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（二）

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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。本研究計劃將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，個體自發性活動及對環境熟悉度均可能對由安非它命或古柯鹼引發制約性場地偏好之行爲有不等程度的影響。實驗一操弄不同空間大小引發不等程度之自發性活動對安非他命引發制約性場地偏好之影響，其結果發現這兩者之間並無顯著相關。實驗二操弄個體對先前曝露於制約環境所造成不等熟悉程度，結果發現這項操弄並未對安非它命引發制約性場地偏好造成顯著影響。基於中腦的度巴胺神經傳導作用一直被認爲是主導制約性場地偏好的中樞所在，實驗三將安非他命或古柯鹼直接注射入大腦內與度巴胺神經系統有關之解剖部位，測試其是否得以引發制約性場地偏好行爲，其結果發現安非他命注入依核的core區域而非shell區域得以引發制約性場地偏好行爲，古柯鹼則注入依核的shell區域而非core區域得以引發制約性場地偏好行爲。當兩藥被注入前額葉皮質區時，古柯鹼較安非他命易於引發制約性場地偏好行爲。本研究的實驗的結果除了進一步確認心理性興奮劑引發制約性場地偏好之機制專屬性外，並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，自發性活動，環境熟悉度，中樞微量注射，度巴胺系統，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. This study intended to further reveal the neurobehavioral mechanisms of CPP induced by AMP and COC in the rat. From behavioral perspective, the degrees of locomotion was manipulated by employing two different sizes of conditioning compartment, whereas the familiarity to environment was manipulated by the pre-exposure to the conditioning compartment. Experiment 1 found that the locomotor activity related spatial size are not involved in the formation of CPP of AMP. Experiment 2 demonstrated that the CPP of AMP was not affected by the familiarity to CPP apparatus. In terms of neural mechanisms, Experiment 3 was designed to examine whether the CPP can be induced by central microinjection of AMP or COC into the DA related areas in the brain. Microinjection of amphetamine into the core, but not the shell, area of the nucleus accumbens produced a marked CPP. Conversely, such CPP was solely observed when cocaine with higher dose was infused into the shell area. The cocaine infused into the medial prefrontal cortex is more prone to induce CPP than amphetamine. Together, in addition to verifying the specificity of drug-induced CPP, these results indicate important differences between the neural substrates for the reward effects of amphetamine and cocaine in the CPP task.

Key Words: conditioned place preference, amphetamine, cocaine, locomotor activity, environmental familiarity, microinjection, dopamine 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 intended to further reveal the neurobehavioral mechanisms of CPP induced by AMP and COC in the rat. From the behavioral perspective, factors such as drug induced locomotor activity and the experience related to the test environment can potentially be affecting AMP- or COC- induced CPP effects which have not been fully attended in the past. In regarding the mesolimbic dopamine (DA) systems previously recognized to mediate the general reward effects (Koob & Bloom, 1988; Le Moal & Simon, 1991; Wise & Rompre, 1989), the present work used microinjection to investigate the specific location of DA-related areas in the mediation of CPP induced by AMP and COC. There were three experiments designed and executed in this study. Experiment 1 aimed to concurrently manipulate the locomotor activity related spatial size to examine how this factor would be involved in the formation of CPP of AMP. Experiment 2 aimed to examine the different degrees of familiarity to CPP apparatus to affect the CPP of AMP. Experiment 3 was designed to examine whether the CPP can be induced by central microinjection of AMP or COC into several subareas in the nucleus accumbens (NAC) and the medial prefrontal cortex (MPFC). The data collected from this project were expected to elucidate the neurobehavioral mechanisms for the effects of AMP and COC on CPP and to provide valuable information in clinical.

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. 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 during experimental sessions, 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. Time 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 (eg. 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: Two groups (n=8 each) were assigned to test with different sizes of the CPP apparatus. The major different sizes were based on the floor area of the side chamber. One of which is the regular size as aforementioned, whereas the other one is about one quarter to that. The drug treatment used to initialize CPP was amphetamine (2 mg/kg, IP) in this experiment.

Experiment 2: Two groups (n=8 each) were assigned to test whether the exposure to the saline-paired side was as equally essential as that with the drug-paired side. The procedure for the control group was just as those described above (amphetamine 2mg/kg, IP). In contrast to the control subject, the subjects of the experimental group were placed in their home cage for 30 min right after the saline administration during in the conditioning phase. Another group (n=8) was examined its CPP acquisition after a period of exposure to the CPP apparatus before conditioning. This pre-exposure was manipulated by placing the subject into the saline-paired chamber and the drug-paired chamber for 16 days, with unequally numbers of session.

