國立政治大學心理學研究所碩士論文

探討大白鼠之

風險選擇行為之神經機制

Investigation of neural mechanisms of

risky choice behavior in the rat

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中文摘要

「風險決策」行為非常普遍的存在於吾人之日常生活中，而選項所帶來的風險和獎勵是吾人進行決策時的重要考量因素。風險選擇的適當與否，對於個體的生存扮演著相當重要的角色。在以往的文獻中，對於決策的行為歷程已有所關注及探討，但對於風險選擇行為的神經生理機制迄今未明。本研究藉由大白鼠於 T 字迷津中，選擇確定之低酬賞或高不確定性之高酬賞的行為表現，進行風險選擇行為的探討。本研究中以兩項主要實驗，探討風險選擇行為之神經行為機制。實驗 1a 中，確定之低酬賞端固定呈現 1 顆食物粒，而高不確定性之高酬賞端則同時操弄酬賞物機率(50%、25%及 12.5%)以及酬賞物的量(2、4 及 8 顆)，以系統性地檢驗期望值(0.5、1 和 2)於此風險選擇行為中扮演的角色。行為結果顯示當風險較低時，大白鼠會選擇高不確定性之高酬賞端；而風險較高時，則轉為選擇確定之低酬賞端。實驗 1b 中，系統性地施打不同劑量之安非他命，探討多巴胺系統在此風險選擇行為中之機制。實驗結果顯示施打安非他命後，大白鼠表現出相對地追求風險之行為，亦即選擇高不確定之高酬賞端之比例顯著高於控制組。實驗 2 中，藉由毀除大腦特定部位(依核、背外側之紋狀體、眶前額皮質、內側之前額皮質)，檢驗風險選擇行為之神經基礎。毀除後之結果顯示，僅有依核受到毀除之大白鼠表現出相對地趨避風險之選擇行為。綜合以上結果，本研究建立之風險選擇行為與多巴胺有關，而依核在此行為歷程中扮演重要的調節角色。

關鍵字：決策，風險選擇，期望值，中腦多巴胺系統，神經毒素毀除，大白鼠

Abstract

Many decisions people make every day involve uncertainty where both risks and rewards associated with each option need to be considered. Behavioral performance associated to risk-based choice appears wildly over the lifespan, and the fitness of risky choice behavior plays an important role in individual survival. Despite a growing body of research has focused to investigate the neurobiology of decision making, little is known about the neurobehavioral mechanisms of risky choice behavior. Based on a pilot work, this study used a T-maze to study decision under a probability-based risk in the rat. The subject was assessed on making choice to obtain either a large reward associated with risk of non-reward "empty" or a small reward ensured for every entry. Two experiments were conducted in this project to investigate neurobehavioral mechanisms of probabilistic risky choice behavior. In Experiment 1a, probabilistic risky choice behavior was systemically assessed under three expected values (0.5, 1.0, and 2.0) by manipulating the probabilities of reward presence (50%, 25%, and 12.5%) and the reward magnitude (2, 4, or 8 pellets) in the probabilistic high reward (PHR) arm. Behavioral data showed that the subject chose the probabilistic high reward in a lower risk condition but would shift to the choice of certain low reward (CLR) as the risk is increased. In Experiment 1b, the dose effects of amphetamine on this probabilistic risky choice task was tested to verify whether the dopaminergic mechanism was involved. Amphetamine, presumably activating brain dopamine systems, produced a relatively risk-seeking effect on the present behavioral task. In Experiment 2, the excitoneurotoxic lesion was conducted in the nucleus accumbens, the dorsolateral striatum, the orbitofrontal cortex, and the medial prefrontal cortex to examine the neural substrates for this probabilistic risky choice behavior. The results showed that the lesion of the nucleus accumbens significantly produced a relatively risk-averse effect on the present behavioral task, as compared to

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the lesions made on the other three brain areas. In conclusion, the probabilistic risky choice behavior established in the present study is dopamine dependent. And, the nucleus accumbens plays a major role of mediating this behavioral processing.

Chapter One: Introduction

The issue of decision making has received considerable attention in a fashion of continuous growth recently.   To examine decision making, the choice behavior could be simply measured when an individual makes decision among different options each with a certain value of outcome and a specific probability for the outcome.   However, in the real-world, the choice behavior is more complex than it was thought.   For example, buying stuffs, investing stocks, taking tests and choosing a job for career development, choice behavior with uncertainty is involved in almost everything that people engage to do.

It is now clear that decision making is a higher order of cognitive function. Substantial data have been accumulated to elucidate decision making from psychology and other disciplines of social science.   How the brain mediates or modulates the processes or components involved in decision making remains inconclusive.   Despite this, from clinical studies, behavioral patterns of decision making observed in the patient with certain types of neurological or psychiatric disorder is different from that of normal individual.   For instance, impulsivity for choosing the immediate reward rather than the delayed reward with a magnified amount has been observed in the subject with the diagnosis of pathological gambling or drug addiction.   Such a case is in common cross societies and cultures.   Thus, the investigation of neural mechanisms of decision making becomes an important research topic and can enhance our understanding how the brain drives for decision making.

Economical viewpoints

According to the economical principles, the expected outcome values and the uncertainty of options are two factors being considered when people make decision or choose an option for the specific purpose (Schultz, Preuschoff, Camerer, Hsu, Fiorillo,

Tobler, & Bossaerts, 2008).   In general, expected value (EV) can be calculated by knowing the outcome probability and the possible outcome value of a specific option. From the economical viewpoint, the subject should reasonably choose the option with higher EV in order to get more profits.   However, the subjective value which differed between individuals has neglected in the basic concepts of EV.   Thus, "expected utility" has been proposed and taken the individual subjective values into account (von Neumann and Morgenstern, 1947).   The differences between expected utility and EV are the expected utility argued that individual's subjective value is not a linear correlation with the outcome values.   That is, if an outcome value increased, the higher outcome values would became less worthy for the decision makers, such phenomenon shows a concave utility function (Schultz et al. 2008).

In the past, many studies indicated that most people would not so rational in some specific conditions.   In prospect theory (Kahneman & Tversky, 1979), the individual's attitudes toward gain and loss are not the same.   In a monetary gain condition, most people were risk-averse.   But in a monetary loss condition, most people are risk-seeking.   For example, a choice between certain $9,000 and 50% of $20,000 dollars, most people would choose $9,000; in contrast, a choice between certain loss $9,000 and 50% of loss $20,000 or loss nothing, most people would bet on the 50% of loss nothing.   Moreover, the results from the experiments of framing effect (Tversky & Kahneman, 1981) reveal a perception of decision problem and the evaluation of probabilities and outcomes produce predictable shift of preference when the same problem is framed in different ways.   These findings indicate the deviation from the prediction of EV can be a bias to change the choice made by people.   As addressed in *Neuroeconomics* edited by Glimcher, Camerer, Fehr, and Poldrack (2009), people often make decisions based on the emotional states (Glimcher et al. 2009, chap 16 & 19), social preferences (Glimcher et al. 2009, chap 18), past

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experiences (Glimcher et al. 2009, chap 22) and environmental conditions (Glimcher et al. 2009, chap 28) rather than the purely judgments from outcome values or EV. Although the earlier concepts about EV failed to precisely predict individual's choice or attitude in certain conditions, it is still a key variable for decision making or processing choice behaviors (Schultz, 2010).

Dopamine and decision making

In recent years, the midbrain dopamine system has been argued for playing an important role in decision making and related cognitive processes, such as prediction error and reward valuation (Schultz, 2010).　Several dopamine related areas in the brain have been reported to be critically involved in different types of decision making including the analysis of cost and benefit (Boksem & Tops, 2008; Salamone, Correa, Farrar, Nunes, & Pardo, 2009), delay (or temporal) discounting (Cardinal, 2006; Kobayashi & Schultz, 2008) and probabilistic task (Niv, Duff, & Dayan, 2005; Schultz et al, 2008).　Furthermore, a series of neurophysological studies done by Schultz and his colleagues demonstrated that neuronal activities of ventral tegmental area encode the reward probability and risk (Schultz et al., 2008; Schultz, 2010; Fiorillo, Tobler, & Schultz, 2003; Kobayashi & Schultz, 2008).　With dopaminergic activation, the subject can learn and/or integrate the information which includes reward value, reward probability and reward magnitude among different options in order to making decisions (Schultz et al., 2008).　It is worth to further delineate which area of the midbrain dopamine system is involved in a specific type or process of decision making.

The analysis of cost and benefit

A basic form associated with decision making is the process of the analysis of cost and benefit.　Namely, the subject makes decision to choose an option either high cost/benefit or low cost/benefit.　Neural mechanisms underlying the analysis of cost

and benefit are reasonably inferred from human study of neuropsychology (Boksem & Tops, 2008).   The involvement of the midbrain dopamine system in the choice behavior task related to cost/benefit analysis has also been reported in animal studies (Cousins, Atherton, Turner, & Salamone, 1996; Salamone, Cousins, & Snyder, 1997). In these experiments, two choice options were set on the two arms of T-maze, one arm was the high-cost-high-benefit (HH) arm, and the other was the low-cost-low-benefit (LL) arm.   The "high cost" for rats was designed by a barrier placed on the way to access the high reward.   The rat has to climb and across the barrier, presumably with higher cost, to obtain a lager amount of reward.   In contrast, there was no barrier (low cost) on an arm entry to receive a smaller amount of reward.   The results show that the normal rat chooses the HH arm more than the LL arm, indicating that the subject is willing to pay more effort in order to get more benefit.   In contrast, choice response would be shifted to LL arm in the rat with lesions of the nucleus accumbens (Salamone, Correa, Farrar, & Mingote, 2007) and medial prefrontal cortex (Walton, Bannerman, Alterescu, & Rushworth, 2003).   These data indicate that the rat would not pay more effort to get more benefit under the impairment of midbrain dopamine system.

Probabilistic risky choice behavior

The probabilistic risky choice behavior, as aforementioned, is gaining more and more attention in the field of decision making.   According to Schultz et al. (2008), the definition of risk is the degree of second moment of the probability distribution over possible outcomes.   Risk and ambiguity are two forms of uncertainty.   The difference between these two terms is up to whether the probability is known or not. The probability is known in the risk form, whereas that is not for ambiguity.   While subjects facing risky choice task, the probability will be an important variable that may potentially affect the decision.   The effects of different probabilities on

individual decision making are recently reported in human gambling task and animal study.

Risk-based task in human

The Iowa Gambling Task (IGT) (Bechara, Damasio, Damasio, & Anderson, 1994; Bechara, Damasio, Damasio, & Lee, 1999) is one of the crucial studies about human decision making based on risky choice.   In IGT, the subjects were asked for making choices among four card decks A, B, C, and D which were presented on the screen. Decks A and B were defined as "bad deck" which brought large gain and large loss, with a total loss in the long run.   In contrast, decks C and D were "good deck" which brought small gain and small loss, with a total gain at the end.   In the beginning of IGT, subjects chose "bad deck" more frequent because of the immediately large reward outcomes.   However, the subjects gradually shifted to choose "good deck" after several trials.   That the subject makes decision, in the long run, would intend to avoid the loss indicate a perception of risk existed and a nature of risk avoidance.

The animal experiments related to probabilistic risky choice issues

The aforementioned IGT studies raise many intriguing issues about probabilistic risky choice behavior.   van den Bos, Lasthuis, den Heijer, van der Harst, and Spruijt (2006) developed a rodent model of IGT.   In modifying from human IGT, the monetary gain was substituted by sugar pellets and monetary loss was conducted by quinine-treated pellets in this model.   There were also four choice options which divided into two "bad arms" and two "good arms."   In which, every 10 choices in "bad arms" contained a chance of winning big reward (3 sugar pellets) but rest of 9 trials were losses (quinine-treated pellets).   In "good arms," every 10 trials contained 8 trials of small reward (1 sugar pellet) among quinine-treated pellets.   Regardless to the strain, gender or housing condition, the response pattern of rodent performed was similar to that performed by human subjects on IGT.

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Another animal model associated with probabilistic risky choice behavior was established on the basis of a probabilistic discounting task by using the operant chamber (Cardinal & Howes, 2005).   In that study, two levers were set as for two different reward options including a large/uncertain reward lever and a small/certain reward lever.   Pressing the small/certain reward lever would present 1 pellet reward for sure, whereas pressing the large/uncertain reward lever would present 4 pellets reward with specific probabilities.   The probabilities to receive a large reward were manipulated from 100% decreased to 50%, 25%, 12.5%, and 6.25%.   When the probability decreased, the percentage of rat's response of choosing the large/uncertain reward lever decreased and in turn shifting to respond on the small/certain reward lever.   In comparing to the sham lesion controls, the rats with lesions of the core subarea of nucleus accumbens showed a relatively risk-averse pattern by significantly decreasing the responses on the large/uncertain reward lever.

Pharmacological treatments on risk-based tasks

The probability discounting task in the rat developed by Cardinal and Howes (2005) led subsequent studies with psychopharmacological approach to investigate the relationship between neurotransmitter and risky choice behavior.   St Onge and Floresco (2008) modified an operant chamber with two levers set for evaluating the probabilistic discounting task.   After initial magazine training, different groups of rats received either dopamine agonists or antagonists via intraperitoneal injection. Nine different dopamine related drugs were tested, including dopamine general agonist amphetamine and specific dopamine receptors (D_1, D_2, D_3, D_4) agonists and antagonists.   The results indicate that the injections of the dopamine D_1 and D_2 agonist increased the percentage of choosing the large/risky reward lever.   However, the injection of dopamine D_3 agonist decreased the percentage of choosing the large/risky reward lever and shifted toward the small/certain reward lever.

Conversely, neither D_4 agonist nor D_4 antagonist produced any significant effect on this task. Thus, these pharmacological data show that different dopamine subtype receptors may be involved in the risky choice behavior.

In considering that the probabilistic risky choice behavior in the subject of drug addiction different from that of normal subject, Floresco and Whelan (2009) examined the effects of repeated amphetamine treatment on a probabilistic and an effort discounting task. The subject was presumably developed a drug induced sensitization by 15 intraperitoneal injections of amphetamine every 2 days with doses increased from 1 mg/kg to 5 mg/kg after every third injection. The results show that this repeated amphetamine administration increases risky choice. However, this amphetamine treatment did not alter effort-based decision making on the effort discounting task. It is thus suggested that the sub-chronic administration of amphetamine impairs decision making based on balancing the risk and reward. It is worthy to further examine what neural mechanisms potentially underlie this behavioral change after the aforementioned drug treatment given by systemic injection.

Neurophysiological approaches applied in risk-base tasks

In addition to pharmacological approach, Fiorillo et al. (2003) used electrophysiological recording for directly investigating the relationships between dopamine neurons and probabilistic risky choice behavior. The electrodes were implanted in Rhesus monkeys' midbrain dopamine related areas A8, A9, and A10. In each trial, subjects were presented by different pictures on the screen as conditioned stimuli. Each picture was correspondingly represented with a specific probability (100%, 75%, 50%, 25%, or 0%) to obtain the reinforcer. In the condition that the monkeys obtain reward under a relatively lower probability (25%), the increased firing responses of dopamine neurons occurred at the reward delivery time

point.   Another interesting result was observed when monkeys obtained reward as presented by relatively higher probability (75% or 100%).   In which, the firing of dopamine neurons increased but with a less magnitude than that elicited in the condition of 25%.   In addition, the peak of these neuronal firings located closely to the time point of the onset of conditioned stimulus (the picture) but not the reward delivery.   Together, the degrees of dopamine neuron firing are depended on the predictability of reward presence, which can be determined by the reward probabilities.   This experiment provided direct evidence in supporting that dopamine neurons play an important role in the probabilistic risky choice task.

Brain functions and lesion studies

Based on a hypothesis that different areas of the brain could potentially form a circuit associated with decision making, an increasing number of studies indicate that several brain areas including the prefrontal cortex (PFC), the orbitofrontal cortex (OFC), the dorsal striatum have involved in the process of decision making (Balleine, Delgado, & Hikosaka, 2007; Lee, Rushworth, Walton, Watanabe, & Sakagami, 2007; Murray, O'Doherty, & Schoenbaum, 2007).

Based on that the OFC is contained with neurotransmitters of dopamine and serotonin, Walker, Robbins, and Roberts (2009) conducted neurotoxins to induce dopamine or serotonin depletions in the marmosets' OFC area.   The marmosets were trained to choose one of two stimuli on the touch sensitive screen for reward.   After initial training, subject underwent Pavlovian training for learning an association between the stimuli (picture) and conditioned stimuli (sound).   Then, an extinction task was conducted.   The results show that the subjects with dopamine, but not serotonin, depletion in the OFC kept responding in the extinction phase.   These data imply that reinforcement function have been impaired after the neurotoxin induced dopamine depletion.   Walker et al. (2009) suggested that the OFC is critical for

representing reward value and is also necessarily for learning and updating information.

Further, some other brain areas are suggested to link with the function of probabilistic risky choice behavior, including anterior cingulated cortex and OFC (Mobini, Body, Ho, Bradshaw, Szabadi, Deakin, & Anderson, 2002; Walton, Kennerley, Bannerman, Phillips, & Rushworth, 2006). Moreover, as mentioned earlier, the nucleus accumbens core also played an important role on probabilistic risky choice behavior (Cardinal & Howes, 2005). For example, when subjects making decisions on large/uncertain reward or small/certain reward, the rat with lesion of nucleus accumbens core chose small/certain reward more likely and showed relatively risk-averse attitude as compared to sham lesion group. As for the OFC, Mobini et al. (2002) tested the lesion effects of the OFC on both delayed and probabilistic reinforcement conditions. In the probabilistic task, one of the levers presented one reward pellet with 100% probability, while the other lever presented two reward pellets with specific probabilities (88%, 72%, 48%, 32%, 20%, and 8%) according to each phase. The results show that the rat with OFC lesion declined their preference for the larger but less probable reinforcer.

With a modification on the task of Cardinal and Howes (2005), St. Onge and Floresco (2009) investigated the role of the PFC on the probabilistic risky choice. The microinfusion of overdosed GABA agonist was used to directly inactivated rats' medial PFC, OFC and anterior cingulate respectively. The results show that the rat with medial PFC inactivation increased the choices toward the large/risky lever. However, the OFC and anterior cingulated inactivation had no effect on choice behavior but the response latencies did longer than control. Based on these findings, the medial PFC is thought to play an important role in mediating risk-based decision.

EV as a key variable in probabilistic risky choice task

The probability to present reward and the reward magnitudes are two variables concerned by the subject in the probabilistic discounting task. To choose, both probability and magnitude are crucial information for the decision making. Following the EV being defined as a summation of each reward probability multiple with its reward magnitude, it is important to investigate the neural mechanism underlying the processes relevant to EV during decision making.

