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以制約場地偏好行為模式探討藥癮復發的行為神經機制

Investigation of neurobehavioral mechanisms of drug relapse by the use of conditioned place preference model

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Abstract

Accumulative evidence supports the idea that the addicted drugs act as reinforcers of drug-taking and drug-seeking behaviors. From the past, it is argued that the mesolimbic dopamine (DA) systems play a major role of the underlying neural mechanisms for drug reward. The dopaminergic mechanisms mediating the drug reward and/or addiction are more complicated than what were thought in the past. There is a bottleneck to reveal the behavioral mechanisms for a sophisticated delineation of drug addiction. Despite the aforementioned progress made on in psychopharmacology of drug reward, it is still not clear about the underlying neural mechanisms for drug reinstatement also known as the relapse in clinic. Therefore, this 2-year project investigated the neurobehavioral mechanisms of drug reinstatement with a focus of using amphetamine CPP model in the rat. In the first year, the major work was to establish an animal behavior model of drug reinstatement in amphetamine conditioned place preference. With several experiments, the extinction protocol was verified to work, that consisted of 4 cycles of 2 days (exposure to CPP context without any injection in one day and CPP re-testing in the other day) and followed by a 3-day withdrawal (staying in home cage). In the second year, experiments with pharmacological manipulations will be conducted to study the neural substrates for the drug reinstatement on aforementioned place conditioned behavior. A dose dependent effect of amphetamine to prime the extinguished CPP was obtained based on the aforementioned extinction protocol. Regarding the role of dopamine subtype receptors involved in drug reinstatement, D1, but not D2, receptor agonist reactivates amphetamine CPP. Furthermore, preliminary data show such effect is mediated by brain-derived neurotrophic factor (BDNF) expression ih the medial prefrontal cortex. Together, the current data provide a further step in revealing the neurobehavioral mechanisms underlying drug reward and reinstatement of amphetamine. Throughout executing this project, one SCI paper publication and at least five conferences paper presentations have been completed among other relevant academic accreditations obtained.

Keywords: drug reward, drug reinstatement, conditioned place preference, brain dopamine systems

中文摘要

越來越多的證據顯示上癮的藥物對「嗑藥」與「求藥」行為扮演增強物的角色，過去的研究證據顯示大腦多巴胺系統對這種藥物酬賞行為的增強扮演重要的角色。最近的研究持續的關注多巴胺與藥物酬賞之間的關係，主要的評論認為大腦多巴胺不應只是產生酬賞增強作用而已。行為層面的探討就如同神經機制的探討一樣，目前都有待突破瓶頸。行為層面的重要課題之一，便是有關停用藥物一段時日後的再復發現象。本項二年期專題研究計畫預計執行實驗內容是：第一年建立以安非他命引發制約性場地偏好行為之再復發的動物模式；第二年對此再復發行為進行藥理的操弄測試。實驗結果首先在於確認一個有效的「消除」步驟，其包括四個兩天的週期（含第一天放入 CPP 兩側配對箱與第二天的 CPP 再測，均無任何注射），及三天留滯在各自的飼養籠。利用這項步驟將所習得的 CPP 消除，再用較低的安非它命劑量引燃 CPP 的效果，實驗結果得到顯著的藥物反應劑量。這項安非它命的引燃 CPP 效果，可以被多巴胺 D1（而不是 D2）的致效劑取代。另外，安非它命 CPP 的再犯行為與前額葉皮質的大腦神經滋養因子（BDNF）的表現有關。這個計劃的成果進一步的解析安非它命藥物復發行為的神經機制，執行過程中的學術論著發表包括一篇 SCI 期刊論文及至少五篇的國際會議論文，同時還有一些其它間接的學術成果。

