行政院國家科學委員會補助專題研究計畫成果報告

利用跑爬跑行為工具探討中腦多巴胺系統的神經行為機制(3/3)

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中文摘要

中腦至皮質及邊緣系統的多巴胺被認爲是主導個體對酬賞的主觀感受(包括自然界的酬賞物質及上癮的藥物)，它並且支配與酬賞有關的行爲學習增強歷程。唯這個多巴胺系統是如何主導上述行爲功能仍有疑慮且值得再行探究，既使已有很多証據顯示拮抗或破壞多巴胺會導致行爲表現受損，但進一步去確認這個腦與行爲關係的議題是極重要的研究工作。本研究建置跑爬跑行爲作業去探討之，它是一種新的且敏感的行爲測試工具。紋狀體、依核、內側前額葉皮質是這個系統軸突終端聯會區，它們應對這種可操作不同作業要求的行爲工具有不相等的機制參與其中。因之，本研究的目的即在於驗証這個多巴胺系統影響或參與跑爬跑行爲的神經行爲機制。這行爲工具被設計成探討不同的行爲內涵，包括利用不等的爬繩長度反映行爲成本，利用不等質量的酬賞物反映增強誘因的利益，及成本與利益兩因子的交互作用。另外，這行爲工具亦可進行制約增強的實驗步驟；更特別的是其可以反映一種經由成本利益分析的行爲反應分配或選擇的內涵，即對以高成本行爲反應得到高利益酬賞之選項有反應的偏好傾向。這些是不同行爲內涵的跑爬跑作業被有效的建置後，本計畫利用神經毒物破壞前述多巴胺軸突終端區，以驗証這些區域的參與機制爲何。實驗結果發現依核（而非背側紋狀體）參與成本利益分析的跑爬跑選擇行爲，另外相似的結果也發現在背側前額葉皮質（而不是腹側前額葉皮質）。另外，本研究進一步建立一種風險選擇行爲，以進一步佐證多巴胺與成本利益分析的關係。本研究亦藉由 Fos 蛋白質免疫分析法，檢驗不同行爲作業內涵對大腦神經細胞的活性之影響，結果發現在多巴胺系統的相關部位確實有相對應的反應。

關鍵詞:　酬賞動機，增強，選擇行爲，成本與利益，中樞破壞，Fos 蛋白質免疫分析法

Abstract

The mesocorticolimbic dopamine (DA) system is known as the best candidate for the common neural substrate for mediating the subjective rewarding action of natural rewards and drug of abuse, and also for playing a critical role in the maintenance of reinforcement process. However, how exactly the DA exerts its functions in this brain system for reward-motivated behavior remains uncertain and in debate. Despite considerable evidence showing behavioral impairment under DA antagonism or lesion, it is important to be more precise on parsing the relationship between behavior and brain on this issue. This project set up a sensitive and newly developed measure so-called run-climb-run (RCR) behavioral task to encounter this challenge. It was reasonably presumed that different terminal areas of this DA system are differentially are involved in distinct requirements on RCR behavioral task. As focusing on such a theme, this project aims to investigate the neurobehavioral mechanisms of mesocorticolimbic DA system by the use of RCR behavioral task. This 3-year project was designed to reveal the effects of DA antagonism on several different behavioral components of the RCR task. The behavioral components were measured by manipulating 1) solely on the rope length to determine the response cost required for completing behavior, 2) solely on the reward value of reinforcer to judge the earned benefit, and 3) the interaction between cost and benefit by holding one factor in consistent and varying the other. Another behavioral manipulation was focused on establishing the concurrent choice paradigms based on selecting either high/cost to get high/benefit or low/cost to get low/benefit. Following the establishment of these different tasks on RCR behavior, neurotoxic lesions made in the striatum, the nucleus accumbens (NAC), and medial prefrontal cortex (mPFC) were conducted to determine the role of DA terminal areas involved. The results show the NAC (but not the dorsal striatun) was involved in the present task, such role was also confirmed to dorsal mPFC (but not ventral mPFC). Moreover, the present work further set up a risky choice behavior to determine the core component of cost/benefit analysis in choice. By the use of Fos-like assay, the immunoreactivity tested from several regions within the mesocorticolimbic DA system were correlated with the different requirements on RCR behavior.

