & Goeders, 1989). The present report also shows the failure to induce CPP for COC locally infused into either the amygdala or the hippocampus. This finding indicate that the role of either one area of the limbic systems is not as critical as that of the NAC of the DA system for psychostimulant to induce CPP.

In summary, the effects of mild stressors on the CPP induced by AMP and COC were examined in three experiments. Experiment 1 using tail-pinch showed that this mild stressor significantly disrupted the acquisition, rather than the expression, of CPP induced by systemic injection of AMP. Tail-pinch applied in both stages of acquisition and expression significantly suppressed the CPP of systemic COC. The results of Experiment 2 demonstrated that the stressful experience from group housing significantly impaired the formation of CPP by systemic AMP. The results of Experiment 3 demonstrated that the stressful experience from inconsistent feeding enhanced the magnitude of CPP of systemic AMP or COC. In terms of neural mechanisms, Experiment 4 was designed to examine whether the CPP can be induced by central microinjection of AMP or COC into the DA related brain areas other than theNAC. Microinjection of COC into the MPFC, but not the amygdala or hippocampus, produced a marked CPP. In addition, a CPP was solely observed when a higher dose of AMP locally infused into the infra-limbic area of MPFC. Together, these results verify how the stressful experience affects drug-induced CPP and continuously highlight important differences between the neural substrates for the reward effects of AMP and COC in the CPP task.

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