關鍵詞：藥物酬賞、藥物（癮）再復發、制約式場地偏好行為、大腦多巴胺系統。

I. Background

Accumulative evidence supports the idea that the addicted drugs act as reinforcers of drug-taking and drug-seeking behaviors. In other words, those drugs produce rewarding effects can then be characterized with abuse potential, such as psychostimulant drugs (e.g. amphetamine, cocaine) and opioid compounds (e.g. morphine, heroine). From the past, it is argued that the mesolimbic dopamine (DA) systems play a major role of the underlying neural mechanisms for drug reward. The use of traditional operant conditioning paradigm on self-administration and self-stimulation highlights that the release of dopamine in the brain serves as the reinforcement contingency for establishing the drug addictive behavior. However, the dopamine hypothesis for drug reward and addiction behavior has been continuously debated since the last decade. The dopaminergic mechanisms mediating the drug reward and/or addiction are more complicated than what were thought in the past. Similarly, as the challenge appeared on investigating neural mechanisms, there is a bottleneck to reveal the behavioral mechanisms for a sophisticated delineation of drug addiction. Despite the aforementioned progress made on in psychopharmacology of drug reward, it is still in obscure about the efficient anecdote or medical treatment for drug abuse. One of the critical concerns on behavioral characteristics of this syndrome is regarding to the resumption of drug seeking or drug consumption after a protracted abstinence, so-called relapse in clinical. Accumulating data have demonstrated the drug reinstatement in the rodent animals, but most of the previous studies tested the reinstatement developed by cocaine or morphine, rather than amphetamine. Moreover, most of previous studies applied the behavioral task of self-administration. Relatively less data were reported by the study using conditioned place preference (CPP) as the behavioral task. Therefore, this project investigated the neurobehavioral mechanisms of drug reinstatement of

amphetamine. The project was approved to conduct those essential experiments over the last two years. In the first year, the main theme is to establish an animal behavior model of drug reinstatement by the use of amphetamine induced CPP. In the second year, experiments with pharmacological manipulations were conducted to study the neural substrates for the drug relapse on aforementioned place conditioned behavior.

II. Methodology

The Wistar rat**s,** with food and water in *ad libitum*, were used as the subjects. Each subject was handled for 3 to 5 min daily for 2 weeks before entering experimental protocol.

The conditioned place preference (CPP) apparatus was made of Plexiglas and consisted of 3 different compartments. The start box was separated from other two chambers by a Plexiglas plate door. One chamber was painted white wall and had a wire-meshed floor, while the other one was painted with black and white vertical stripes (4 cm each) and had a grid floor made of stainless steel rods running in parallel. The entrance of each side chamber was partitioned by Plexiglas plates during the conditioning sessions, but left open for free access pre-conditioning exploration and post-conditioning test sessions. The apparatus doses not induce any unconditional preference for wither side chamber on a group. The CPP apparatus was located in an isolated room with a dim light.

The protocol of amphetamine induced CPP was similar to what has been used in the lab (e.g. Liao et al, 2000). In brief, it was consisted of three phases including 1) the pre-conditioning phase, 2) the conditioning phase, and 3) the post-conditioning test of CPP. Each subject, in the pre-conditioning phase, was allowed to explore the whole contextual environment of CPP apparatus for 10 min. In the subsequent 3

days, as for the conditioning phase, the rat received 3 saline injects paring to one compartment and 3 amphetamine injection (1 mg/kg, IP) pairing to the other compartment in alternate. Each rat was given the saline treatment in the morning and the amphetaine treatment in the afternoon. The rat was confined to the corresponding compartment for 30 min 5 min after saline or amphetamine injection,. The post-conditioning test of CPP was conducted 24 hr after the last conditioning session. During this test, rat was allowed to freely access to either compartment for 10 min. Time spent in each compartment during post-conditioning test was recorded by an automated system with video taping.

An extinction protocol was introduced in the next day of the post-conditioning test of CPP. In which, eight daily sessions were consisted of 4 cycles of two days. In the first day of each cycle, each rat was confined into each of the two compartments of CPP apparatus which procedures were same as the conditioning days but without any injection treatment. In the second day of each cycle, each rat was allowed to freely access either compartment for 10 min which procedure was same as that described for the post-conditioning test of CPP. This procedure of repeatedly testing the CPP in the rat provided the data determining whether the CPP was extinguished or not. The rat was then left without any experimental treatment in it home cage, simulating as the withdrawal stage. Subsequently, the rat received the reinstatement test with the priming injection of amphetamine or dopamine related agents.

While conducting the aforementioned experiment as proposed, the present study also carried some experiments relevant to this drug induced place conditioning behavior. These data are worthy and informative for PI to continuously exploring the neurobehavioral mechanisms of drug reward and drug seeking behavior.