Recently, a few studies using human subjects to examine how the EV may alter in risk related decision making. By using electroencephalography (EEG) technique, Polezzi, Sartori, Rumiati, Vidotto and Daum (2010) recorded the EEG changes when human subjects making decisions between zero EV and positive EV of 2.5. In the results, there was not significant difference between positive EV options and zero EV options on the EEG components of P300 wave and feedback related negativity (FRN). However, by using specific techniques to analyzed electrical sources, the results indicate that the midbrain dopamine system, especially striatum, was highly activated while subjects doing this decision making task. Rolls, McCabe, and Redoute (2008) using functional magnetic resonance imaging (fMRI) to study human decision making under different EV's. They aimed to locate the brain areas that represent EV or reward magnitude (RM). With different EV (5, 9, 10 and 30) and RM (0, 10 and 30), the specific brain regions that correlated to EV or RM were found by the brain imaging analysis. The results show that activations of the medial OFC were correlated with both EV and RM, the activations of ventral striatum were correlated with RM but not the EV and the anterior insula were correlated negatively with EV. Moreover, another fMRI study by Tobler, Christopoulos, O'Doherty, Dolan, and Schultz (2009) indicate that the EV signals were risk-attitudes dependent between individuals. Four levels of EV range from 50 to 200 were used, each of them had a

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high- and low- risk variant with the same EV.   The results of fMRI analyses revealed that the EV signals in lateral prefrontal cortex reduced by risk in risk-averse individuals, but increased in the risk-seeking individuals.   Also, Tobler et al. (2009) suggested that the EV and risk were coding in the striatum.   These findings suggest that the dopamine system may also relate to the EV concepts on the human subjects. However, the data from EEG and fMRI are still weak on explanation of the causal effect of the brain and the behavior.

In summary, a growing number of studies in the neuroscience of decision making have started focusing on the issue of the probabilistic risky choice behavior. However, most of the animal model studies focused on investigating or manipulating the reward probabilities but neglected the issue of EV.   The importance of EV on decision making has been highlighted by several studies (Rolls et al., 2008; Polezzi et al., 2010; Schultz, 2010).   That is, the subject making decisions could indeed be influenced by different EV.   Moreover, considerable evidences suggest that the dopaminergic activation encoded EV and responded to the stimulus associated to EV. Thus, the present study emphasized on the manipulations of different EV and investigated the neurobehavioral mechanisms of the rats' probabilistic risky choice behavior.

Rationale

From literature reviewed above, a few studies examined the effects of reward probabilities on risky choice in animal models (Cardinal & Howes, 2005; Mobini et al., 2002; St. Onge & Floresco, 2008, 2009).   These previous studies manipulated the reward probabilities but holding the reward magnitude in constant on each lever, in consequence, the EV changed with these different reward probabilities.   However, the issue of EV has not been manipulated and discussed in these studies.   Following the argument that the EV was related to the desirability of subjects in the decision

making (Rolls et al., 2008; Schultz, 2010), it is worthy to conduct a systemic examination on the EV in the probabilistic risky choice behavior.   Thus, present study manipulated different EV based on the adjustment of the probability to present reward and the reward magnitude.

Through a pilot work (Yang, Lin, & Liao, 2007), the present animal model were constructed by using a T-maze.   One of the arms was designated as probabilistic high reward (PHR) arm and the other one as certain low reward (CLR) arm.   Choosing CLR arm would present 1 pellet reward for sure.   In contrast with CLR arm, higher reward would be found in the PHR arm but with specific probabilities.   Preliminary data showed that the rats shifted their preference from the PHR arm toward the CLR arm while PHR arm's reward probability decreased.   The results confirmed the risk-related decision making in this behavioral task.   Another pilot study of the pharmacological test on the effects of acute amphetamine was conducted in this lab (Lin, Yang, Yen, & Liao, 2008).   The results show that the rat with amphetamine treatment would choose the PHR arm more than that under saline control.

The aforementioned data were collected only from the condition of EV=1 on both CLR and PHR arm.   It is possible that the performance on the probabilistic risky choice behavior can be altered under conditions with different EV.   The present study systemically examined three conditions of EV in 0.5, 1.0, and 2.0 on PHR arm, but EV on CLR arm was kept constant at 1 in all three conditions.   If rats' probabilistic risky choice behavior performance appears differently under three EV conditions, it is then inferred that the subject has basic perception of EV for making choice or decision.

Finally, the present study evaluated the lesion effects of the probabilistic risky choice behavior in order to reveal the neural substrates for this behavior.

Chapter Two: Methods

Subjects

Wistar rats (250-300g) were the subjects of this study, as purchased from BioLASCO Taiwan.　Each rat was housed individually and maintained on a 12h of light/dark cycle.　The temperature was kept constant around 22±2℃ in the animal colony.　During the experimentation, the subjects were maintained in a food deprivation regimen, which were about 85% of their normal weight.　The water was provided in *ad libitum*.　All experiments were regulated by the local animal care committees of National Cheng Chi University.

Apparatus

For testing the probabilistic risky choice, a T-maze was used in this study which was consisted of one start arm (55 cm × 15 cm × 25 cm) and two goal arms (55 cm × 15 cm × 25 cm each).　Chocolate pellets, approximately 0.15 g per pellet, were used as the reward put in the end of the goal arms.

A rectangle box (80 cm × 25 cm × 35 cm) was used in the discrimination test. One end of this rectangle box was separated by an opaque plate (35 cm × 25 cm) into two compartments for baiting different amounts of reward

For measuring the general locomotion, a box (35 cm × 35 cm × 55 cm) with an infrared camera set 150 cm above the central point of box floor was used in the locomotor activity test.

Drugs

The drug used in Experiment 1b was the dopamine general agonist amphetamine. The amphetamine was dissolved in physiological 0.9% saline and protected from light. The drug doses, 0.5 and 1.0 mg/kg are referred to those used in Lin, Yang, Yen, and Liao (2007).

Discrimination test

A simple discrimination test on reward magnitude was conducted in Experiment 2. In a rectangle box, each side compartment was baited either 1 pellet or 2 pellets for every trials. If the rat performs the ability to distinguish the reward magnitude between 1 and 2 pellets, the rat was presumably have the ability to distinguish further differences of the reward magnitude ratios of 1:4 and 1:8 set in the probabilistic risky choice task. The criterion set for each rat to learn this basic discrimination capability was determined by entering the larger reward compartment for successive 10 trials.

Locomotion activity test

To secure the subject not being affected by lesion, the locomotion activity test was conducted in Experiment 2. Each rat was allowed to freely explore the box for 30 minutes. The traveling distance of each rat was measured.

Procedures of Experiment 1a

All the rats were randomly assigned into 3 groups. Each group was corresponding to each EV condition. Before training, there was a one-day habituation session of 15 min. In which, the subject was allowed to freely explore the whole area of T-maze without any reward baited.

In the subsequent training of probabilistic risky choice behavior, one arm of the T-maze was designated as certain low reward (CLR) arm and the other arm was the probabilistic high reward (PHR) arm. In the CLR arm, 1 pellet reward was delivered for sure in every entrance. As for the PHR arm, the reward magnitude and the probability of its delivery were varied in the conditions with different degrees of risk for obtaining the reward. The magnitude of CLR arm versus that of PHR arm was set in three reward ratios including 1:2, 1:4, and 1:8. In the condition of reward ratio 1:2, the reward magnitude of 2 chocolate pellets was presented with 50% of probability in the PHR arm. In the condition of reward ratio 1:4, the reward

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magnitude of 4 chocolate pellets was presented with 25% of probability in the PHR arm. Also, in the condition of reward ratio 1:8, the reward magnitude of 8 chocolate pellets was presented with 12.5% of probability in the PHR arm. Either CLR or PHR arm in any of three reward ratios was under a premise that the EV was kept constantly at 1. In a pilot study, we found that the rats in the reward ratio 1:16 condition performed with a "floor effect." That is, the rats chose the CLR arm mostly and showed a significant aversion of entering the PHR arm. Therefore, to prevent a confounding effect potentially affected by the floor effect, the reward ratio of 1:16 was not proposed to test in this project.

There were totally three reward ratio conditions 1:2, 1:4, and 1:8, the rats were randomly assigned to accomplish all of these three conditions under a Latin square design. Protocols for each reward ratio were divided into 3 phases: forced choice phase, free choice phase, and rest phase. Each reward ratio condition was run in 14 days, and 16 trials per day with an exception of the rest phase. After 3 days of rest phase, next reward ratio was examined continuously. The following illustration shows the behavioral test on probabilistic risky choice maintained in the condition of reward ratio of 1:2.
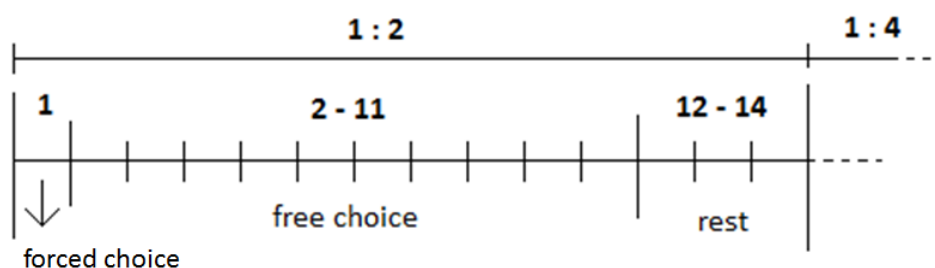
The first phase of each reward ratio condition was the one-day forced choice phase. The purpose of the forced choice phase is to ensure the rat knowing and to distinguish the difference of reward magnitude between both arms. In the forced choice phase, one of the T-maze's arms was blocked by a barrier, so that the rats were

forced to choose the arm without the barrier.   Each rat in the first 8 trials was only

allowed to choose the CLR arm, and subsequent 8 trials set for choosing the PHR arm.

The probability to obtain reward was 100% for every entrance no matter choosing

CLR or PHR arm in this forced choice phase.   It is inferred, the rats would prefer to

go to the PHR arm after the forced choice phase.   Next, in the free choice phase of

10 days, the rat was allowed to freely choose either the CLR or PHR arm.   There

were 16 trials per day in this phase.   For each trial in the free choice phase, the

probability component of obtaining reward was set into the PHR arm only.   After

finishing the free choice phase, there were 3 days of rest phase.   In which, the rat

stayed in its home cage without any training treatment.

The major aim of this experiment was to investigate whether different EV's other

than 1, such as 0.5 or 2, set in the PHR arm would affect this probabilistic risky

choice behavior.   The manipulations of EV were conducted by different reward

probabilities in the PHR arm only.   In contrast, the CLR arm was always keep

constantly presenting 1 pellet for every entrance across all different reward ratio

conditions or different EV conditions.   The following table lists the reward

probability conducted in the PHR arm under these reward ratios of 1:2, 1:4 and 1:8,

across three EV of 0.5, 1 and 2.

Reward probability (PHR arm)		Reward ratio		
		1:2	1:4	1:8
Expected value (set in PHR arm)	0.5	25%	12.5%	6.25%
	1	**50%**	**25%**	**12.5%**
	2	100%	50%	25%

In the condition of EV=0.5 set in PHR arm, the reward probability was reduced

by half to that of the condition of EV=1.   Such that, to keep EV at 0.5, the reward

probabilities were decreased from 50% to 25% with 2 pellets in reward ratio of 1:2 ($25\% \times 2 = 0.5$), from 25% to 12.5% with 4 pellets in reward ratio 1:4 ($12.5\% \times 4 = 0.5$), and from 12.5% to 6.25% with 8 pellets in reward ratio 1:8 ($6.25\% \times 8 = 0.5$). Regarding to condition of EV=2 in PHR arm, the reward probability was doubled as compared to that of the condition of the EV=1.   To keep EV at 2, the probabilities were be increased from 50% to 100% with 2 pellets in reward ratio 1:2 ($100\% \times 2 = 2$), from 25% to 50% with 4 pellets in reward ratio 1:4 ($50\% \times 4 = 2$), and from 12.5% to 25% with 8 pellets in reward ratio 1:8 ($25\% \times 8 = 2$).

In regarding to the experimental design, EV conditions were arranged in the between-subject manner.   That is, three separate groups were assigned for three EV conditions.   Each group of rats was engaged only one EV condition.

Procedures of Experiment 1b

This experiment was to test the effects of dopamine general agonist amphetamine on the probabilistic risky choice behavior.

All the rats used in Experiment 1a were continuously subjected to the Experiment 1b.   There were also three EV conditions (0.5, 1, and 2), the assignment of three groups of rats into specific EV condition was the same in the Experiment 1a. For example, the rat that experienced EV=0.5 condition in Experiment 1a was continuously entering the same EV=0.5 condition in the Experiment 1b.

The protocols of probabilistic risky choice behavior with three reward ratios (1:2, 1:4, and 1:8) conducted in Experiment 1b were similar to those used in Experiment 1a. But, each of the reward ratios contained an additional drug treatment phase of four days.   The following illustration shows the drug treatment on probabilistic risky choice behavior maintained in the condition of reward ratio of 1:2.
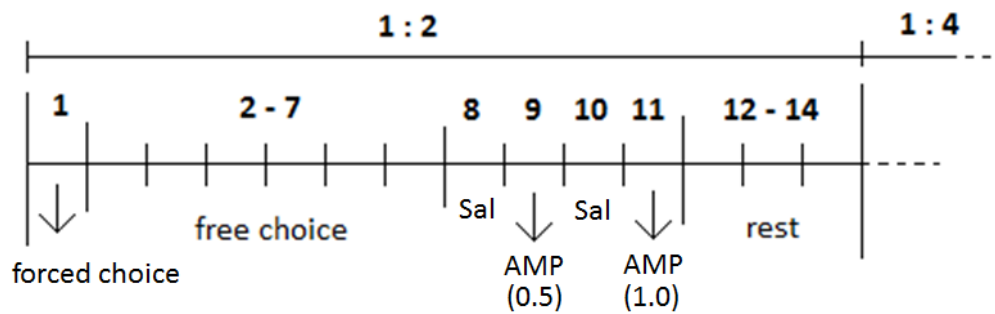
The drug treatment phase was starts from day 8 to 11 (also from day 7 to 10 of free choice). The rat were intraperitoneally injected by dosing with the order of saline, amphetamine 0.5 mg/kg, saline, and amphetamine 1.0 mg/kg over four days. Every drug administration was given in 15 minutes before behavioral test. The injection volume was kept in consistently in 1 ml/kg of body weight.

In completing the drug test of the first reward ratio condition, the other two reward ratio conditions were subsequently preceded. In other words, every subject assigned in a specific condition of certain EV was received drug tests across all three reward ratio conditions in Experiment 1b.

Procedures of Experiment 2

The aim of this experiment was to investigate the neural substrates of the probabilistic risky choice behavior by the use of excitotoxic lesion technique. The main lesion sites in this experiment were nucleus accumbens (NAC) and orbitofrontal cortex (OFC). In considering to the heterogeneity of anatomy and function in the subareas of striatum and prefrontal cortex, the dorsolateral striatum (DLS) and medial prefrontal cortex (mPFC) were targeted as anatomical control for NAC and OFC respectively.

Surgery: Subjects were provided food and water *ad libitum* and subjected to surgery when their body weight stabilized at about 280-300 g. Each rat was

anaesthetized by intraperitoneal injection of Zoletil 50 (Virbac, Carros, France) in a volume of 1 ml/kg.   After anesthesia, the rat was positioned in a stereotaxic apparatus (DKI-900) and drilled with two small holes in the skull over each hemisphere for microinjection of the ibotenic acid (Tocris, USA) into one of the following brain targets: (1) NAC: [AP] = +1.2 mm, [ML] = ±2.0 mm, [DV] = -7.1 mm; (2) DLS: [AP] = +0.7 mm, [ML] = ±3.6 mm, [DV] = -5.0 mm; (3) OFC: [AP] = +3.9 mm, [ML] = ±2.6 mm, [DV] = -2.9 mm; (4) mPFC: [AP] = +3.4 mm, [ML] = ±0.7 mm, [DV] = -2.8 mm.   Injection was given via the 23-gage stainless steel cannula (Shineteh, Taipei, Taiwan) which connected by a polyethylene PE20 tube (Plastics One, Roanoke, VA, USA) by using a 2.0 µL Hamilton syringe (Hamilton, NV, USA).

In the lesion group, 0.5 µL of ibotenic acid was injected into lesion site at a rate of 0.1 µL per 15 sec.   The cannula was left in position for 3 minutes after the completion of the injection in each site.   In the case of the sham lesion group, the surgery procedure was identical except for the vehicle infused only.

After surgery, each rat was subjected to a 7-day recovery.   During which, the rat was allowed to freely access to food and water.   The rat was gradually resumed to the food deprivation regimen before the behavioral tests.

In terms of behavioral task applied in Experiment 2, only the condition of EV=1 equally set in both CLR and PHR arm was conducted.   This behavioral protocol was referred by the results obtained from Experiments 1a and Experiment 1b.   In brief, the conditions of EV=0.5 and EV=2 set in PHR arm produced a "floor effect" and a "ceiling effect" respectively on rats' choice performance (See the results of Experiment 1a and Experiment 1b for more details).   Naive subjects were used in this experiment.   There were totally eight groups of rats (n = 9 in each group).   Four of the groups were the lesion groups: NAC, OFC, DLS, and mPFC.   And, the other

four groups were the sham control groups in corresponding to each of four brain lesion groups.

The behavioral procedures were similar to the Experiment 1a, which also include three reward ratios and each of them has three phases.   The details of experimental procedures to test the probabilistic risky choice behavior are the same as those described in Experiment 1a.

Histology

After behavioral measure finished in Experiment 2, all the subjects were sacrificed by overdose of chloralhydrat (Ferak, Berlin, Germany) and perfused intracardially with normal saline followed by 24% formalin.   The brain was removed and placed in a sucrose/formalin mixture for at least 48 hours.   The brain was sectioned at 40 μm with a freezing microtome and mounted on polysine slides (Menzel-Glaser, Berlin, Germany) and stained with cresyl violet for further histological assessment.

Data analysis

All data were analyzed by analysis of variance (ANOVA) with appropriate experimental designs.   The *post hoc* comparison test was conducted when appropriate.   All the tests with statistical significance were determined by a criterion of $p < 0.05$.

Chapter Three: Results

Experiment 1a: Probabilistic risky choice in different EV conditions

The results of Experiment 1a are presented in Figure1, Figure2, and Figure 3, respectively covering the data collected when the EV set on 1, 0.5, and 2 on the PHR arm. In Figure 1, the results of a two-way ANOVA showed a significant main effect of reward ratio ($F(2, 10) = 14.613$, $p < 0.01$) and a significant reward-ratio-by-day interaction ($F(18, 90) = 2.679$, $p < 0.01$). A test of simple main effect revealed a significant difference of reward ratio factor on the last nine days (from day 2 to 9, in the orders of $F(2, 4) = 8.133$, 31.778, 21.128, 28.299, 29.000, 22.991, 31.452, 30.117, and 45.408, $p < 0.05$). Further *post hoc* comparisons revealed that the percentage of choosing PHR significantly higher in reward ratio 1:2 than reward ratio 1:4 from day 2 to 10 ($p < 0.05$) and reward ratio 1:8 from day 2 to 10 ($p < 0.05$) but no difference between reward 1:4 and 1:8 in each of all nine days ($p > 0.05$).