Experiment 3: Several groups were assigned to test if amphetamine or cocaine cane be directly infused into a specific brain area 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. As determined by Paxinos and Watson (20), the coordinates for the final injection sites of the NAC in the core area and the shell area were AP = 1.7 mm, L = $\pm$1.8 mm, D = -4.8 mm, and AP = 1.7 mm, L = $\pm$1.8 mm, and D = -6.2 mm, respectively. In addition, a border area between core and shell (AP = 1.7 mm, L = $\pm$ 1.8 mm, D = -5.7 mm) and a caudal NAC (AP = 1.0 mm, L = $\pm$ 1.8 mm, D = -5.2 mm)

were also verified to assess the specificity of CPP effects elicited from the other two sites. The coordinates for two subareas of MPFC are listed as followings: the anterior cingulate/ prelimbic area AP = 2.7 mm, L = $\pm$ 0.5 mm, D = - 2.5 mm, the infralimbic area AP = 2.7 mm, L = $\pm$ 0.5 mm, D = - 3.5 mm. 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 ul 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 verfication 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.

Statistical analyses: An one-tailed t-test for dependent designs was conducted to verify the effects of CPP under each experimental treatment. Statistic significance was determined by the value of $p<0.05$.

Results and Discussions

Experiment 1: Both groups, tested in different sizes of the CPP apparatus, significantly acquired the amphetamine-induced CPP. It was assumed that different degrees of locomotion could be derived from the different sizes of the test area. These data suggest that the magnitude of locomotion can not be correlated with the degree of CPP performance.

Experiment 2: While the CPP was significantly shown in the control group following the regular procedure of place conditioning, it was not the case for the experimental group with the conditioning only for the drug-pair side (see Figure 1). These results indicate that the contrast, in terms of the conditioning of injection with a specific context, between the saline-paired and the drug-paired sides can be critical in determining the CPP acquisition. A group manipulated with the pre-exposure to CPP apparatus still significantly acquired the CPP. Thus, the familiarity induced by the pre-exposure was not as expected to affect the CPP acquisition.

Experiment 3: The results of CPP test after amphetamine or cocaine locally infused into the subregions of NAC are presented in Figure 2. For the subjects with cannulae aimed at the NAC core area shown in Figure 2(A), both groups of rats treated with two doses of amphetamine produced significant place preferences for their amphetamine paired chambers ($t(11)= -5.3$ and $t(7)= -5.5$, both $p<0.05$). No such CPP effect was reveled in the groups treated with cocaine in the NACC core area. As shown in Figure 2(B), subjects conditioned with intra-NACC shell only with the high dose of cocaine developed the significant preference to for the drug-paired chamber, $t(7)= -2.05$, $p<0.05$. Two control groups of animals respectively receiving saline vehicle microinjection into the core and shell areas showed no preference for either side chamber ($p>0.05$), indicating that the CPP apparatus employed here is balanced. In Figure 3, the effective doses of amphetamine (10 ug) and cocaine (100 ug) found in the preceding were used to infused in either the border area of core and shell or the caudal NACC. Neither amphetamine nor cocaine injected into the caudal NAC produced

CPP. Although no CPP effect approaching significance was observed for the cocaine in the border area, animals with amphetamine infused into that site were effectively exhibit CPP ($t(8)= -2.7$, $p<0.05$). Of the 4 sites of the NAC tested, the core and shell areas are respectively for amphetamine and cocaine infusion to produce CPP. Figure 4 presents the photomicrographs of the placements of microinjections into these two sites of NAC.

The results of CPP test after amphetamine or cocaine locally infused into the subregions of MPFC are presented in Figure 5. The CPP was significantly induced only when the microinjection of higher dose of cocaine (100 ug) conducted into either the anterior cingulate/ prelimbic area or the infralimbic area, $t(10)= -4$, $t(10)= -2.7$, both $p<0.05$. No such effect was seen for the group treated with lower dose of cocaine or those groups of amphetamine. Figure 6 presents the photomicrographs of the placements of microinjections into these two sites of MPFC.

The major finding of this experiment indicates that the NAC appears to be heterogeneous with regard to the acquisition of psychostimulant-induced CPP. Microinjection of amphetamine into the core, but not the shell, area produced a marked CPP. Conversely, such CPP was solely observed when cocaine with higher dose was infused into the shell area. Our results complement previous evidence of CPP initiated by microinjection of amphetamine into the NACC (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 amphetamine administered in both systemically and centrally produces a more robust CPP effect than cocaine (Hoffman, 1989), these findings suggest an important discrepancy between the neural substrates for amphetamine and cocaine CPP. Regarding the MPFC, the CPP was only induced by cocaine but not by amphetamine. These results indicate that the action of cocaine in MPFC is more sensitive to produce a rewarding stimulus as associated with the environmental cues than that of amphetamine. Cocaine is suggested to be more readily to induce psychopharmacological effects than amphetamine (Johanson & Fichman, 1989). The differences of MPFC microinjections of cocaine and amphetamine on the CPP was not compatible to that both drugs could be self-administered into the MPFC (Koob & Goeders, 1989).

Conclusion

The CPP can be specifically induced by amphetamine in association with a whole perspective of environmental cues. This type of behavior is not correlated with either the locomotion relevant to the size of association compartment or the familiarity to the pre-exposure to the conditioning environment. In terms of neural mechanisms, the NAC is a heterogeneous structure with regards to the place conditioning effect induced by amphetamine or cocaine. The cocaine infused into the MPFC is more prone to induce CPP than amphetamine. Together, these results indicate important differences between the neural substrates for the reward effects of amphetamine and cocaine in the CPP task.

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