III. Data Collected

1. In the first year of this project, this project spent much longer time than expected in establishing a reliable protocol of the extinction in order to test the drug reinstatement. The protocol later being verified as successfully working is described in the Methodology above. In this report, as for a record, it is worthy to briefly describe what had been tried with the negative outcomes. Such that, the procedures of amphetamine induced CPP established in this lab were first tried by extending into the extinction sessions in several controls for the extinction procedure being tested. One of the controls was to stay in the home cage over the extinction sessions (10 days). The other was simply putting the subject into the conditioned compartments in alternate over 10 days, and without any injection of amphetamine or saline. Moreover, the third control group was treated with saline in the compartment where the subjects were previously given with amphetamine. Although the results showed that all three type of extinction (or withdrawal) treatments did impair the appearance of CPP, the statistical tests did not confirm the significant effect of extinction. It can be attributed to the relative large variation. And, this negative result might be attributed to the inadequate length of extinction sessions (10 days in this case). A new batch of subject was later testes with the longer extinction, 20 days as set, but still in failure. This part of experiment was failed might be due to the lack of automated CPP measures. The CPP tests conducted by manual recording took a high cost of man power that slowed down the progress of data collection. To encounter this shortage, this lab fortunately received an extra financial support from the University setting up additional CPP apparatus that can be recorded in automated. The extinction and the drug reinstatement

2. Besides running the aforementioned experiment, this lab has been continuing to collect data to deal with the animal model of place conditioning. Two lines of

studies with several experiments were done and presented below with title and abstract for each (labeled by 2.1 2.2 & 2.3).

2.1 Dose effects of Dopamine Receptor Antagonists on Stressor Induced Conditioned Place Preference in Rats

An immediate and robust release of dopamine appears in several brain regions under acute stressor, but it remains uncertain about how this enhancement of dopamine is involved in behavioral process of learning and memory. Conditioned place preference (CPP) is a behavioral task based on classical conditioning paradigm, which has been frequently used to measure the motivation and its relevant learning. Accordingly, this study manipulated the after-effect of elevated platform or restraint stressor was used as unconditioned stimulus (US) to be associated with a specific context. A place conditioning test given 24 h later, CPP was determined if subjects significantly showed conditioned approach behavior toward the stressor paired chamber more than the other one (non-stressor-paired). Such CPP effect was significantly induced by two types of stressor from placing the subject on an elevated platform and a restraint holder. Additional experiments were conducted to test the involvement of dopamine in this type of CPP by administering selective dopamine D1 and D2 receptor antagonist, SCH23390 (0.025 and 0.05 mg/kg) and raclopride (0.05 and 0.1mg/kg) respectively, before each stressor manipulation. The results showed that both dopamine receptor antagonists attenuated the formation of stressor induced CPP. Together, the stressor can serve as a valid US to facilitate the conditioned approach response to form a CPP, and such behavior is dopamine dependent. This part of data has been published in a SCI journal (Shen et al., 2010).

2.2 The dopamine receptors in the medial prefrontal cortex involved in place conditioning as an acute stressor given simultaneously in associative context.

The medial prefrontal cortex (mPFC) is activated under stressor, but the

functional role of this area in stress remains unclear. Our previous studies demonstrated the conditioned place preference (CPP) induced by restraint stressor. Moreover, when mPFC was temporally inactivated before the manipulation of stressor, the formation of such a CPP was attenuated. It is still not known whether the mPFC is involved in either the phase of stress manipulation or the place conditioning. The present study tried to solve this issue by temporally inhibiting the mPFC right after the stressor manipulation and before the commencement of place conditioning. The general procedure was that rat in the Stress group was removed from its home cage and placed into a conditioning chamber of CPP task for 30 min. Four hours later, the subject was restrained in a plastic tube for 30 min before placing into the other conditioning chamber for the stressor-pairing session of 30 min. Subjects in the Control group experienced the same procedure but without stressor manipulation. The rats in experimental groups were immediately microinjected with lidocaine (3%) or saline into the mPFC after the stressor. A place conditioning test conducted in the following day (24 hr later) and revealed that the subject of the Stress group receiving saline significantly spent more time lingering in the conditioned chamber, indicating that CPP was formed by the association of the restraint stressor and a specific context. Such CPP effects were significantly attenuated by the treatment of licocaine into the mPFC. Together, the present study confirmed the effects of restraint stressor in the formation of the presentCPP, and the mPFC was involved. These data alone with others in regarding to the involvement of medial prefrontal cortex have presented in conferences (Shen & Liao, 2009 & 2010).