Figure 1

In Figure 2, the results of ANOVA showed a significant main effect of reward ratio ($F(2, 10) = 6.988$, $p < 0.05$), a significant main effect of day ($F(9, 45) = 35.66$, $p < 0.001$), and a significant reward-ratio-by-day interaction ($F(18, 90) = 3.592$, $p < 0.001$). A test of simple main effect revealed a significant difference of reward ratio factor only on the second day ($F(2, 4) = 7.484$, $p < 0.05$). Further *post hoc* comparisons revealed the percentage of choosing PHR significantly higher in reward ratio 1:4 than reward ratio 1:2 ($p < 0.05$) and significantly higher in reward ratio 1:8 than reward 1:2 ($p < 0.05$).

Figure 2

In Figure 3, the results of ANOVA showed a significant main effect of reward ratio ($F(2, 10) = 8.406$, $p < 0.01$) and a significant main effect of day ($F(9, 45) = 21.836$, $p < 0.001$).   *Post hoc* comparisons of reward ratio factor revealed the percentage of choosing PHR significantly higher in reward ratio 1:2 than reward ratio 1:8 ($p < 0.01$) but no differences between reward ratio 1:2 and 1:4 neither reward ratio 1:4 and 1:8 ($p > 0.05$).   And *post hoc* comparisons of day factor revealed the percentage of choosing PHR significant lower in day 1 than day 2 to 10 ($p < 0.01$), day 2 lower than day 4 to 10 ($p < 0.01$), and day 3 lower than day 4 and day 5 ($p < 0.05$).

Figure 3

Figure 4 shows the mean values of choosing PHR over the last five days of free choice phase on each of three reward ratios.   The results of a two-way ANOVA revealed a significant main effect of EV ($F(2, 10) = 77.406$, $p < 0.001$), a significant main effect of reward ratio ($F(2, 10) = 15.406$, $p < 0.01$), and a significant EV-by-reward-ratio interaction ($F(4, 20) = 9.214$, $p < 0.001$).   A test of simple main effect revealed a significant difference of EV factor on reward ratio 1:2 ($F(2, 4) = 2688.648$, $p < 0.001$) and 1:4 ($F(2, 4) = 1607.175$, $p < 0.001$) and a significant difference of reward ratio on EV=1 ($F(2, 4) = 35.664$, $p < 0.01$).   Further *post hoc* comparisons of EV factor revealed that in reward ratio 1:2, the percentage of choosing

PHR significant higher in EV=2 than EV=0.5 ($p < 0.001$) and EV=1 higher than

EV=0.5 ($p < 0.001$); and in reward ratio 1:4, EV=2 was significantly higher than

EV=1 ($p < 0.01$) and EV= 0.5 ($p < 0.001$).   The *post hoc* comparisons of reward

ratio factor revealed that in EV=1, the percentage of choosing PHR significantly

higher in reward ratio 1:2 than reward ratio 1:4 ($p < 0.01$) and 1:8 ($p < 0.01$).

Figure 4

Experiment 1b: Effects of amphetamine on probabilistic risky choice behavior

The results of Experiment 1b are presented, based on the condition of EV set in

PHR arm as 1, 0.5 and 2, in Figure5, Figure 6, and Figure 7 respectively.   In each of

these three figures, only the data collected in the last four days are relevant to the drug

treatment.

Figure 5

Figure 6

Figure 7

The effect of amphetamine on the present probabilistic risky choice behavior is shown in Figure 8.　For the condition of EV=1 set in both PHR and CLR arm, as shown in the top panel of Figure 8, the main effects of dose and reward ratio, $F(2, 10) = 5.798$ and $F(2, 10) = 6.883$ respectively, are significant ($p < 0.05$).　*Post hoc* comparisons of main effect of dose revealed that the higher dose of amphetamine (1 mg/kg) significantly increased the percentage of choosing PHR compared to saline treatment ($p < 0.01$).　Notably, the effect of lower dose amphetamine (0.5 mg/kg) was only marginal significantly increase the percentage of choosing PHR compared to saline control group ($p = 0.051$).　The *post hoc* comparisons of main effect of reward ratio revealed the percentage of choosing PHR significantly higher in reward ratio 1:2 than reward ratio 1:4 ($p < 0.01$) and 1:8 ($p < 0.01$).

Figure 8

The data analyses for those shown in the intermediate panel, as revealed by a two-way ANOVA, neither the main effect of dose nor the main effect of reward ratio were significant ($p > 0.05$).　Also, the test of EV-by-reward-ratio interaction was not significant ($p > 0.05$).　For the data presented in the bottom panel of Figure 8, none of the tests of two-way ANOVA was significantly detected ($p > 0.05$, see Table 1 of Appendix for the details).

Table 1

Experiment 2: Effects of lesion manipulations on probabilistic risky choice

Histology

The results of histological analyses of bilateral lesions in NAC, DLS, OFC, and mPFC were presented in Figure 9, Figure 10, Figure 11, and Figure 12 respectively. These rats showed extensive cell collapse and gliosis in the location of the lesion sites.

In Figure 9, the diagram shows the histological examination of NAC.   The top panel shows the diagram of the extension of NAC lesions (n = 9).   The black area represents the most intensive of lesions area made for all the subjects, whereas the grey area represents the maximum lesion area.   The sketch diagram was adopted from the Figure 23 of the rat brain atlas by Paxinos and Watson (2007).   Bottom panel shows the representative photographs of coronal sections, and the red circle remarked areas highlight the location of NAC lesions (right photo) and sham lesion (left photo) respectively.   The areas of damage in the ventral part of striatum and did not extended to the lateral ventricle.

Figure 9

In Figure 10, the diagram shows the histological examination of DLS.   The top panel shows the diagram of the extension of DLS lesions (n = 9).   The black area represents the most intensive of lesions area made for all the subjects, whereas the grey area represents the maximum lesion area.   The sketch diagram was adopted from the Figure 27 of the rat brain atlas by Paxinos and Watson (2007).   Bottom panel shows the representative photographs of coronal sections, and the red circle

remarked areas highlight the location of DLS lesions (right photo) and sham lesion (left photo) respectively.

Figure 10

In Figure 11, the diagram shows the histological examination of OFC.   The top panel shows the diagram of the extension of OFC lesions (n = 9).   The black area represents the most intensive of lesions area made for all the subjects, whereas the grey area represents the maximum lesion area.   The sketch diagram was adopted from the Figure 9 of the rat brain atlas by Paxinos and Watson (2007).   Bottom panel shows the representative photographs of coronal sections, and the red circle remarked areas highlight the location of OFC lesions (right photo) and sham lesion (left photo) respectively.

Figure 11

In Figure 12, the diagram shows the histological examination of mPFC.   The top panel shows the diagram of the extension of mPFC lesions (n = 9).   The black area represents the most intensive of lesions area made for all the subjects, whereas the grey area represents the maximum lesion area.   The sketch diagram was adopted from the Figure 10 of the rat brain atlas by Paxinos and Watson (2007).   Bottom panel shows the representative photographs of coronal sections, and the red circle remarked areas highlight the location of NAC lesions (right photo) and sham lesion (left photo) respectively.

Figure 12

Effects of NAC lesion

The results of post-lesion tests on locomotor activity and discrimination test are shown in Figure 13.　In the top panel of Figure 13, the locomotor activity of the NAC lesion group was significantly higher than that of the sham lesion control group ($t(11) = 2.94$, $p < 0.05$).　As the intermediate panel and bottom panel of Figure 13 shows, the results of independent t-test applied on the two measures of the discrimination task showed no significant difference between the NAC lesion and the sham control groups ($p > 0.05$).

Figure 13

Figure 14 shows the effects of NAC lesion on probabilistic risky choice behavior. The results of a three-way ANOVA revealed a significant main effect of lesion ($F(1, 16) = 8.954$, $p < 0.01$), a significant main effect of reward ratio ($F(2, 32) = 20.568$, $p < 0.001$), and a significant main effect of day ($F(9, 144) = 28.026$, $p < 0.001$).　Also, for the two-way interaction tests, there was a significant reward-ratio-by-day interaction ($F(18, 288) = 10.023$, $p < 0.001$) and a significant lesion-by-day interaction ($F(9, 144) = 2.441$, $p < 0.05$).　However, the lesion-by-reward-ratio-by-day interaction was not significant ($F(18, 288) = 1.242$, $p > 0.05$).　In Figure 15, the tests of simple main effect of lesion-by-day interaction revealed significantly difference in lesion factor, further *post hoc* revealed a decrease

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in NAC lesion group on day 4, day 5, day 7, day 8, day 9, and day 10 ($p < 0.05$).

The of day factor in lesion-by-day interaction revealed significant difference in NAC lesion group ($F(9, 8) = 5.701$, $p < 0.05$).　Further *post hoc* comparisons revealed that the percentage of choosing PHR significantly higher in day 1 than day 2 to day 9 ($p < 0.05$), day 2 higher than day 3 to day 10 ($p < 0.001$), day 3 higher than day 4 to day 10 ($p < 0.01$), day 4 higher than day 7 to day 8 ($p < 0.05$), day 5 higher than day 7 to day 8 ($p < 0.05$), day 6 higher than day 8 and day 9 ($p < 0.05$), and day 7 higher than day 10 ($p < 0.05$).　Also, a test of simple main effect of reward-ratio-by-day interaction revealed a significant difference of reward ratio factor on each of all ten days (in the orders of $F(2, 15) = 12.462$, 6.051, 14.865, 10.875, 14.092, 14.042, 21.581, 19.108, 14.510, and 27.064, $p < 0.05$) and a significant difference of day factor on reward ratio 1:4 ($F(9, 8) = 5.364$, $p < 0.05$) and 1:8 ($F(9, 8) = 44.287$, $p < 0.001$).　Further *post hoc* comparisons of reward ratio factor revealed the percentage of choosing PHR was significantly higher in reward ratio 1:2 than reward ratio 1:4 from day 2 to 10 ($p < 0.05$) and higher than reward ratio 1:8 in the first 2 days ($p < 0.05$).　Also, reward ratio 1:8 was significantly higher than reward ratio 1:4 in the first 2 days ($p < 0.05$). The *post hoc* comparisons of day factor revealed that in reward ratio 1:4, the percentage of choosing PHR significantly higher in day 1 than other 9 days ($p < 0.05$), day 2 higher than day 3 and day 5 to 10 ($p < 0.05$), day 3 higher than day 10 ($p < 0.05$), day 5 higher than day 10 ($p < 0.05$), day 6 higher than day 8 ($p < 0.05$), day 7 higher than day 8 and day 10 ($p < 0.05$), and day 9 higher than day 10 ($p < 0.05$). And, in reward ratio 1:8, the percentage of choosing PHR significantly higher in day 1 than day 2 to 10 ($p < 0.001$), day 2 higher than day 3 to 10 ($p < 0.05$), day 4 higher than day 5 to 10, ($p < 0.05$), day 5 higher than day 7 and day 10 ($p < 0.05$), and day 8 higher than day 10 ($p < 0.05$).

Figure 14

Figure 15

Figure 16 shows the effects of NAC lesion on probabilistic risky choice behavior in the first 3 days, the intermediate 4 days, and the last 3 days of ten-day free choice test.   The main effect of reward ratio was significant in all three blocks ($p < 0.01$) and would not be mentioned below.   In the first 3 days (top panel), there was no significant main effect of lesion ($F(1, 16) = 0.2$, $p > 0.05$) nor significant lesion-by-reward-ratio interaction ($F(2, 32) = 0.34$, $p > 0.05$).   The results of a two-way ANOVA revealed a significant main effect of lesion ($F(1, 16) = 8.792$, $p < 0.01$) in the intermediate 4 days and a significant main effect of lesion ($F(1, 16) = 6.939$, $p < 0.05$) in the last 3 days (See Table 2 of Appendix for the details).

Figure 16

Table 2

Effects of DLS lesion

The effects of DLS lesion on locomotor activity and discrimination test are shown in Figure 17.   In the top panel of Figure 17, there were no differences on

locomotor activity between DLS lesion group and sham lesion group ($t(10) = 0.349$, $p > 0.05$).   Also, as lower two panels of Figure 17, there were no differences on the two measures of the discrimination between two groups ($p > 0.05$).

Figure 17

Figure 18 shows the effects of DLS lesion on probabilistic risky choice behavior. The results of a three-way ANOVA revealed a significant main effect of reward ratio ($F(2, 32) = 24.687$, $p < 0.001$), and a significant main effect of day ($F(9, 144) = 18.684$, $p < 0.001$).   Also, for the two-way interaction tests, there was a significant reward-ratio-by-day interaction ($F(18, 288) = 18.487$, $p < 0.001$).   However, the lesion-by-reward-ratio-by-day interaction was not significant ($F(18, 288) = 0.904$, $p > 0.05$).   A test of simple main effect of reward-ratio-by-day interaction revealed a significant difference of reward ratio factor on each of last nine days (from day 2 to 9, in the orders of $F(2, 15) = 9.369$, 15.947, 12.846, 21.426, 25.833, 45.589, 51.143, 65.990, and 71.946, $p < 0.01$) and significant difference of day factor on reward ratio 1:4 ($F(9, 8) = 3.988$, $p < 0.05$) and 1:8 ($F(9, 8) = 8.093$, $p < 0.01$).   Further *post hoc* comparisons of reward ratio factor revealed the percentage of choosing PHR was significantly higher in reward ratio 1:2 than reward ratio 1:4 from day 2 to 10 ($p < 0.05$) and higher than reward ratio 1:8 from day 3 to 10 ($p < 0.05$).   Also, reward ratio 1:8 was significantly higher than reward ratio 1:4 in the first 2 days ($p < 0.05$). The *post hoc* comparisons of day factor revealed that in reward ratio 1:4, the percentage of choosing PHR significantly higher in day 1 than other 9 days ($p < 0.05$), day 2 higher than day 3 and day 5 to 10 ($p < 0.05$), day 3 higher than day 4 ($p < 0.05$),

day 6 higher than day 8 ($p < 0.05$), and day 7 higher than day 8 ($p < 0.05$).   And, in reward ratio 1:8, the percentage of choosing PHR significantly higher in day 1 than day 2 to 10 ($p < 0.05$), day 2 higher than day 3 to 10 ($p < 0.001$), day 3 higher than day 5 to 10, ($p < 0.01$), day 4 higher than day 6 to 10 ($p < 0.05$), day 5 higher than day 7 and day 10 ($p < 0.05$), day 6 higher than day 7 to 10 ($p < 0.05$), and day 7 higher than day 10 ($p < 0.05$).

Figure 18

Figure 19 shows the effects of DLS lesion on probabilistic risky choice behavior in the three blocks of the first 3 days, the intermediate 4 days, and the last 3 days of ten-day free choice test.   Only the main effect of reward ratio was significant in all three blocks ($p < 0.01$).   None of the lesion effect or the lesion-by-reward-ratio interaction was significant in all three blocks ($p > 0.05$, see Table 3 of Appendix for the details).

Figure 19

Table 3

Effects of OFC lesion

The effects of OFC lesion on locomotor activity and discrimination test are shown in Figure 20.   In the top panel of Figure 20, the results of independent t-test

revealed a significant increase on locomotor activity in OFC lesion group than that of sham lesion group ($t(16) = 2.827$, $p < 0.05$).   As for the discrimination ability, as the lower two panels of Figure 20, no differences on both of two measures of the discrimination between two groups were detected ($p > 0.05$).

Figure 20

Figure 21 shows the effects of OFC lesion on probabilistic risky choice behavior. The results of a three-way ANOVA revealed a significant main effect of lesion ($F(1, 16) = 9.725$, $p < 0.01$), a significant main effect of reward ratio ($F(2, 32) = 17.831$, $p < 0.001$), and a significant main effect of day ($F(9, 144) = 10.257$, $p < 0.001$).   Also, for the two-way interaction tests, there was only a significant reward-ratio-by-day interaction ($F(18, 288) = 8.437$, $p < 0.001$).   The lesion-by-reward-ratio-by-day interaction was not significant ($F(18, 288) = 1.322$, $p > 0.05$).   A test of simple main effect of reward-ratio-by-day interaction revealed a significant difference of reward ratio factor on last nine days (from day 2 to 9, in the orders of $F(2, 15) = 6.952$, 11.594, 9.539, 10.971, 16.946, 19.050, 26.919, 49.429, and 34.195, $p < 0.01$) and significant difference of day factor in reward ratio 1:8 ($F(9, 8) = 6.042$, $p < 0.01$). Further *post hoc* comparisons of reward ratio factor revealed the percentage of choosing PHR was significantly higher in reward ratio 1:2 than reward ratio 1:4 from day 2 to 10 ($p < 0.05$) and higher than reward ratio 1:8 from day 4 to 10 ($p < 0.05$). Also, reward ratio 1:8 was significantly higher than reward ratio 1:4 in day 2 and day 3 ($p < 0.05$).   The *post hoc* comparisons of day factor revealed that in reward ratio 1:8, the percentage of choosing PHR significantly higher in day 1 than day 2 to 10 (p

< 0.05), day 2 higher than day 3 to 10 ($p < 0.05$), day 3 higher than day 5 to 10 ($p < 0.05$), day 4 higher than day 7 to 10 ($p < 0.05$), day 5 higher than day 8 to 10 ($p < 0.05$), day 6 higher than day 7 to 10 ($p < 0.05$), day 7 higher than day 8 to 10 ($p < 0.05$), and day 8 higher than day 10 ($p < 0.05$).

Figure 21

Figure 22 shows the effects of OFC lesion on probabilistic risky choice behavior in the three blocks of the first 3 days, the intermediate 4 days, and the last 3 days of ten-day free choice test.　The main effect of reward ratio was significant in all three blocks ($p < 0.01$).　The results of two-way ANOVA revealed only a significant main effect of lesion in the intermediate 4 days ($F(1, 16) = 5.592, p < 0.05$).　None of two-way interaction was significant ($p > 0.05$, see Table 4 of Appendix for the details).

Figure 22

Table 4

Effects of mPFC lesion

In the sham lesion group, one rat died during surgery, and the other one rat was excluded from analyses of probabilistic risky choice behavior due to persistent of choosing left arm of the T-maze (> 93%) across all three reward ratios.

Figure 23 shows the effects of mPFC lesion on locomotor activity and discrimination test.　　In the top panel of Figure 23, there were no difference on locomotor activity between mPFC lesion group and sham lesion group ($t(15) = 0.539$, $p > 0.05$).　　Also, as lower two panels of Figure 23, there were no differences on both of two measures of the discrimination between two groups ($p > 0.05$).

Figure 23

Figure 24 shows the effects of mPFC lesion on probabilistic risky choice behavior.　　The results of a three-way ANOVA revealed a significant main effect of reward ratio ($F(2, 28) = 7.331$, $p < 0.01$), and a significant main effect of day ($F(9, 126) = 8.055$, $p < 0.001$).　　Also, for the two-way interaction tests, there was a significant reward-ratio-by-day interaction ($F(18, 252) = 13.656$, $p < 0.001$). However, the lesion-by-reward-ratio-by-day interaction was not significant ($F(18, 252) = 1.062$, $p > 0.05$).　　A test of simple main effect of reward-ratio-by-day interaction revealed a significant difference of reward ratio factor on day 1 and day 4 to 10 ($F(2, 15) = 4.001$ in day 1; from day 4 to 10, in the orders of $F(2, 15) = 6.254, 7.842, 7.386, 13.158, 17.914, 37.756$, and 27.067, $p < 0.05$) and significant difference of day factor in reward ratio 1:8 ($F(9, 6) = 37.610$, $p < 0.001$).　　Further *post hoc* comparisons of reward ratio factor revealed the percentage of choosing PHR was significantly higher in reward ratio 1:2 than reward ratio 1:4 from day 4 to 10 ($p < 0.05$) and higher than reward ratio 1:8 in day 1 and day 4 to 10 ($p < 0.05$).　　But there was no significant difference between reward ratio 1:4 and 1:8 in day 1 and day 4 to 10 ($p > 0.05$).　　The *post hoc* comparisons of day factor revealed that in reward ratio 1:8, the percentage of

choosing PHR significantly higher in day 1 than day 2 to 10 ($p < 0.05$), day 2 higher than day 3 to 10 ($p < 0.05$), day 3 higher than day 4 to 10 ($p < 0.01$), day 4 higher than day 7 to 10 ($p < 0.05$), day 5 higher than day 7 to 10 ($p < 0.01$), day 6 higher than day 7 to 10 ($p < 0.05$), day 7 higher than day 10 ($p < 0.05$), and day 9 higher than day 10 ($p < 0.05$).