Key Words: reward motivation, reinforcement, choice behavior, cost/benefit, lesion, intracranial microinjection, c-Fos

The mesocorticolimbic dopamine (DA) system is known as the best candidate for the common neural substrate for mediating the subjective rewarding action of natural rewards and drug of abuse, and also for playing a critical role in the maintenance of reinforcement process. However, how exactly the DA exerts its functions in this brain system for reward-motivated behavior remains uncertain and in debate. Despite considerable evidence showing behavioral impairment under DA antagonism or lesion, it is important to be more precise on parsing the relationship between behavior and brain on this issue. This project was originally plan to set up a sensitive and newly developed measure so-called run-climb-run (RCR) behavioral task to encounter this challenge. It is reasonably presumed the different DA terminal areas are differentially involved in distinct requirements on RCR behavioral task. As focusing on such a theme, this project has been focusing to investigate the neurobehavioral mechanisms of mesocorticolimbic DA system by the use of RCR behavioral task. Proposed to complete in three years, this project was designed to reveal the effects of DA antagonism on several different behavioral components of the RCR task. Manipulation on the behavioral components was including 1) solely on the rope length to determine the response cost required for completing behavior, 2) solely on the reward value of reinforcer to judge the earned benefit, and 3) the interaction between cost and benefit by holding one factor in consistent and varying the other. In addition, reinforcers with different values were conditioned with neutral visual stimuli and then determine the effects of conditioned reinforcement. Another behavioral manipulation focused on establishing the concurrent choice paradigms based on selecting either high/cost to get high/benefit or low/cost to get low/benefit. Following the establishment of these different tasks on RCR behavior, the excitotoxic lesions were conducted in the striatum, the nucleus accumbens, and medial prefrontal cortex (mPFC) to determine the potential DA mechanisms involved. In this final brief report of this 3-year project, the results from three major studies were presented in the followings. Some of these data have submitted to present in two international neuroscience conferences, International Brain Research Organization (IBRO) World Congress of Neuroscience, in July 2007 (at Melbourn, Australia) as well as the Society for Neuroscience Annual Conference (at San Diego, CA, USA, in November, 2007). The rest of the data will soon be presented to the conferences in domestic and/or international. Subsequently after the data presentation in conference, these data will be put into several manuscripts submitted to journal for the official publication. At present, one manuscript is under 2nd review, and two manuscripts were in preparation.

I. Tests of benefit-up and cost-down on a run-climb-run behavior in rats with striatal

lesions

Using a RCR behavioral task, previous studies reported behavioral deficits induced by systemic injection of DA receptor antagonists. Accordingly, the present study further investigated how lesions in two striatal subareas would affect RCR behavioral performance. Also, behavioral manipulations on either increasing reward magnitude (benefit-up) or decreasing the work requirement for response (cost-down) was evaluated in the striatal-lesion subject. Food-deprived rats were trained to traverse a floor alleyway (150 cm), climb a vertical rope (70 cm), and run across an upper runway board (100 cm) to access a single piece of chocolate as reward. Following baseline training, ibotenic acid was used to produce excitotoxic lesions in the nucleus accumbens and the ventrolateral striatum. Post-lesion test was conducted on the regular RCR task over 3 consecutive days. Extended tests by increasing reward magnitude (as 3 pieces of chocolate provided) and decreasing task-required effort (by shortening the climbing rope length from 70 cm to 30 cm) were conducted thereafter. Lesion of the nucleus accumbens significantly disrupted the RCR behavioral performance as revealed by the increased time to complete the task. Although the time to complete the task was increased by lesion of the ventrolateral striatum, such effect was not statistically confirmed. While behavioral impairment produced by striatal lesions was not attenuated by the reward increment, it was significantly reversed by the shorter rope treatment. These data indicate that the nucleus accumbens is a critical striatal subarea for driving the motivation of RCR behavior. The reduced demanding effort (or cost) to complete task can reverse the impairment of RCR behavior induced by the nucleus accumbens lesion.

II. Nucleus accumbens, but not dorsolateral striatum, involved in the choice response on a run-climb-run behavioral task.

Many behaviors perform under the analysis of cost to exert and benefit to obtain. How the brain gets involved in this processing remains unknown. Previous studies of the brain reward indicate the dopamine systems are relevant to behavioral choice based on cost/benefit analysis. The present study was designed to investigate the lesion effects of nucleus accumbens (NAC) and dorsolateral striatum (DLS) on a choice behavior of RCR task. Rats were trained to traverse an uncovered floor alleyway (150 cm), climb a vertical rope (35 or 140 cm represented as the short or long rope), and run across an upper board (100 cm) to access chocolate for the reinforcement. All subjects were trained to climb the short rope for obtaining a smaller amount of reward. As reaching a stable baseline of performance, they were further trained to climb the longer rope for receiving a larger amount of reward and reached a stable baseline of performance. Subsequently, they were introduced to the

concurrent choice test for 5-day pre-lesion test, in which the subjects significantly chose the long rope to obtain 4 pieces of chocolate rather than the short one to obtain only one piece of chocolate. They were then separated into 4 groups: 2 groups received excitotoxic lesion in either NAC or DLS, while the other 2 groups served as the sham controls. The post-lesion data showed that NAC, rather than DLS, lesion significantly shifted the choosing from the long rope into the short one. Also, the mean time to complete RCR task with the long rope was significantly increased by NAC lesion, but not by DLS lesion. Microstructural analysis on behavioral performance on different segments of RCR task with the long rope revealed that the most apparent impairments induced by NAC lesion were the shifted motion from the end of the floor alley way to the rope when hopping or to initiate climbing and the rope climbing. These data suggest that the NAC is critically involved in RCR behavior and essential for the choice made between high-cost-high-reward and low-cost-low-reward options.