2.2 Adenosine receptor agonists infused into striatal subareas blocking the expression of conditioned place preference induced by amphetamine

Adenosine has been suggested to play a pivotal role in modulating central

reward functions. A growing body of evidence indicates that adenosine receptors in different brain regions can affect the releasing level of several major neurotransmitters. Previous work showed that systemic injection of adenosine agonists influences the rewarding effects of psychostimulant drugs. However, the central mechanism of these interactions between the adenosine agonists and the psychostimulants are still unclear. It is presumed that different subtypes of adenosine receptors exert heterogeneous behavioral functions. The present study was designed to examine the role of adenosine subtype receptors in amphetamine (AMP) induced conditioned place preference (CPP) which is a widely used animal model to testify the drug addiction. Selective adenosine A1 and A2 receptor agonists, CPA and CGS21680 respectively, were locally infused into the lateral striatum and nucleus accumbens (NAC) on the expression of AMP CPP. Male Wistar rats with chronic cannulae implanted in the lateral striatum or NAC were subjected to CPP protocol with 3 AMP and 3 saline pairing trials in alternate. To induce CPP, the subjects were intraperitoneal injected either AMP (1.0 mg/kg) or saline shortly before conditioning sessions. The test session was conducted after 24 hrs after the last conditioning trial. On the post-conditioning test day, the subject was infused a dose of adenosine agonist or saline 20 min prior to the test session. The results showed that AMP significantly elicited CPP at the present dose tested. In which, the subject spent more time in the AMP paired context than the saline paired one on the post-conditioned test. Microinjections of both CPA and CGS21680 into lateral striatum suppressed the expression of AMP CPP at all doses tested. Similar results were revealed from the treatments of CPA and CGS21680 infused into NAC. These results show that local infusion of selective adenosine A1 or A2 agonists into either lateral striatum or NAC attenuates the expression of CPP induced by AMP. It suggests that activation of adenosine A1 and A2 subtype receptors in the striatal areas is involvement in blocking

the rewarding properties of AMP. This part of data has been presented in an international conference (Yang & Liao, 2009).

3. Priming effects of amphetamine and dopamine D1 receptor agonist on the reinstatement of amphetamine induced conditioned placed preference

This study using the amphetamine induced CPP investigated the dose effects of amphetamine on the reinstatement. Further experiments tested whether the dopamine D1 receptor agonist, SKF38393, would be able to prime the reinstatement of amphetamine CPP. Following our previous work (e.g. Liao et al, 2000), CPP was formed by amphetamine with dosing given at 1 mg/kg (IP). As described in the Methodology, the rats were then subjected to the extinction phase, which was consisted of 8-day exposure to the CPP test apparatus and following by a 3-day of staying in the home cage as the withdrawal. No injection was conducted in the extinction phase. The drug reinstatement tests were conducted 24 hr after the end of extinction phase. The results, consistent to previous work, amphetamine CPP was significantly demonstrated in all groups. The data, as measured over the 2^{nd}, 4^{th}, 6^{th}, and 8^{th} day of extinction, show that the amphetamine CPP was significantly extinguished after the present regimen of extinction. The doses of amphetamine (0, 0.5, and 0.75 mg/kg) were then evaluated in the drug-induced reinstatement test. A dose-related fashion was confirmed for amphetamine reactivating the extinguished CPP. In a separate experiment, SKF38393 (0, 0.1, 0.5 mg/kg) was tested for its priming effect on the reinstatement of amphetamine CPP. Only the high dose of SKF38393 could reinstate the amphetamine CPP. These results, together, suggest that the reinstatement of amphetamine CPP is depended in dopamine D1 receptors. This part of data will be presented in an international conference to be held in next year (Liao et al, 2011)

4. Priming effects of dopamine D2 receptor agonist on the reinstatement of amphetamine induced conditioned placed preference