Figure 24

Figure 25 shows the effects of mPFC lesion on probabilistic risky choice behavior in the three blocks of the first 3 days, the intermediate 4 days, and the last 3 days of ten-day free choice test.   In the first 3 days, a two-way ANOVA revealed only a significant lesion-by-reward-ratio interaction ($F(2, 28) = 3.755$, $p < 0.05$).   In the intermediate 4 days and last 3 days, the main effect of reward ratio was significant, $F(2, 28) = 11.509$, $F(2, 28) = 17.693$ respectively ($p < 0.001$, see Table 5 of Appendix for the details).

Figure 25

Table 5

Chapter Four: Discussion

In this present study, by the establishment of a probabilistic risky choice model in the rat, the neural substrates of risk-based decision making were systemically investigated by the use of excitotoxic lesion technique.

The results of Experiment 1a show that the manipulations of different EV's set in CLR and PHR arm significantly affected the probabilistic risky choice made by the rat. When two chosen options had the same EV = 1 set for both CLR and PHR arm, the rat exhibited different patterns of choice behavior following different reward probabilities and magnitudes under each reward ratio. In addition, given in options with different EV's (EV 0.5 vs. EV 1 or EV 2 vs. EV 1), the subject apparently chose the option which had higher EV. This finding implies that the rat may have a "sense" to process basic EV in this probabilistic risky choice behavior.

The effects of amphetamine tested in Experiment 1b, showed a drug induced relatively risk-seeking choice behavior when the condition of EV set in both CLR and PHR arms at 1. These results provide an evidence to support the role of dopamine system is involved in the present behavioral task of probabilistic risky choice.

In Experiment 2, the results show that the lesion of NAC produced a relatively risk-averse choice behavioral effect. By contrast, the lesion of DLS as an anatomical control, had no such an effect on probabilistic risky choice behavior. These findings indicate the heterogeneity of behavioral function existed between the NAC and DLS on probabilistic risky choice. Regarding to the subarea of prefrontal cortex as manipulated, the OFC lesion produced a tendency of relatively risk-averse choice behavior by the rat showing a marginal significant decrease on the percentage of choosing PHR in the last 3 days of free choice phase. By contrast, lesion of mPFC did not alter the probabilistic risky choice behavior in this study.

Manipulations of different EV conditions on probabilistic risky choice behavior

In the condition of EV=1 set in both CLR and PHR arm, the rat showed different preferences of choices over three different reward ratios. The rat preferred to choose PHR arm in the reward ratio of 1:2 but preferred to choose CLR arm in the reward ratio 1:4 and 1:8. In terms of the risk, the reward ratio 1:2 leads a relatively lower risky condition whereas the reward ratio 1:8 generates a higher risky condition (Tobler, O'Doherty, Dolan, & Schultz, 2007; Tobler et al., 2009). The results indicate that the subject may respond to the different degrees of risk set on the basis of the probabilities of the reward presence. The rat performed in a relatively risk-seeking fashion in the lower risky condition, but became responding toward a more relatively risk-averse manner in the higher risky condition on the present task. Thus, this study demonstrates that the probabilistic risky choice behavior made by the rat can be risk dependent.

Further, to our knowledge, this is the first study emphasizing EV given in animal model of probabilistic risky choice behavior. The results suggested that the rat would choose the option which had relatively higher EV if the EV's were set in different among the chosen options. For instance, in the condition of EV=0.5 set in PHR arm and EV=1 set in CLR arm, the rat significantly preferred to choose CLR arm. Conversely, in the condition of EV=2 set in PHR arm and EV=1 set in CLR arm, the rat significantly preferred to choose PHR arm. These results indicate that the rat may have a cognition-like function to process the EV.

However, whether the rat actually had an internal representation of EV is still a controversial issue. In this present study, a between-subject design was used on the EV factor. The comparisons of internal representation of different EV within a rat are then limited, because of the rat only experienced one of three EV's conditions.

Thus, further investigations by a within-subject design may be helpful to verify the notion of rats' internal EV representation.

As mentioned earlier, Tversky and Kahneman (1979) suggested that people show risk-averse attitude while in a monetary gain condition. In the present study, the rat chose the option of 2-pellet with 50% rather than 1-pellet with 100%, this result implied a relatively risk-seeking choice behavior in reward ratio 1:2 (see Figure 1). Although there are several discrepancies in methodology between animal and human studies, the aforementioned result of the present study is somewhat intriguing. In this specific condition of reward ratio set on 1:2, but not on1:4 or 1:8, the subject may still prefer to take a relative lower risk for obtaining the reward that can reduce its hunger drive. The motivational state for the present animal subject is different from that of the human subject in the test of a monetary gain condition with probabilistic risk. It should also be noted that the risk-averse response was appeared in the reward condition of 1:4 and 1:8 in the present study. In human study, in contrast to the monetary gain condition, risk-averse response appears in the monetary loss condition. Together, it may be an interesting issue to be further investigated with a more sophisticated experimental design.

Linear functions for EV representation

According to Cardinal and Howes (2005), the rat's internal representation of probabilities may be possibly evaluated by "indifference probability." By using a linear regression function, Cardinal and Howes (2005) suggested that the core of NAC-lesioned rats had higher indifference probabilities, the value of 1-pellet is equal to 4-pellet with 70%. Namely, the rat would not choose large/uncertain lever when the probability of obtained 4-pellet was below 70%. Thus, the estimated value was used to infer the rat with NAC lesion showing a relatively risk-averse choice behavior as compared to sham lesion group.

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In this present study, there will be three distinct indifference probabilities if we apply this linear function into three reward ratios. For example, in the reward ratio 1:2, there will be an indifference probability for the rat by regress the 2-pellet with probabilities of 25%, 50%, and 100%. Also, another two indifference probability values would be calculated for reward ratio of 1:4 and 1:8. In addition to reward ratio, the present study manipulated probability to adjust the EV. Thus, it can be a more complex linear function for representing the EV. In which, both reward magnitude and probability would be necessarily included within the regression model. Nevertheless, in considering the internal representation of EV, it may be possibly assessed in current data. While EV=1 was equally set in both CLR and PHR arm, the rat chose PHR arm significantly more than CLR arm in reward ratio 1:2. This result implies that the rat's internal representation of EV toward 2-pellet with 50% probability is higher than 1-pellet. In contrast, the internal EV toward 4-pellet with 25% probability and 8-pellet with 12.5% probability is lower than 1. Based on this implication, further study using linear regression model will be helpful for investigating the rat's internal representation of EV.

The roles of reward probability and reward magnitude within EV

EV is defined as a summation of the probability to obtain each of reward multiple with the reward magnitude as received. It is interesting to figure out which of the components may play the major role on the probabilistic risky choice behavior. In most of previous studies that investigated probabilistic risky choice task, the reward magnitude was kept in constant but changing the probabilities with sessions for the test (Mobini et al., 2002; Cardinal & Howes, 2005; St. Onge & Floresco, 2008, 2009). These results indicated that the rat decreased choices of large but risky lever with the reward probabilities decreased (as the risk increased). In contrast, the results from the tests set by keeping the reward probabilities in constant but changing the reward

magnitudes revealed that the rat chose the to respond for the option containing the larger reward (Cardinal & Howes, 2005; Zeeb, Robbins & Winstanley, 2009).   A separate statistical analysis was conducted to clarify this issue for the current data. As shown in the following table, three thicken frames represent the experimental conditions are characterized by three different reward magnitudes presented in the same reward probability (25%) set in the PHR arm.

Reward probability		Reward ratio					
		1:2		1:4		1:8	
		CLR arm	PHR arm	CLR arm	PHR arm	CLR arm	PHR arm
EV	0.5	**100%**	**25%**	100%	12.5%	100%	6.25%
	1	100%	50%	**100%**	**25%**	100%	12.5%
	2	100%	100%	100%	50%	**100%**	**25%**

Figure 26 shows the results of this assigned condition.   As indicated by a significant main effect on the reward magnitude from a two-way ANOVA ($F_{(2, 15)} = 5.691$, $p < 0.05$), the percentage of choosing PHR was higher given in 8-pellet reward condition than that given in 2-pellet reward.

Figure 26

Together, with all of these results from the present studies and the others, it is still difficult to declare which of the two components within EV plays the key role in this kind of risk-based decision making.   Because of both factors of the probability to

obtain reward and the reward magnitude as received are crucial for making a risky

choice.   Thus, the rat chooses among different EV options are likely based on the

integration of both probabilities and magnitudes of the presented reward (Zeeb et al.,

2009).   This argument is in agreement with the notion that EV is a key factor as

indicated by human fMRI studies showing the subject's making choices under

uncertainty (Schultz et al. 2008; Tobler et al. 2009).

<u>The risk of "get nothing" versus the risk of "punishment"</u>

   The behavioral mechanisms to elucidate the risky choice may be more complex

than what being thought with a certain experimental design used in the animal study

(e.g. Cardinal and Howes, 2005).   In present study, the risk could be regarded as "get

nothing" rather than "loss something."   Namely, only the "gain" domain was

considered in the present task.   However, this type of operational definition may not

be the only way to delineate the risk.   There were several other studies examining the

risk by simultaneously manipulating both the "gain" and the "loss" in the design of

animal behavioral tasks.   As mentioned earlier, in a rodent model of IGT (van den Bos

et al., 2006), the loss were represented for the animal by a bitter-tasted quinine-treated

pellet.   Another behavioral task developed by Simon, Gibert, Mayse, Bizon, and

Setlow (2009) was set up to assess a larger reward but with the risk of punishment

with footshock on the probabilistic discounting task.   The results indicated that the

rat, in a condition with a higher intensity of shock, chose to press more on the small

reward and safe lever than the large reward but risky lever.   Also, with the

probabilities of the punishment increased (ascending from 0%, 25%, 50%, 75%, to

100%), the rat decreased their preference toward large reward lever.   From these

studies, it is suggested that the concept of "gain and loss" can be assessed in animal

models.   And, the risk to obtain a punishment can be addressed as a "loss" and is

indeed with the impact to influence the subject's choice.   Despite this highlight, there

is a further concern in terms of EV to this issue.   Namely, it would then be difficult

to measure the values of punishments contrast to the values of reward.   For example,

as suggested by van den Bos et al. (2006), the starving rat still ate the quinine-treated

pellet.   Also, in Simon et al. (2009), the rat showed a wide individual variation

toward footshocks.

Regardless whether only the gain domain or both the gain and loss being

manipulated, a similarity of behavioral choice pattern is found in between the

aforementioned studies and the present study using a probabilistic risky choice

without presenting any punishment for the "loss."   The rat shifted their preferences

from choosing large reward toward small reward as the probabilities of obtaining

large reward decreased or the probabilities of punishment increased in the large

reward site.   Thus, "get nothing" of the present task is presumably regard as a kind

punishment for the subject, which is true if it is starved under a food-deprived

condition.

Effects of amphetamine on probabilistic risky choice behavior

The effects of amphetamine on probabilistic risky choice behavior were

systemically assessed in Experiment 1b.   In the conditions of EV=0.5 or EV=2 set in

PHR arm, the amphetamine did not affect any behavioral responding on probabilistic

risky choice.   One way to explain the negative results of amphetamine treatment may

be attributed to the "floor effect" and "ceiling effect" derived from the EV set in PHR

arm.   Namely, the risk perception in either condition is too rigid to influence

behavior response on this task.   In the condition of EV=1 equally set in both CLR

and PHR arm, the results indicate that the rat injected with the high dose of

amphetamine treatment (1.0 mg/kg) showed a relatively risk-seeking fashion of

choice behavior.   That is, psychostimulant drug alter the choice behavior showing

relatively more risk-seeking compared to saline control group.

From those reported in a previous study by St. Onge and Floresco (2008), amphetamine induced increases in probabilistic risky choice were significantly higher than saline control group on the reward probabilities of 25% and 12.5%.   Thus, the rat with amphetamine treatment showed relatively risk-seeking only when the risk was relatively higher.   In this present study, we found a similar tendency on reward ratio 1:8 which is a relatively higher risky condition (the top panel of Figure 8). However, based on our results, this notion is limited due to the dose-by-reward-ratio interaction effect was not significantly confirmed.   In this regard, if a further reward ratio 1:16 or a higher dose of amphetamine could be extendedly tested, it would then be able to verify the effects of amphetamine affected risky choice behavior only on higher risky condition.

In addition, a chronic effect of repeated amphetamine treatment on probabilistic risky choice behavior had increased risky choice after drug exposure, whereas these treatments did not affect effort-based decision making (Floresco & Whelan, 2009).

The effects of amphetamine induced relatively risk-seeking on this probabilistic risky choice may attribute to the drug effects on the mesolimbic dopamine systems because amphetamine is a general dopamine agonist in terms of pharmacology (St. Onge & Floresco, 2008).   Another supportive evidence to this notion is that cocaine, as one of the psychostimulants but known as a serotonin transporter blocker, had no effects on this kind of behavior (Simon et al., 2009).   Moreover, the different dopamine subtype receptors were involved in the probabilistic risky choice behavior (St. Onge and Floresco, 2008) and in a five-choice serial reaction time task (Zeeb et al., 2009; Winstanley, Zeeb, Bedard, Fu, Lai, Steele, Wong, 2010) in distinctive manners.   In addition to dopamine subtype receptors, the role of dopamine transporter (DAT) is also critical on the risky choice behavior.   In a series of investigations with DAT associated treatments (Adriani, Boyer, Gioiosa, Macri,

Dreyer, & Laviola, 2009; Adriani, Boyer, Leo, Canese, Podo, Perrone-Capano, Dreyer, & Laviola, 2010), the effects of DAT over-expression increased the choices of "large/luck-linked" reward rather than "small/sure" reward.   Taken together, the midbrain dopamine system is highly involved in the probabilistic risky choice behavior.   That the dopamine pre- and post-synaptic mechanisms potentially dissociable on risk-based decision making deserves for further investigations.

<u>The lesion effects of striatal subareas on probabilistic risky choice behavior</u>

In this study, one of the major findings is the lesion of the NAC, but not DLS, affect probabilistic risky choice behavior.   As compared to the sham lesion control, the rat with excitotoxic lesion in the NAC became a relatively risk-averse to respond on this probabilistic risky choice task, namely choosing less PHR even in the lower risk condition.   But such a behavioral alteration was not found in the subjects with DLS lesion.   These results further suggested that the subareas of striatum contain heterogeneity to mediate behavioral functions.

From previous studies, the effects of DLS lesion are shown to impair the habit formation (Yin, Knowlton, & Balleine, 2004) and reduce the resistance to extinction (Castane, Theobald, & Robbins, 2010).   Also, the DLS has been argued to be crucial for stimulus-response (S-R) learning (Horvitz, 2009; Anselme, 2010).   To the best of our knowledge, the present study is the first to investigate the role of DLS on probabilistic risky choice behavior.   The current results show that the lesion of DLS had no effect on this choice task.   This negative result cannot be attributed to those potential side effects derived by the excitotoxic lesion applied in the DLS.   Since there were no differences between the DLS lesion group and the sham lesion control group on locomotor activity or discrimination test.   However, with a further analysis, the average response time of completing a trial on the risky choice was longer in the DLS lesion group (3.41 ± 0.11 s) than sham lesion group (2.60 ± 0.04 s).   The

increase of response time on DLS lesion group may be due to the impairment of motivation (Anselme, 2010), suggesting that the rat could be less motivated to obtain the reward. Another explanation for the lesion of DLS could be the impaired S-R association which leads to behavioral outcome with the increase of the response time (Horvitz, 2009). Despite these arguable confounding effects, it should be noted that the lesion of DLS did not significantly affect the probabilistic risky choice behavior in this present study.

As for the NAC-lesioned induced relatively risk-averse choice behavior, it may not due to the impairment of motor function because a hyperactivity reaction to an open field was revealed by a post-lesion locomotor activity test for the subjects. The average response time of completing a trial was no difference between NAC lesion group (3.00 ± 0.08 s) and sham lesion group (2.90 ± 0.06 s). In addition, there were no omission trials appeared in the probabilistic risky choice tasks. Further, the behavior alteration in the NAC-lesion subjects cannot be attributed to impairment of basic discriminate function. A discrimination test was conducted and the data revealed no differences between NAC lesion group and sham lesion group. The rat with NAC lesion still had the ability to distinguish reward magnitude from 1 pellet and 2 pellets. Whether the lesion effect of NAC or DLS may impair the learning ability to affect the probabilistic risky choice can be an issue to concern. The results of a three-way ANOVA revealed a significant main effect of day and a significant reward-ratio-by-day interaction on both of NAC and DLS analyses (Figure 14 and Figure 18). It is then indicate that the rat with lesion of NAC or DLS did learn by showing dramatic changes on choice in each of reward ratios across ten daily sessions.

Comparing to previous study, Cardinal and Howes (2005) reported that the rat with NAC core lesion showed relatively risk-averse on probabilistic discounting task

in the stable choice sessions of post-surgery.   Consistent with Cardinal and Howes (2005), as revealed by the data presented in the last 3 days of free choice test, the rat with NAC lesion showed relatively risk-averse choice behavior.

Taken together, these data indicate that the NAC, but not DLS, is highly involved in behavioral performance on the probabilistic-based risky choice.

The lesion effects of prefrontal cortex subareas on probabilistic risky choice behavior

The data regarding to the effects of OFC lesion on probabilistic risky choice behavior yielded only a significant main effect of lesion from ANOVA, indicating that the lesion groups decreased the percentage of choosing PHR compared to that of sham lesion group.   As an anatomical control, mPFC lesion produced no difference between lesion and sham lesion groups.   Thus, the rat with lesion of OFC, but not mPFC, showed a tendency of relatively risk-averse fashion on probabilistic risky choice behavior.   Both of the lesions of OFC or mPFC did not impair the motor and basic discriminate function.   In the locomotor activity test, the rat with lesion of OFC showed significant hyperactive behavior compared to sham lesion group.   The OFC-lesioned induced hyperactivity was indicated by de Bruin, van Oyen and van de Pour (1983) on an open field locomotion test.   The response time of completing a trial was no different between OFC lesion group (2.77 ± 0.05 s) and sham lesion group (2.88 ± 0.08 s) neither mPFC lesion group (3.08 ± 0.08 s) and sham lesion group (3.15 ± 0.09 s).