III. The dorsal mPFC, but not the ventral mPFC, involved in the choice response on a run-climb-run behavioral task.

The protocols and materials for this study are similar to those described in the preceding study regarding the nucleus accumbens. It was presumed that the anatomical heterogeneity of mPFC could be true for the present behavior task. The results showed that the lesion applied in the dorsal mPFC rather than in the ventral mPFC significantly shifted the choosing from the long rope into the short one. Also, the mean time to complete RCR task with the long rope was significantly increased by dorsal mPFC lesion, but not by ventral mPFC lesion. These data suggest that the the dorsal mPFC is critically involved in RCR behavior and essential for the choice made between high-cost-high-reward and low-cost-low-reward options.

IV. The development of an animal model of risky choice behavior to further evaluate the cost and benefit involved in behavioral processes.

While the risky choice behavior is common in human, little is known about this kind of behavior in the laboratory animal. An animal model of risky choice behavior has been recently developed in this laboratory by the use of the rat. In a T-maze, a goal arm was designated as certain low reward (CLR) arm providing 1 pellet of chocolate for every entry, whereas the other one was designated as probabilistic high reward (PHR) arm providing 2, 4, or 8 pellets of chocolate to obtain based on a probability of 50%, 25%, or 12.5%. Following the exploration of T-maze, the food-deprived rat was firstly forced to enter each arm set with a distinct amount of reinforcer(s). There were then five daily sessions of free choice conducted for a

certain probabilistic high reward. Under these three probabilities arranged in PHR arm, the rats significantly chose more for CLR than for PHR as the probabilities decreased. These data indicate that the subject would choose CLR as the risk is increased. Moreover, microstructural analyses were conducted to reveal which goal the subject intended to choose after entering a PHR arm with and without reinforcers. It was found that the continuous entrance to the PHR arm was observed in the lower risk condition rather than the higher risk one. Conversely, the subject intended to shift entering the PHR to the CLR in the highest risk condition set in the present study, which was arranged with the lowest probability to obtain reinforcer. Thus, these data indicate the probabilistic-based risky choice behavior can be developed in the laboratory animal. This animal model was further used to investigate the effect of amphetamine on risky choice. In a T-maze, a goal arm was designated as certain low reward (CLR) arm providing 1 pellet of chocolate for every entry, whereas the other was designated as probabilistic high reward (PHR) arm providing 2 (or 8) pellets of chocolate to obtain based on a probability of 50% (or 12.5%). After training, the rats significantly chose more for CLR than for PHR as risk increased. Amphetamine (1mg/kg) treatment significantly produced more PHR arm entries. These data indicate that stimulant drug can facilitate the risky choice behavior. It is highly possible that the mPFC is involved in amphetamine induced behavioral alterations on this behavioral task. Further study is planned to testify this inference.

V. The Fos-like immnoreactivity in the DA-related brain regions in rats under behavioral performances.

The present project has successfully set the immunohistochemical assay of Fos expression in the brain tissues adopted from the rat. This assay has completed in a behavioral task based on a basic motivation issue, which asserted behavior responses to different degrees of rewarding stimuli (1 to 4 pieces of chocolate) leading different amount of Fos expression in distinct brain areas. The results indeed show supportive evidence to this notion, which the striatal subareas are the most sensitive for this manipulation. In addition, Fos expression was examined for the acquisition of a schedule controlled behavior so-called the differential reinforcement for low-rate response (DRL). This behavior contains at least two key components, behavioral inhibition and timing, both could be treated as basic processes underlying the RCR behavior. The results in this part of experiment show distinct areas were involve in mediating these two behavioral components when the subject learn the DRL behavior respectively at an earlier and later stages (i.e. 2 vs. 12 weeks). In brief, the Fos reactivity in mPFC or hippocampus is evident involved in behavioral inhibition, whereas that of striatum is apparently involved in timing process. With this Fos set

up in the lab via this project, it is definitely beneficial for this lab to run behavioral neuroscience work in a more sophisticate way in the future.

In summary, this 3-year project has been under well controlled and completed. In accumulating the work from these 3 years, RCR behavioral model has been constructed for investigating the cost and benefit components of goal-directed behavior. In addition to behavioral study, the brain dopamine areas have been found to be involved in certain types of RCR behavior. How exactly the role for each dopamine area is currently under deliberated with comparing to other literature reports, which work is conducted for preparing the manuscript(s) to submit for journal publication. All this accomplished data will be taken into the solid base for those experiments proposed to initialize the follow-up studies.