The rationale and protocol of this study is similar to those described in the study listed above (no. 3), except testing the dopamine D2 receptor agonist as the priming agent. After the CPP established, the subject went through the extinction phase and showed no preference to initially conditioned compartment. After a 3-day withdrawal, a 0.5 mg/kg of quinpirol was given (IP) to test whether activating D2 receptor can reinstate the extinguished CPP. A negative result was showed for this drug treatment at the dose tested. In considering that this is a single dose test, a higher dose of quinpirol to test is expected before any conclusion can be made in comparing the potential differential effects existed between D1 and D1 receptors involved in the amphetamine CPP reinstatement. .

5. Preliminary tests of brain-derived neurotrophic factor (BDNF) in the amphetamine CPP and its reinstatement.

Accumulating evidence indicates that BDNF is involved in the drug rewarding effect as measured in behavioral sensitization, self-administration and condition place preference. It has been argued that BDNF expression is associated to the neuronal activity and synaptic plasticity. Drug taking and/or seeking behavior may be mediated by neuronal adaptation in the brain dopamine areas with the involvement of BDNF expression. This study quantified the mRNA of BDNF of the amphetamine CPP and the amphetamine reinstatement. From the subjects significantly performed aforementioned behaviors, brain samples of five areas were collected including the medial prefrontal cortex, nucleus accumbens, striatum, amygdala, and hippocampus. Preliminary data showed that BDNF mRNA was not significantly changed in all five

brain areas tested for amphetamine CPP. Following the performance of amphetamine reinstatement, BDNF mRNA was significantly increased in the medial prefrontal cortex but not in the other four areas. These data showed BDNF expression in the rat performing the reinstatement to amphetamine but not for the drug rewarding effect itself. And, this pattern of BDNF expression is different from those reported for cocaine that may indicate the distinctive profiles of underlying neural mechanisms existed between amphetamine and cocaine on drug addiction behavior.

IV. Summary

This 2-year project investigated the neurobehavioral mechanisms of drug reinstatement with a focus of using amphetamine CPP model in the rat. A dose dependent effect of amphetamine to prime the extinguished CPP was obtained based on a special extinction protocol developed in this lab. Regarding the role of dopamine subtype receptors involved in drug reinstatement, D1, but not D2, receptor agonist reactivates amphetamine CPP. Furthermore, preliminary data show such effect is mediated by brain-derived neurotrophic factor (BDNF) expression ih the medial prefrontal cortex. Together, the current data provide a further step in revealing the neurobehavioral mechanisms underlying drug reward and reinstatement of amphetamine. Throughout executing this project, one SCI paper publication and at least five conferences paper presentations have been completed among other relevant academic accreditations obtained.

References cited:

Liao R.-M.*, Chang Y.-H., Wang S.-H., & Lan C.-H. (2000) Distinct accumbal subareas are involved in place conditioning of amphetamine and cocaine. Life Sciences, 67, 2033-2043.

Liao R.-M, Chang Y-C, Yang F-C, & Shen Y.-L (2011) Priming effects of dopamine D1 receptor agonist on the reinstatement of amphetamine induced conditioned placed preference. Conference of The College on Problems of Drug Dependence, June 18-23, 2011, Hollywood, Florida. USA.

Shen Y.-L & Liao R.-M. (2009) The involvement of the medial prefrontal cortex on restraint stressor induced conditioned place preference. Society for Neuroscience, Oct. 17-21, Chicago, IL, USA.

Shen Y-L & Liao R-M (2010) The dopamine receptors in the medial prefrontal cortex involved in place conditioning as an acute stressor given simultaneously in associative context. Annual Conference (49th) of Taiwanese Psychological Association, Nov. 6-7, Ja-Yi, Taiwan.

Shen Y-L & Liao R-M (2010) Post-training infusion of dopamine receptor antagonists in the medial prefrontal cortex did not affect stressor induced place conditioning. Society for Neuroscience, Nov. 13-17, San Diego, CA, USA.

Yang, F.-C. & Liao R.-M. (2009) Adenosine receptor agonists infused into striatal subareas blocking the expression of conditioned place preference induced by amphetamine. Society for Neuroscience, Oct. 17-21, Chicago, IL, USA.