As for the results of the lesion of mPFC had no effect on this task, our findings were inconsistent with those of St. Onge and Floresco (2009).   In which, inactivation of mPFC induced relatively risk-seeking choice behavior, whereas the inactivation of OFC had no effects on the probabilistic risky choice task.

There are four explanations that may clarify these inconsistencies.   First, the experimental procedures were different.   In the present study, all the rats experienced

each of three different reward ratios with 10 daily sessions.　In St. Onge and Floresco (2009), the design which adapted from Cardinal and Howes (2005), the rat experienced all of four reward probabilities within one test day.　The second explanation of these inconsistencies was regarding to the techniques used for brain manipulations.　A more permanent excitotoxic lesion was used in this present study, whereas a reversible inactivation way given by drug of GABA agonist was used in St. Onge and Floresco (2009).　Third, in this study, the naïve rat was subjected to surgery before entering the probabilistic risky choice test rather than a well-trained rat. That is, from the time course of surgery, the lesion effects were supposedly to affect "acquisition" rather than "performance" stage of behavioral measure in this study. Thus, the time point of applying lesion surgery is also a difference in between the present study and that of St. Onge and Floresco (2009).　Fourth, the role of EV was involved in this present study which was different from that of St. Onge and Floresco (2009).　An fMRI study indicated that the activation in medial OFC, but not medial PFC, was positively correlated to both reward magnitude and EV in human subjects (Rolls et al., 2008).　It is then possible that the significant effect of OFC lesion in present study may attribute to the alterations of EV perception.　Namely, while EV=1 set in both CLR and PHR arm, the rat with lesion of OFC exhibited a tendency to behave as if EV is less than 1 while the risks were involved in the PHR arm.

A concern should be made on the inconsistencies among previous studies that investigated the role of OFC on risk-based decision making.　As mentioned earlier, a negative result of the lesion of OFC was reported by St. Onge and Floresco (2009), but a relatively risk-averse choice behavior on a relative long-term task was found in Mobini et al. (2002).　In addition, a relatively risk-seeking choice behavior on an acute 90-trial test was found in Pais-vieira, Lima, and Galhardo (2007).　One of the arguments is the OFC-lesioned induced relatively risk-averse tendencies were

prominent on the relative long-term value between small/certain and large/risky options (St. Onge & Floresco, 2009).   This impairment of learning risk/reward contingencies is supported by a brain imaging (fMRI) study showing that lateral OFC activation is associated with this contingency on the IGT (Lawrence, Jollant, O'Daly, Zelaya, & Phillips, 2009).   Another argument is that, the effect of OFC lesion induced relatively risk-seeking response is associated with the immediate decisions based on the evaluation of the outcome (Pais-vieira et al., 2007).   This notion is supported by the lesion of OFC impaired the "updating" function for the representation of response consequences (Winstanley, Theobald, Cardinal, & Robbins, 2004).   The controversial effects of OFC lesion may be attributed to the exact subareas within OFC made differently across studies.   The coordinates applied to make the OFC lesion from those three studies were different (see the table below).

	Coordinates of lesion sites (relative to bregma)
Mobini et al. (2002)	[AP]: +3.7 mm, [ML]: ±1.2 mm, [DV]: -4.8 mm
	[AP]: +3.7 mm, [ML]: ±2.8 mm, [DV]: -4.4 mm
Pais-vieira et al. (2007)	[AP]: +3.7 mm, [ML]: ±2.4 mm, [DV]: -5.5 mm
St. Onge & Floresco (2009)	[AP]: +3.9 mm, [ML]: ±2.6 mm, [DV]: -2.9 mm

From human fMRI studies, a review by Schultz et al. (2008) suggests that the risk signals in the lateral part of OFC increased with the degree of risk aversion, whereas risk signals decreased with the degree of relatively risk-seeking in the medial part of OFC.   Also, activations of the medial OFC related to both reward magnitude and EV during a decision task (Rolls et al., 2008).   Thus, it is likely that the subareas of OFC along with other prefrontal areas play distinct roles on the risk-related

decision making (Wallis & Kennerley, 2010). It is warranty for the further study to delineate the distinctive mechanisms involved in these different prefrontal subareas.

Limitations of present study and suggestion for future investigation

In terms of probabilistic risky choice task applied in animal studies, most of these studies used operant chambers. In comparison with these studies using fully-automatic operant chambers, the number of trials and sample sizes were limited in this study due to a T-maze was used in present study.

Another limitation is regarding to the forced choice phase design. In present study, every reward ratio had only one day session of running forced choice. In contrast, the forced choice trials were contained in every daily-session in previous studies (e.g. Cardinal & Howes; St. Onge & Floresco, 2008, 2009). Thus, the effects of forced choice were restricted in this study in comparing with the others. For example, some rats chose mostly the CLR arm with the experience of forced choice, it may be a reason why the patterns of first day in each reward ratio have the noticeable variation. Despite this, the rat shows a stable choice behavior in the last few days, suggesting the rat would learn the contingencies of different reward ratios as daily sessions moving on.

Beyond the regions discussed in this present study, a growing body of studies indicates that the mesolimbic dopamine systems are involved in probabilistic risky choice behavior. Thus, there are further candidate brain regions may involved in the process of risks or EV in choice behavior. One of these candidates is the basolateral amygdala (Ghods-Sharifi, St. Onge & Floresco, 2009). Inactivation of the basolateral amygdala induced a relatively risk-averse pattern on probabilistic risky choice behavior. Moreover, as indicated by Roll et al. (2007), insula cortex showed negative correlations with EV in human subject fMRI study, it is possible that insula cortex may involved in the choice behavior in this present study. It is worthy to

further investigate the roles of these brain areas on the probabilistic risky choice with different EV manipulations in the future.

Clinical implications

A number of psychiatric disorders associated with dopamine system that impaired decision making had been identified. Pathological gambling (PG), which had been defined by DSM-IV as a persistent and recurrent maladaptive gambling behavior and was preoccupied with gambling (American Psychiatric Association, 2000). The PG behavior is likely to be triggered by dopamine agonist during a therapy for Parkinson's disease (Ahlskog, 2011; Djamshidian, Cardoso, Grosset, Bowden-Jones, & Lees, 2011). Also, the obsessive-compulsive disorder (OCD) is a psychiatric disorder due to neurobiological abnormalities of OFC and basal ganglia (Cavedini, Gorini, & Bollodi, 2006). Thus, the abnormality of the dopamine related regions in the brain is highly linked to the appearance of these clinical syndromes relevant to the impairment of decision making or habit formation. The present data with regarding to the manipulation of lesion experiments provide comparable results for the study of neurobiological basis of risk-based decision making in clinical. This present study is helpful for understanding the neural substrates underlying in the neurobehavioral mechanisms of risk-based decision making.

Conclusion

The present study established an animal model of probabilistic risky choice based on the EV viewpoint. The results of amphetamine induced relatively risk-seeking behavior indicate that the dopamine system is crucial for the risky choice behavior. From the results of brain lesion experiments, the nucleus accumbens plays a major role of mediating this behavioral processing. In conclusion, the probabilistic risky choice behavior established in the present study is dopamine dependent.

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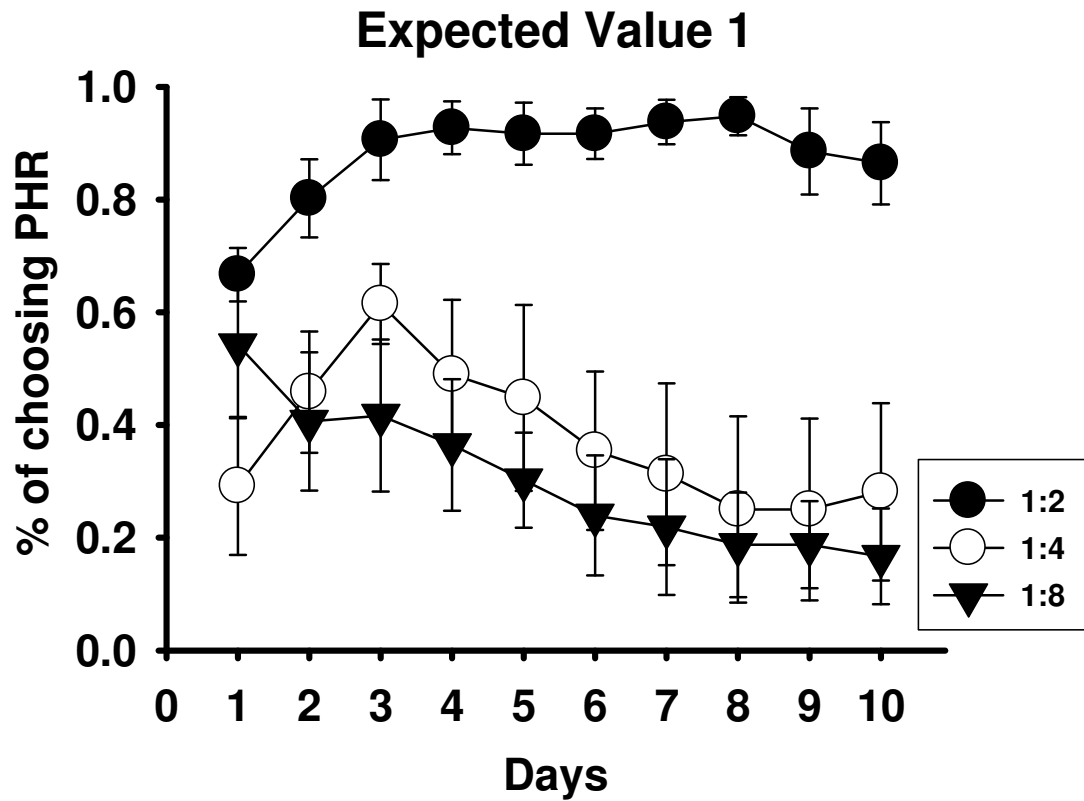
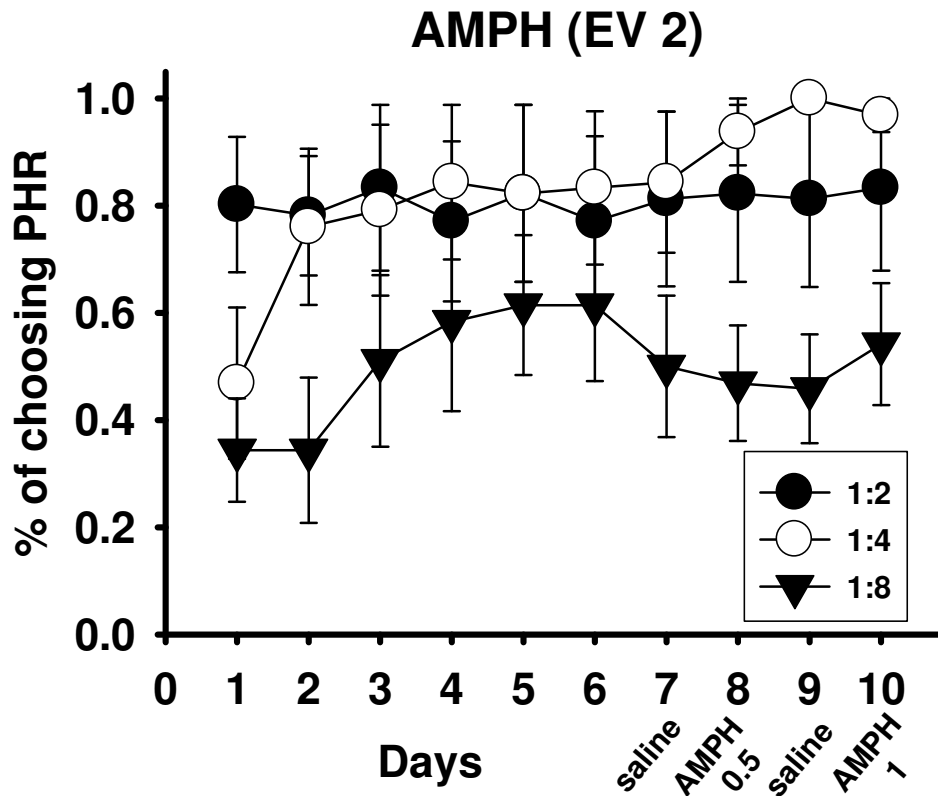**Figure 1**   Percentage of choosing probabilistic high reward (PHR) on three
conditions of reward ratio in the probabilistic risky choice task with the expected
value of 1 equally set for both choice options.   Each reward ratio condition was
tested over ten daily sessions.
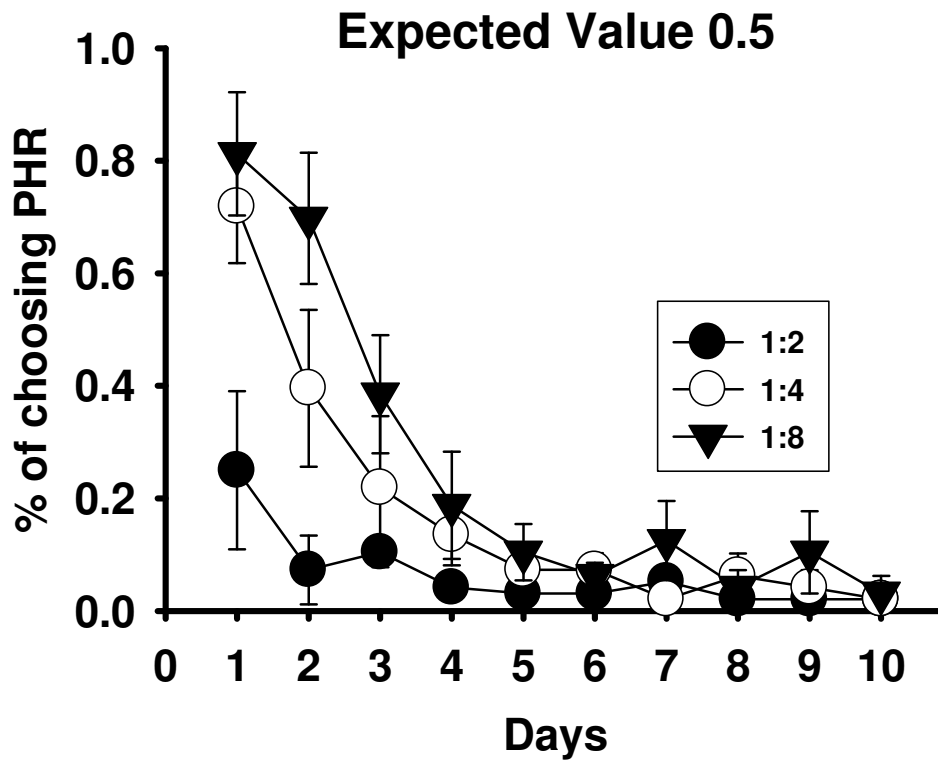
Figure 2   Percentage of choosing probabilistic high reward (PHR) on three conditions of reward ratio in the probabilistic risky choice task with the expected value of 1 set in the certain low reward choice arm and the expected value of 0.5 set in the probabilistic high reward arm.   Each reward ratio condition was tested over ten daily sessions.
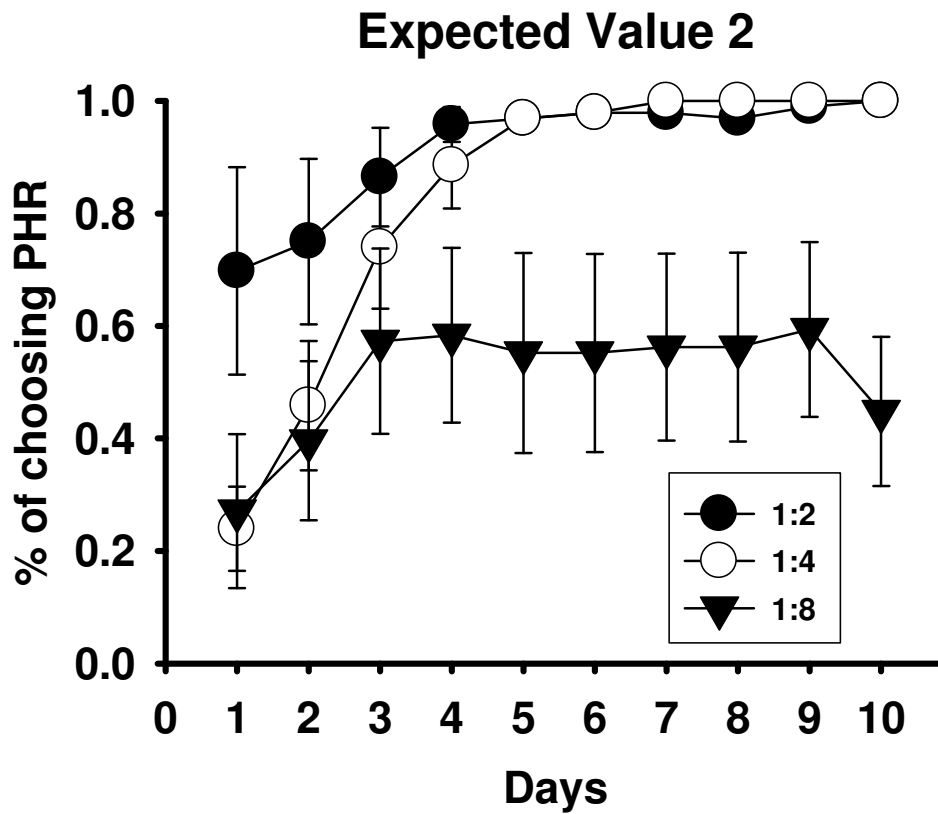
Figure 3 Percentage of choosing probabilistic high reward (PHR) on three conditions of reward ratio in the probabilistic risky choice task with the expected value of 1 set in the certain low reward choice arm and the expected value of 2 set in the probabilistic high reward arm.   Each reward ratio condition was tested over ten daily sessions.
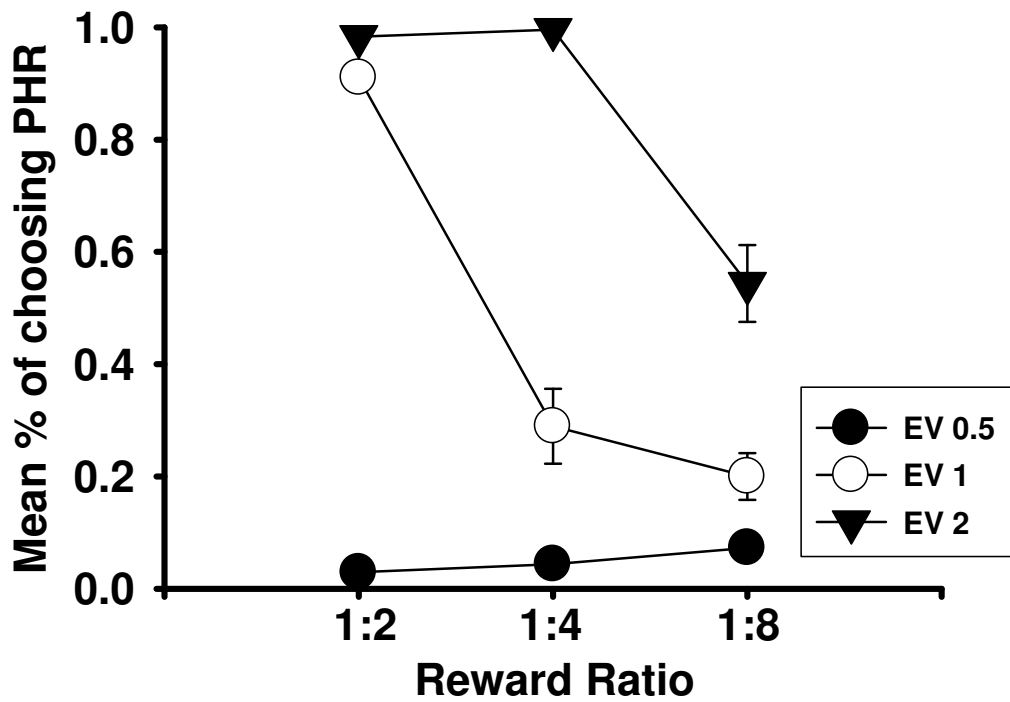
Figure 4 Mean percentage of choosing probabilistic high reward (PHR) on three conditions of reward ratio in the probabilistic risky choice task under three sets of expected value applied in the probabilistic high reward arm. Each data point is the averaged percentage choice of PHR over the last five days of behavioral test given for each condition.
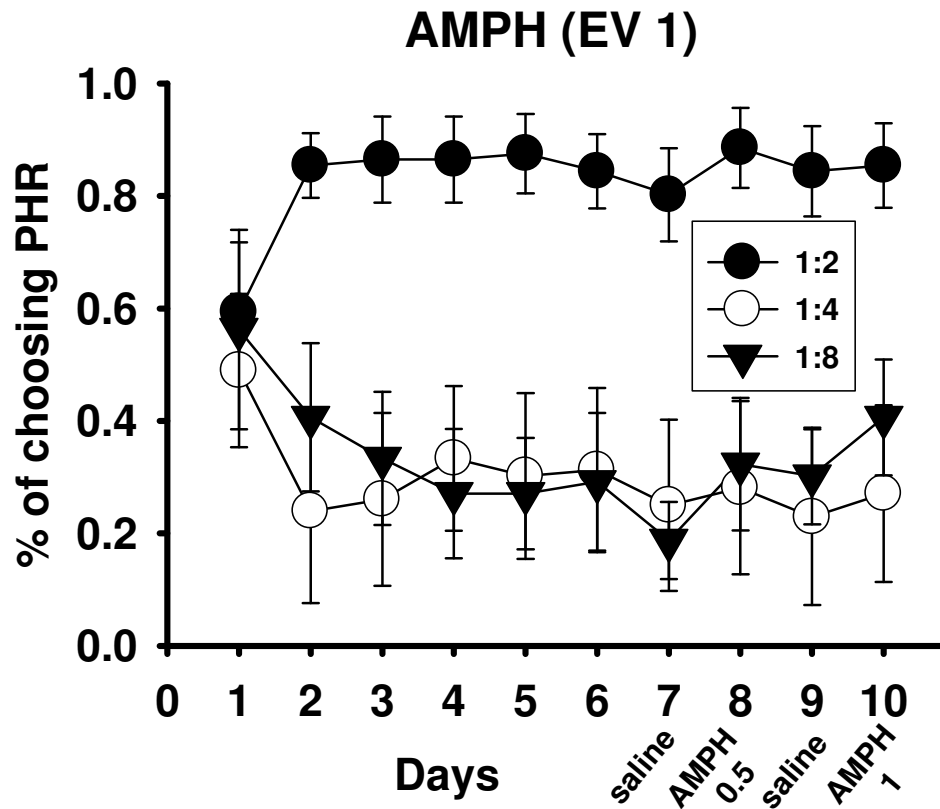
**Figure 5** Effects of amphetamine (AMPH) on percentage of choosing probabilistic high reward (PHR) on three conditions of reward ratio in the probabilistic risky choice task with the expected value of 1 equally set for both choice options. Each reward ratio condition was tested over ten daily sessions, in which saline control treatment was given on the 7th day and 9th day before AMPH treatments of 0.5 mg/kg and 1 mg/kg given on the 8th and 10th day, respectively.
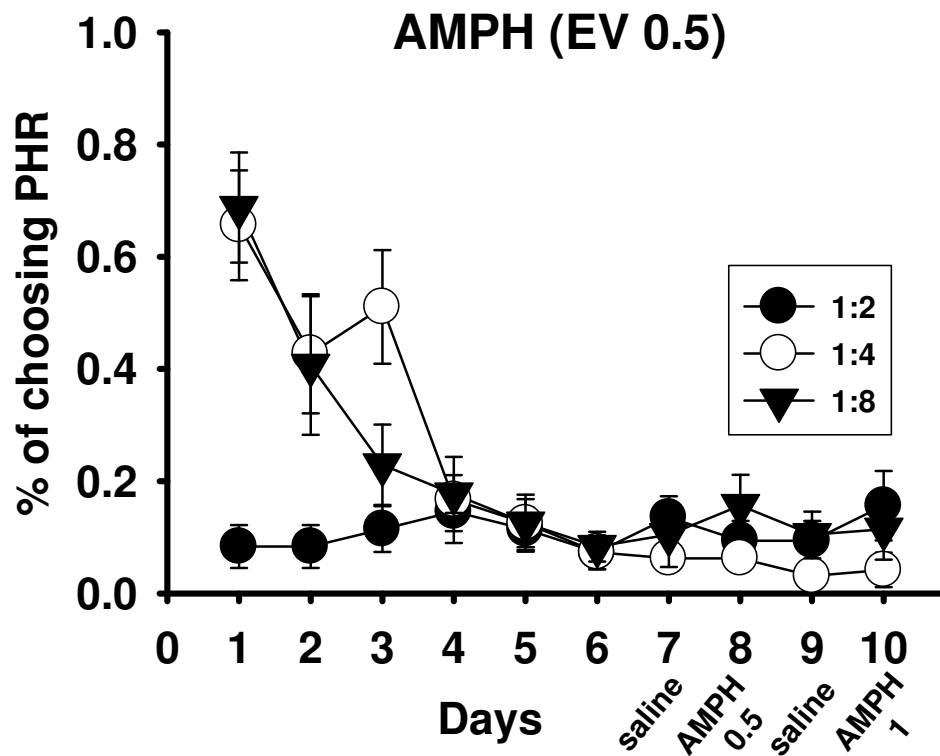
Figure 6   Effects of amphetamine (AMPH) on percentage of choosing probabilistic high reward (PHR) on three conditions of reward ratio in the probabilistic risky choice task with the expected value of 1 set in the certain low reward choice arm and the expected value of 0.5 set in the probabilistic high reward arm.   Each reward ratio condition was tested over ten daily sessions, in which saline control treatment was given on the 7th day and 9th day before AMPH treatments of 0.5 mg/kg and 1 mg/kg given on the 8th and 10th day, respectively.

AMPH (EV 2)

Figure 7 Effects of amphetamine (AMPH) on percentage of choosing probabilistic high reward (PHR) on three conditions of reward ratio in the probabilistic risky choice task with the expected value of 1 set in the certain low reward choice arm and the expected value of 2 set in the probabilistic high reward arm. Each reward ratio condition was tested over ten daily sessions, in which saline control treatment was given on the 7th day and 9th day before AMPH treatments of 0.5 mg/kg and 1 mg/kg given on the 8th and 10th day, respectively.
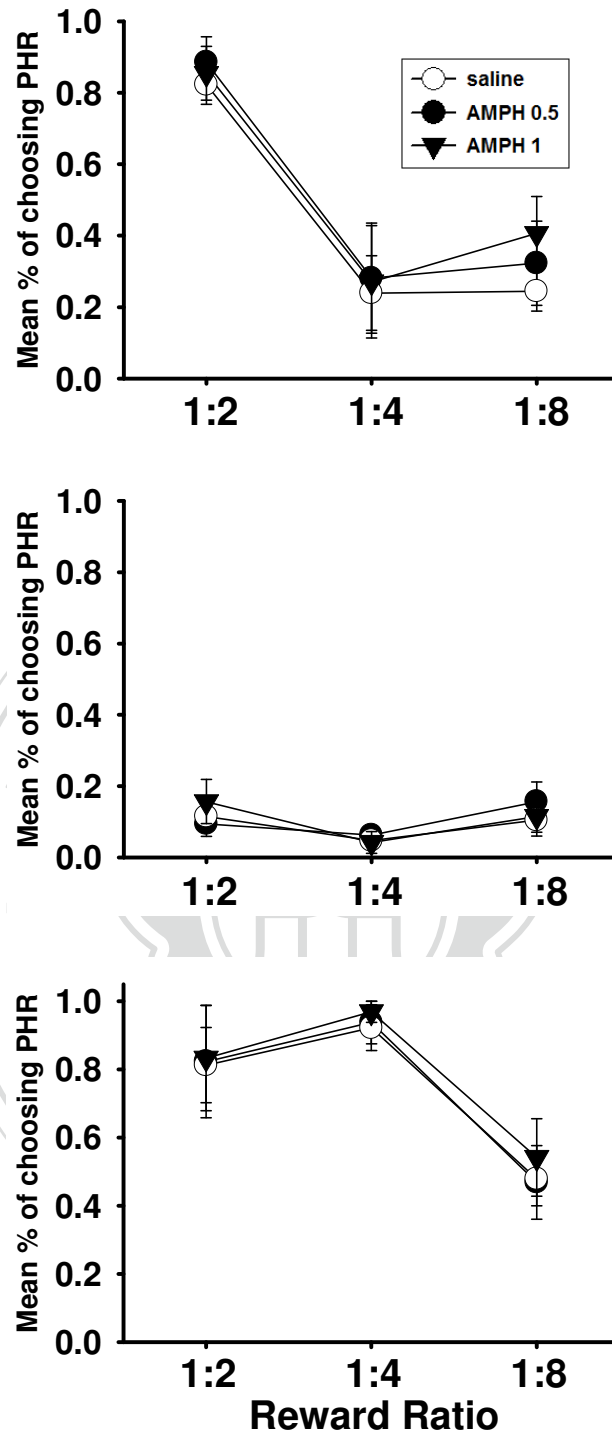
Figure 8 Dose effects of amphetamine (AMPH) on mean percentage of choosing probabilistic high reward (PHR) on three conditions of reward ratio in the probabilistic risky choice task under three sets of expected value applied in the probabilistic high reward arm. Each data point is the averaged percentage choice of PHR over the last five days of behavioral test given for each condition.
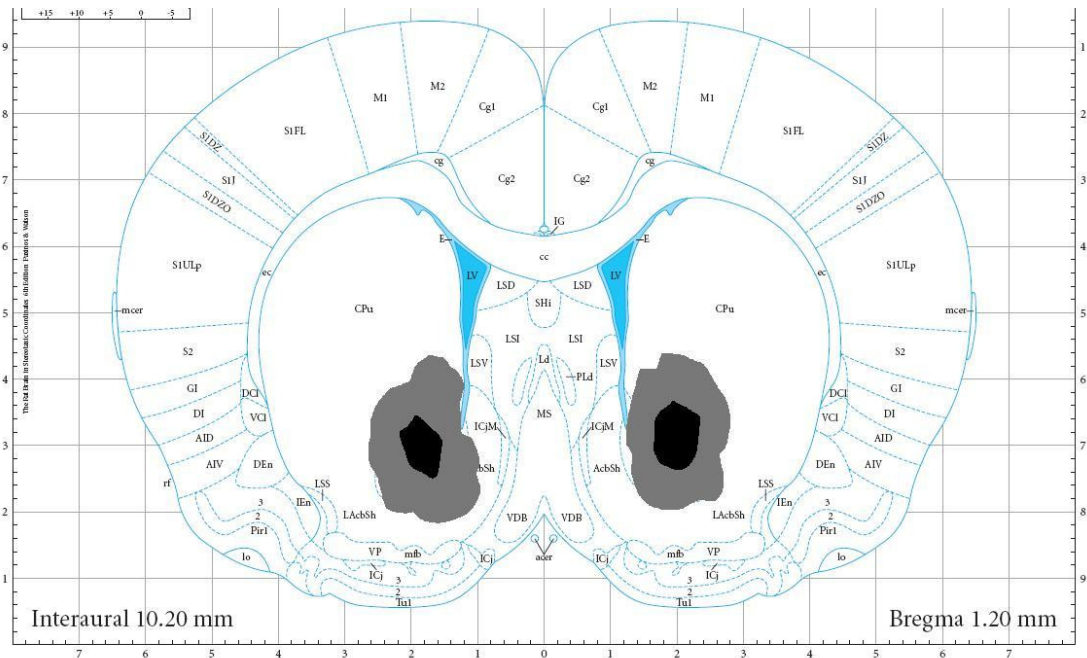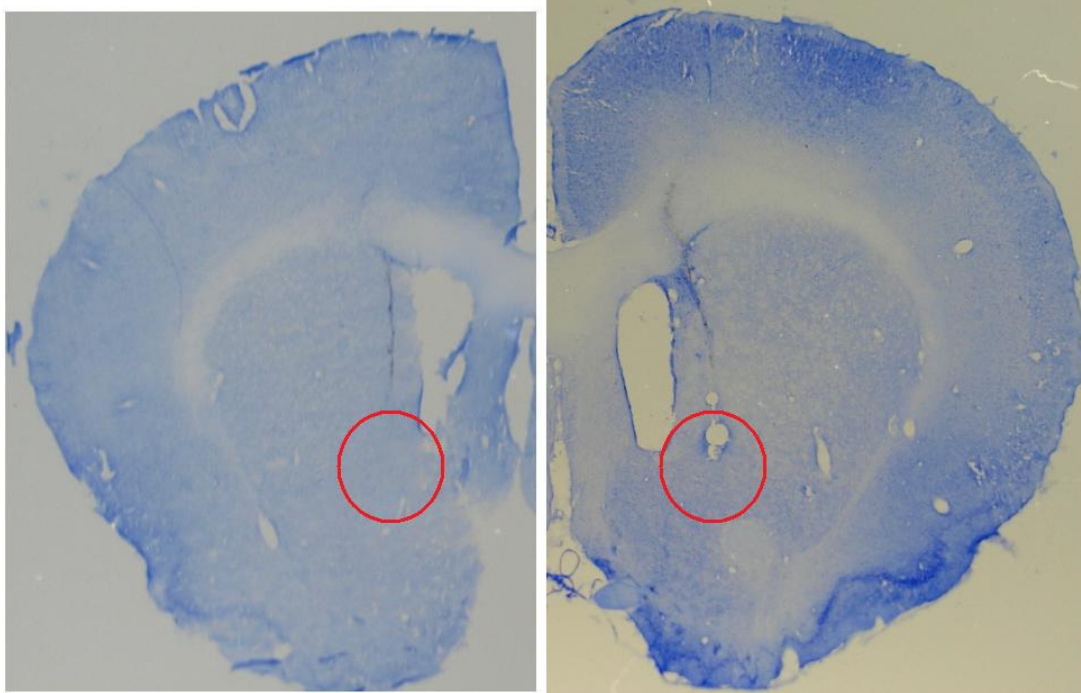
Figure 9 Histological examination of NAC. Top panel shows the diagram (relevant page from Paxinos & Watson, 2007) of the minimum (black) and maximum (grey) extension of NAC lesions (n = 9). Bottom panel shows the photographs of coronal sections with red circle indicated the location of NAC lesions (right panel) and sham lesion (left panel).
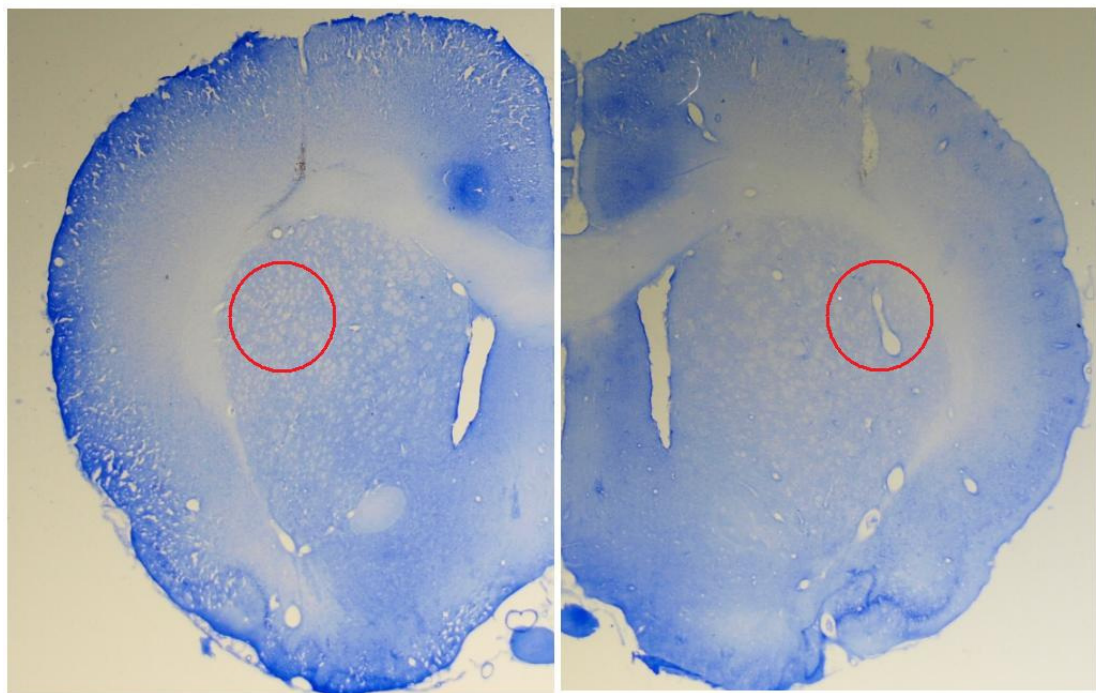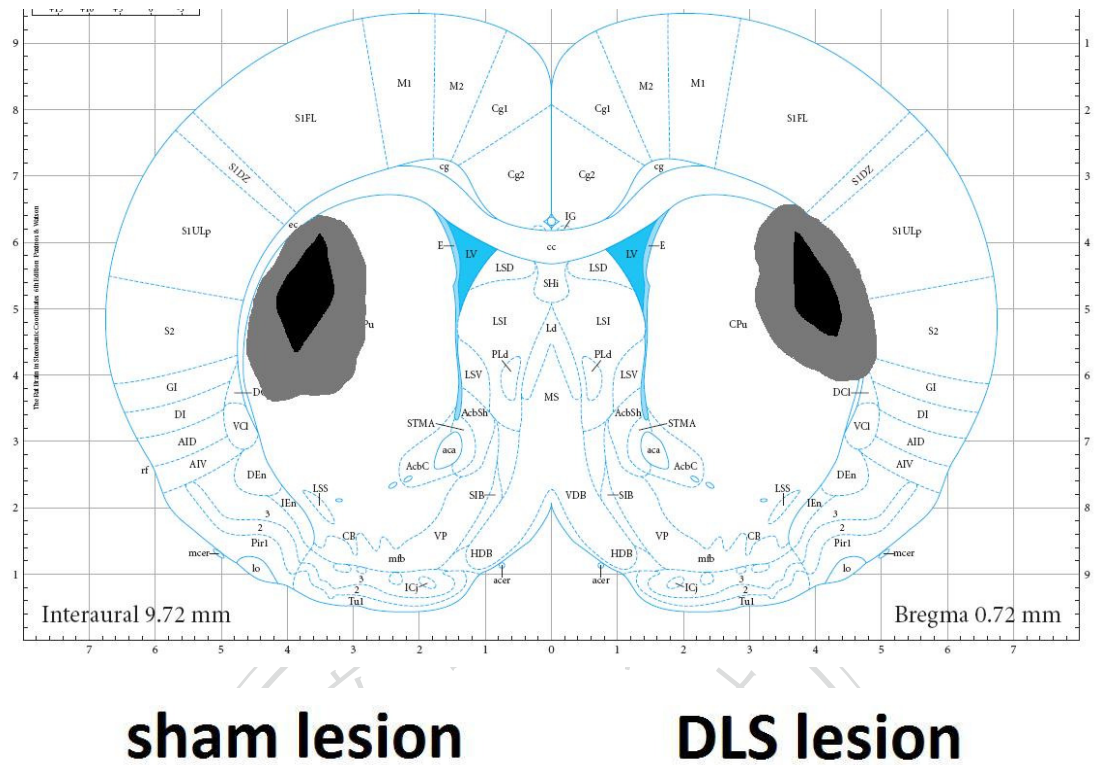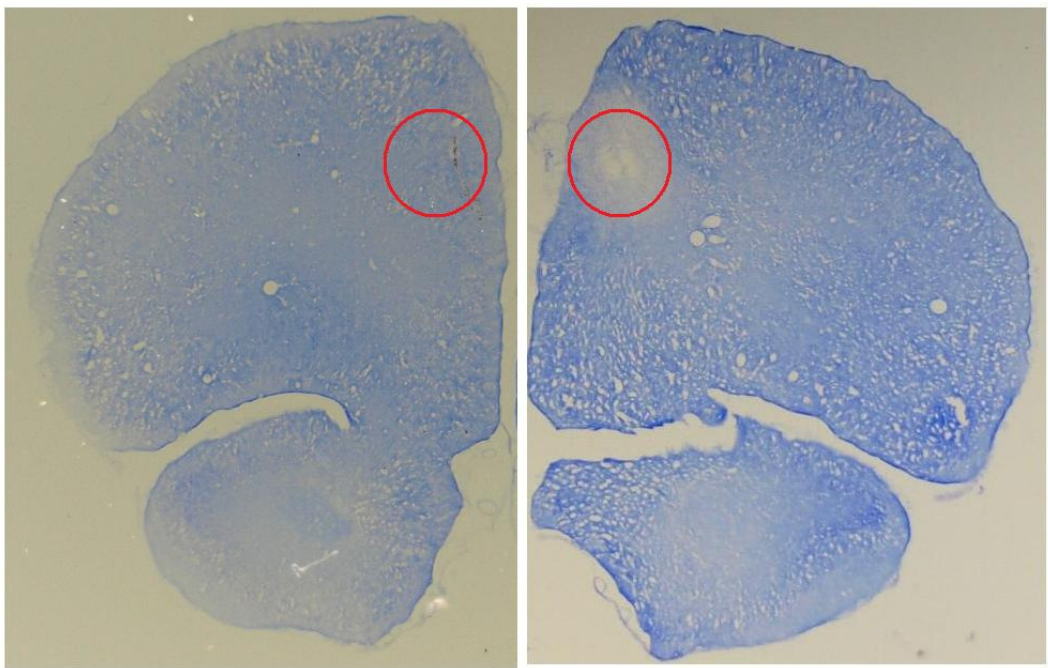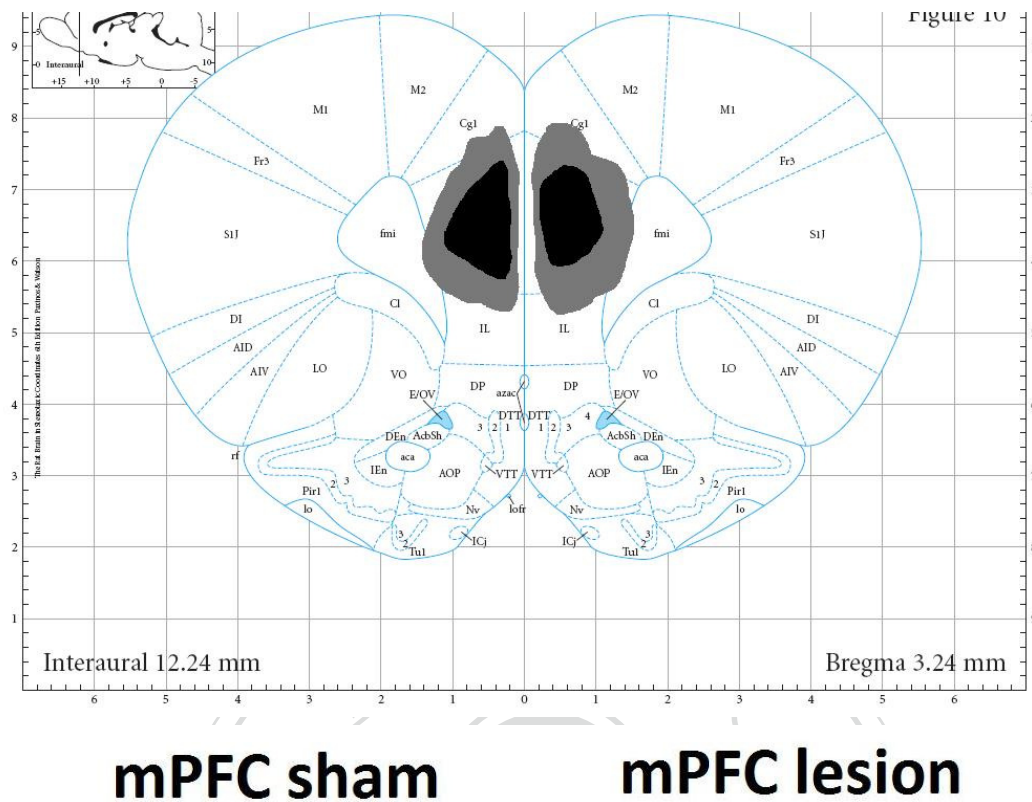
Figure 10 Histological examination of DLS.   Top panel shows the diagram (relevant page from Paxinos & Watson, 2007) of the minimum (black) and maximum (grey) extension of DLS lesions (n = 9).   Bottom panel shows the photographs of coronal sections with red circle indicated the location of DLS lesions (right panel) and sham lesion (left panel).
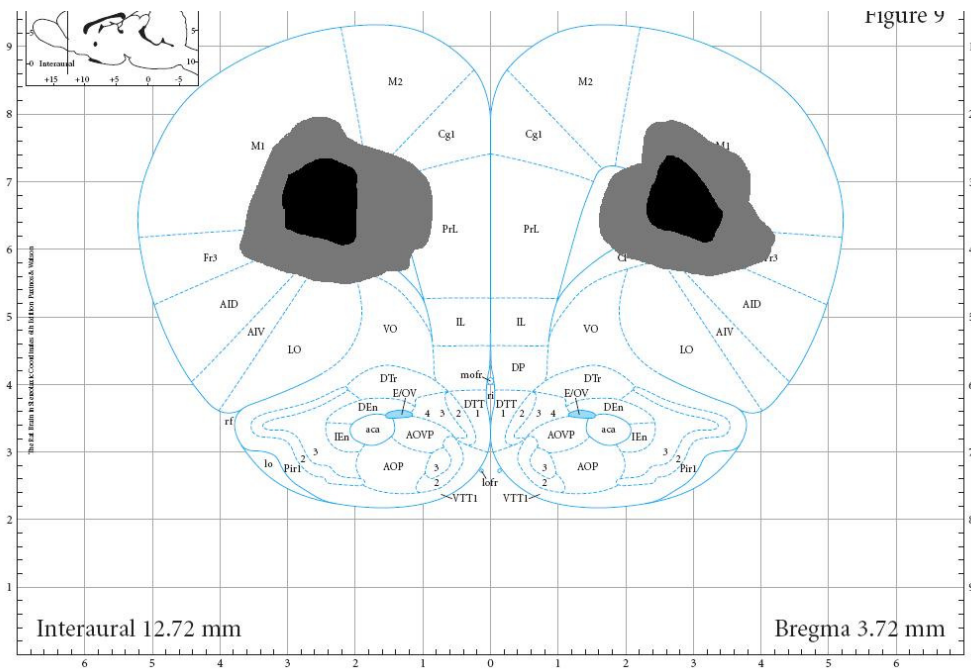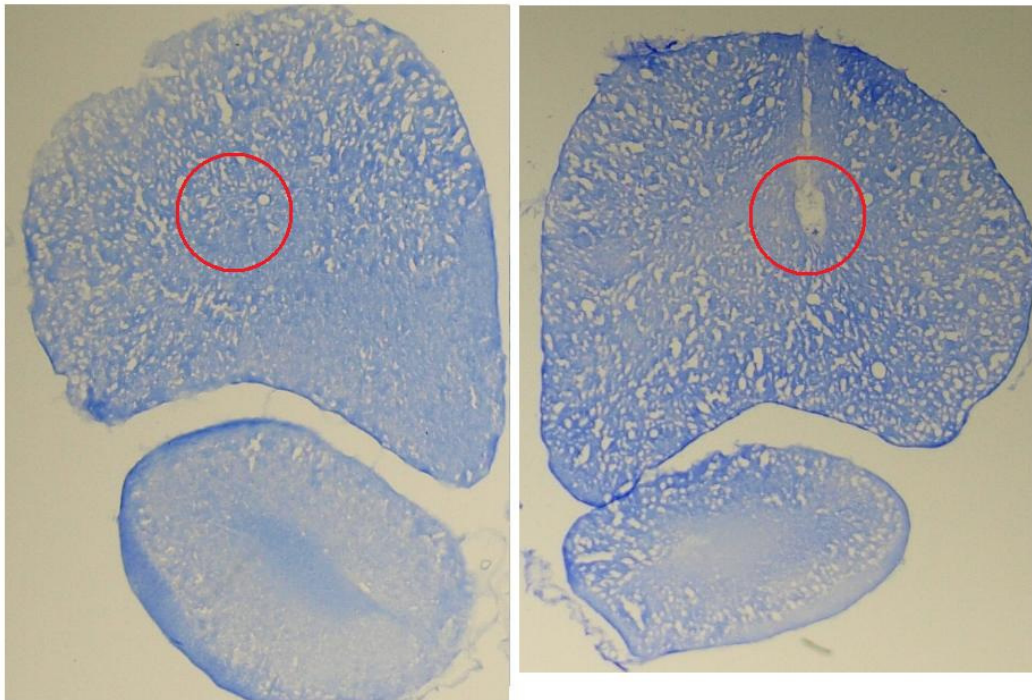
Figure 11 Histological examination of OFC. Top panel shows the diagram (relevant page from Paxinos & Watson, 2007) of the minimum (black) and maximum (grey) extension of OFC lesions (n = 9). Bottom panel shows the photographs of coronal sections with red circle indicated the location of OFC lesions (right panel) and sham lesion (left panel).
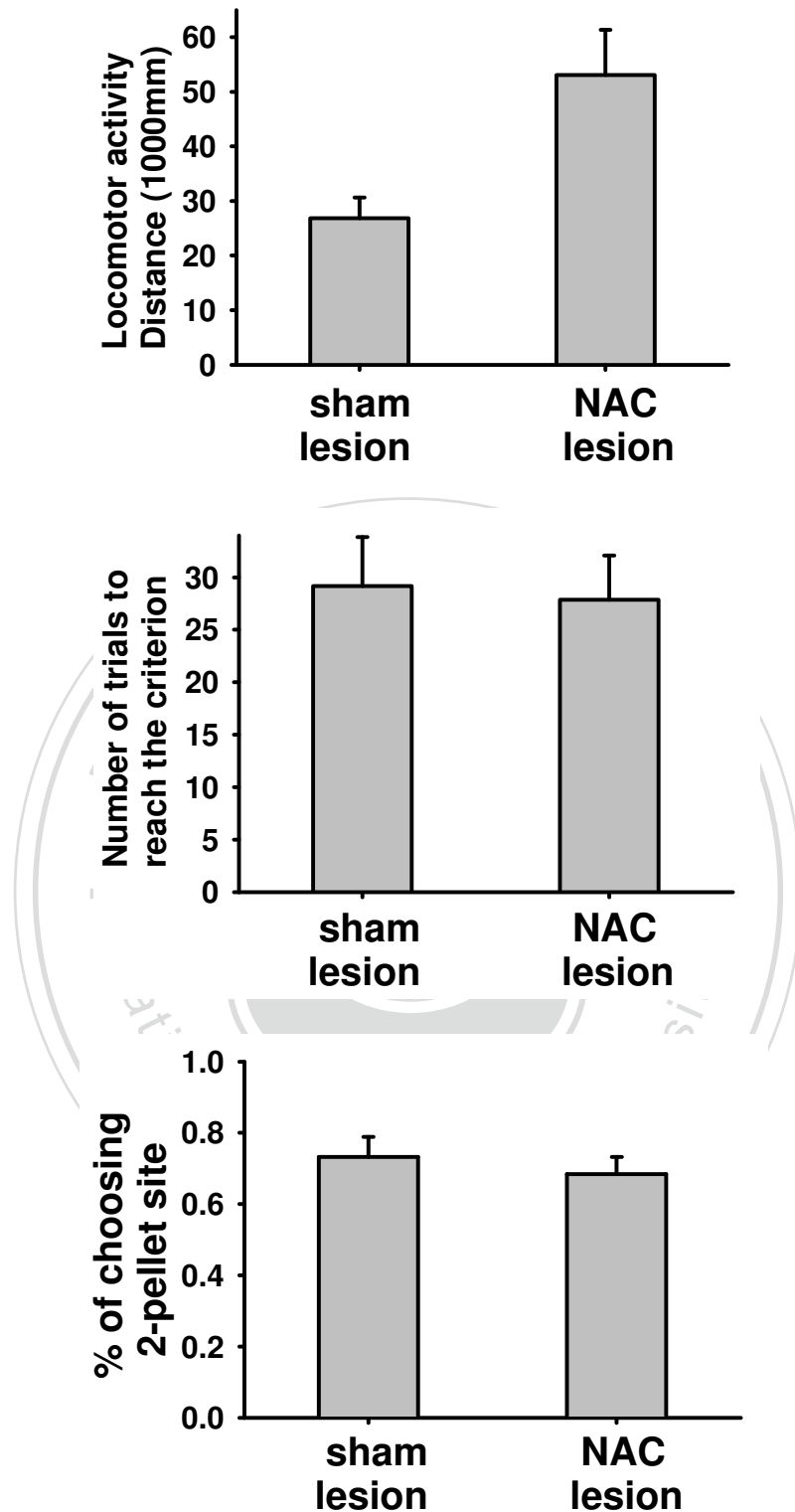
Figure 12   Histological examination of mPFC.   Top panel shows the diagram
(relevant page from Paxinos & Watson, 2007) of the minimum (black) and maximum
(grey) extension of mPFC lesions (n = 9).   Bottom panel shows the photographs of
coronal sections with red circle indicated the location of mPFC lesions (right panel)
and sham lesion (left panel).

Figure 13   Post-lesion tests of locomotor activity (top panel) and the discrimination task of one vs. two chocolate pellets (lower two panels) for the rats with lesion of the nucleus accumbens (NAC) and sham lesion control.   See text for the details regarding the discrimination task.
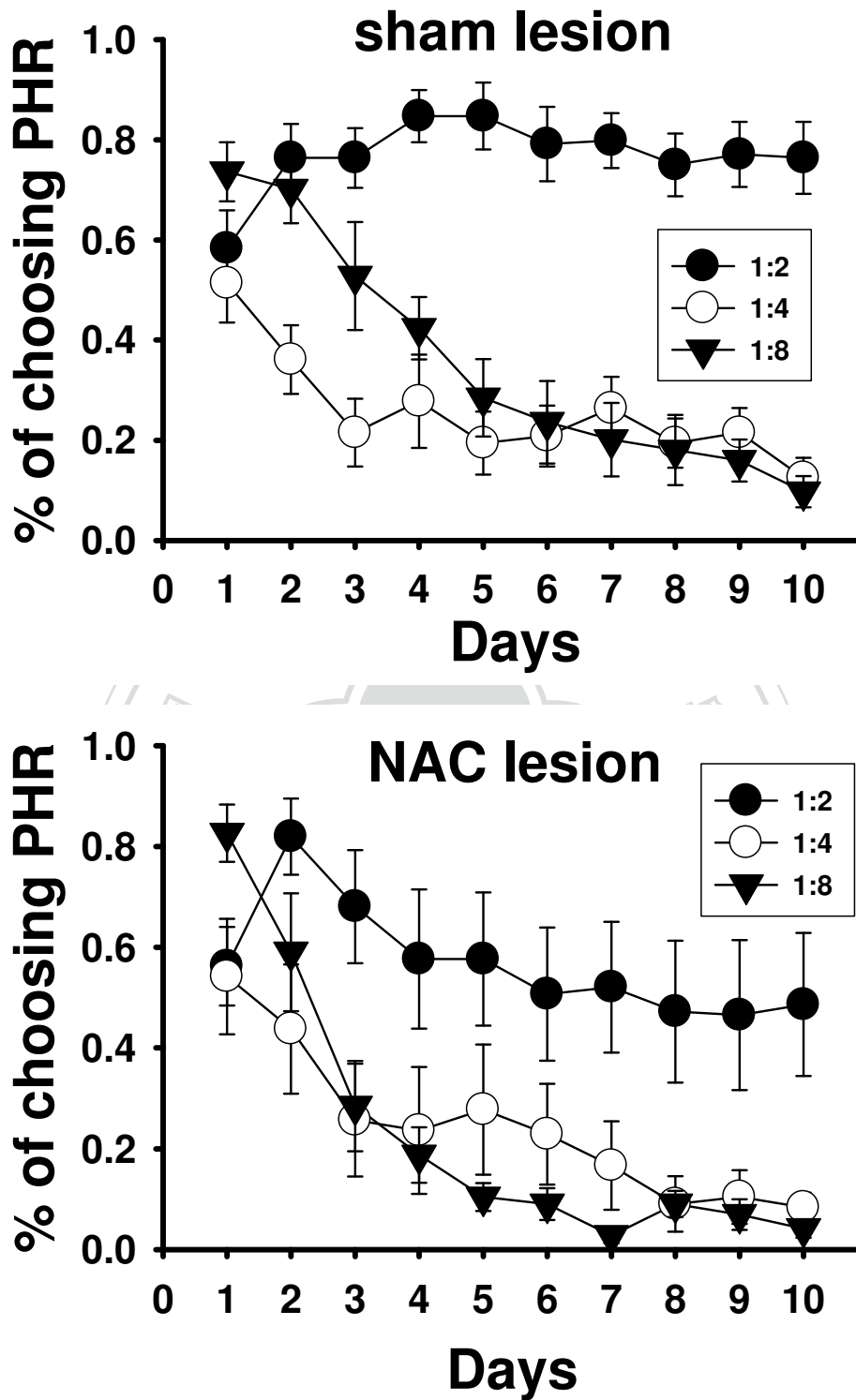
Figure 14 Lesion effects of nucleus accumbens (NAC) on percentage of choosing probabilistic high reward (PHR) on three conditions of reward ratio in the probabilistic risky choice task over a ten-day post-lesion test (bottom panel). The data for the sham lesion control group is presented in the top panel.
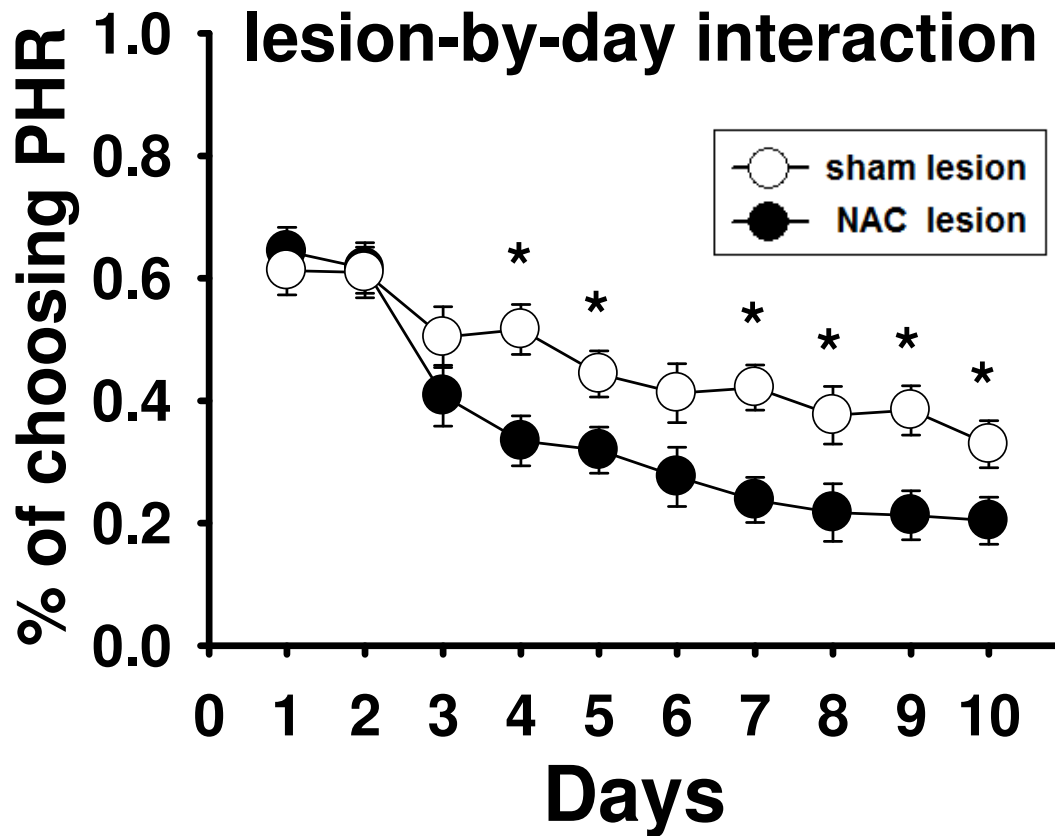
Figure 15 Lesion effects of nucleus accumbens (NAC) on percentage of choosing probabilistic high reward (PHR) on probabilistic risky choice task over a ten-day post-lesion test. Asterisk represents a significant difference from the sham lesion group in each day ($p < 0.05$).
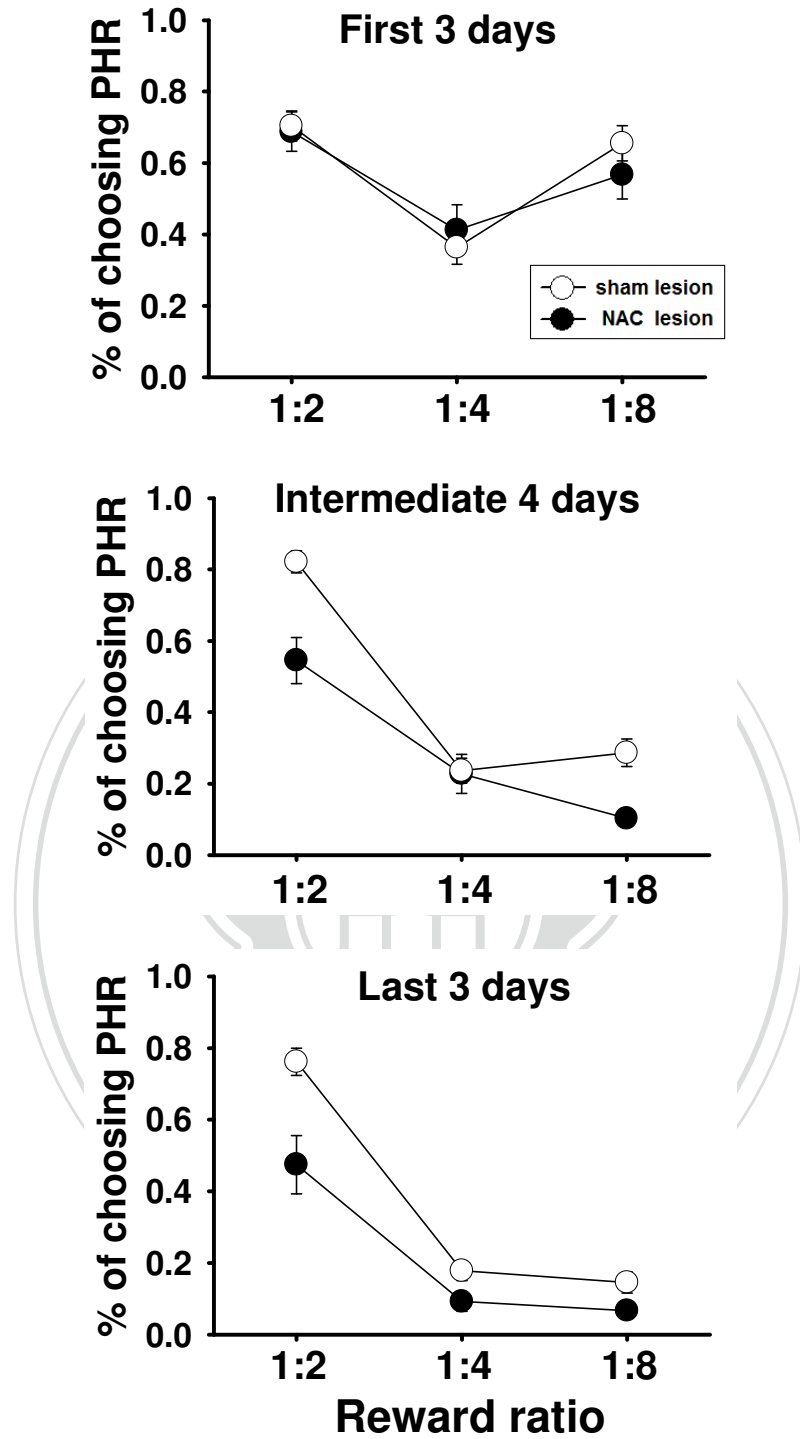
Figure 16   Mean percentage of choosing probabilistic high reward (PHR) on three

conditions of reward ratio in the probabilistic risky choice task in the nucleus

accumbens lesion and sham lesion control groups as behavioral data are separately

analyzed in the first three days (top panel), the intermediate four days (intermediate

panel), and the last three days (bottom panel) of the ten-day post-lesion test.
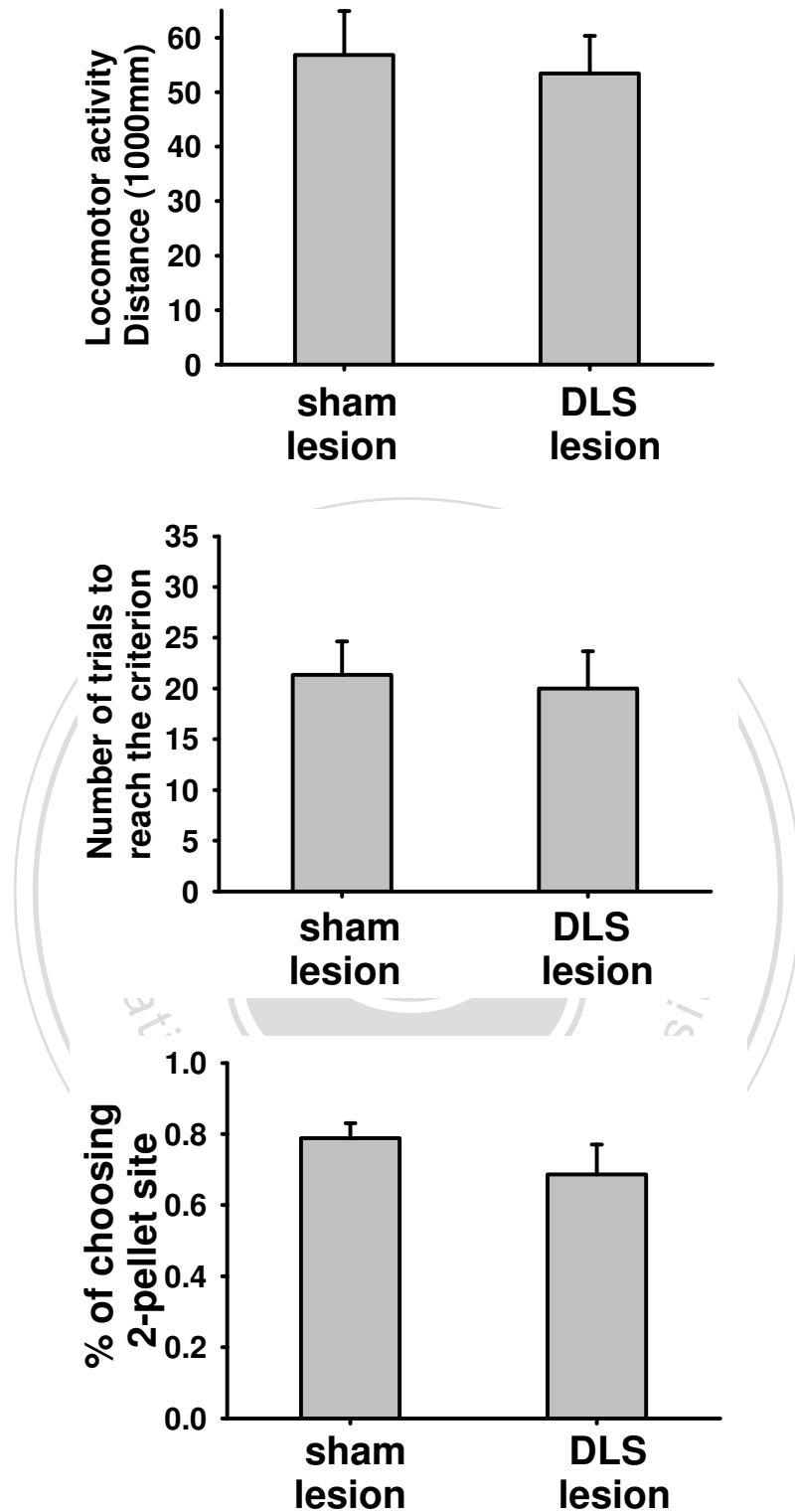
Figure 17 Post-lesion tests of locomotor activity (top panel) and the discrimination task of one vs. two chocolate pellets (lower two panels) for the rats with lesion of the dorsolateral striatum (DLS) and sham lesion control. See text for the details regarding the discrimination task.
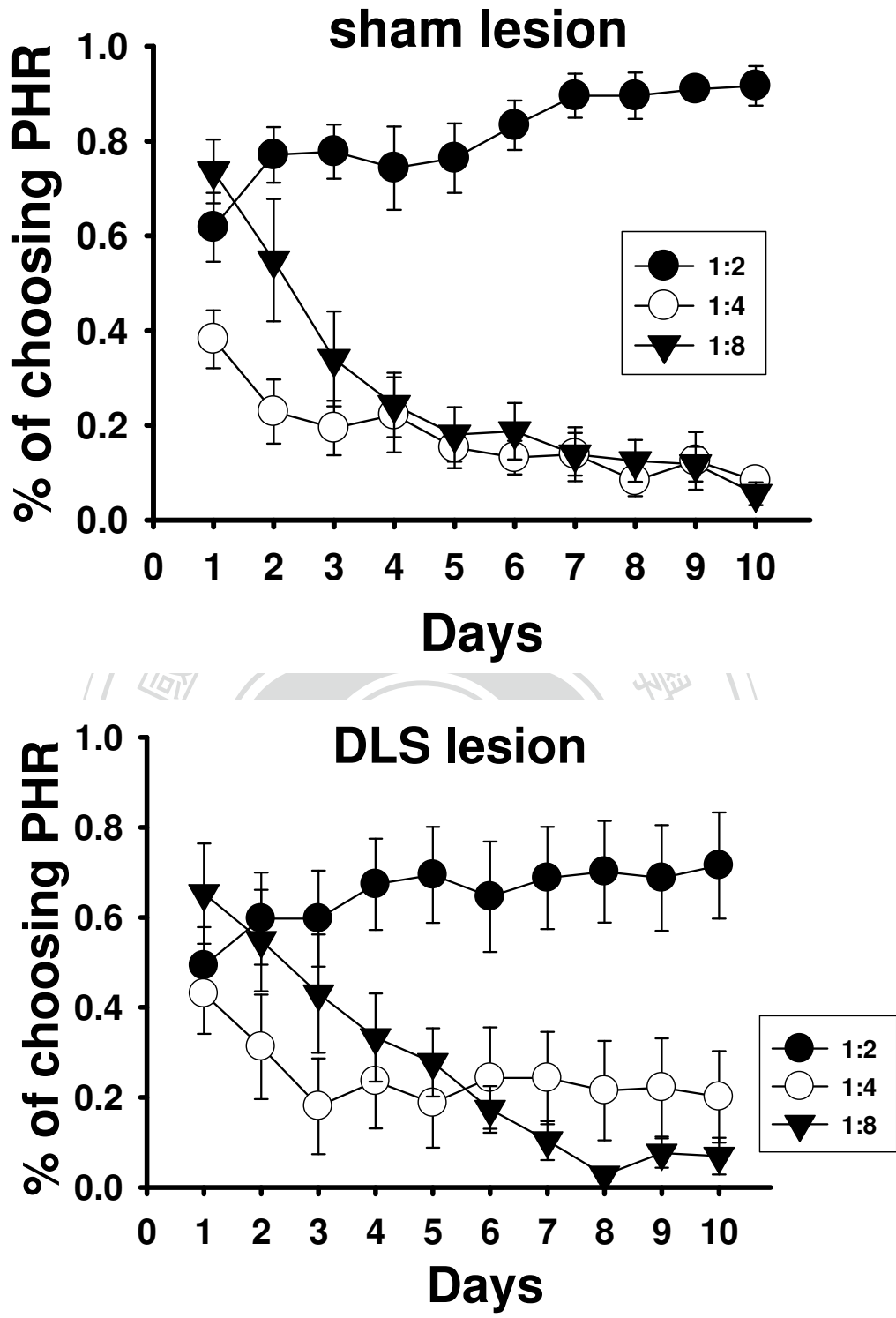
Figure 18   Lesion effects of dorsolateral striatum (DLS) on percentage of choosing

probabilistic high reward (PHR) on three conditions of reward ratio in the

probabilistic risky choice task over a ten-day post-lesion test (bottom panel).   The

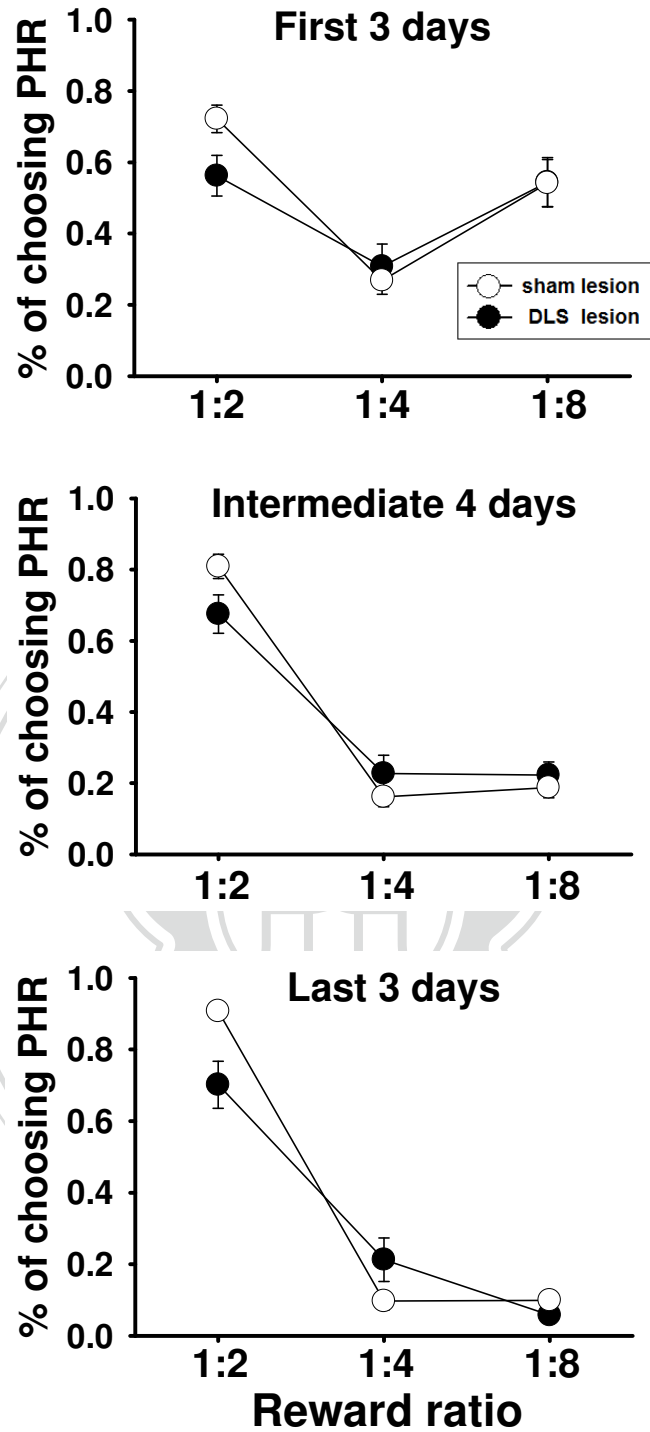data for the sham lesion control group is presented in the top panel.

Figure 19 Mean percentage of choosing probabilistic high reward (PHR) on three conditions of reward ratio in the probabilistic risky choice task in the dorsolateral striatum lesion and sham lesion control groups as behavioral data are separately analyzed in the first three days (top panel), the intermediate four days (intermediate panel), and the last three days (bottom panel) of the ten-day post-lesion test.

Figure 20 Post-lesion tests of locomotor activity (top panel) and the discrimination task of one vs. two chocolate pellets (lower two panels) for the rats with lesion of the orbitofrontal cortex (OFC) and sham lesion control. See text for the details regarding the discrimination task.
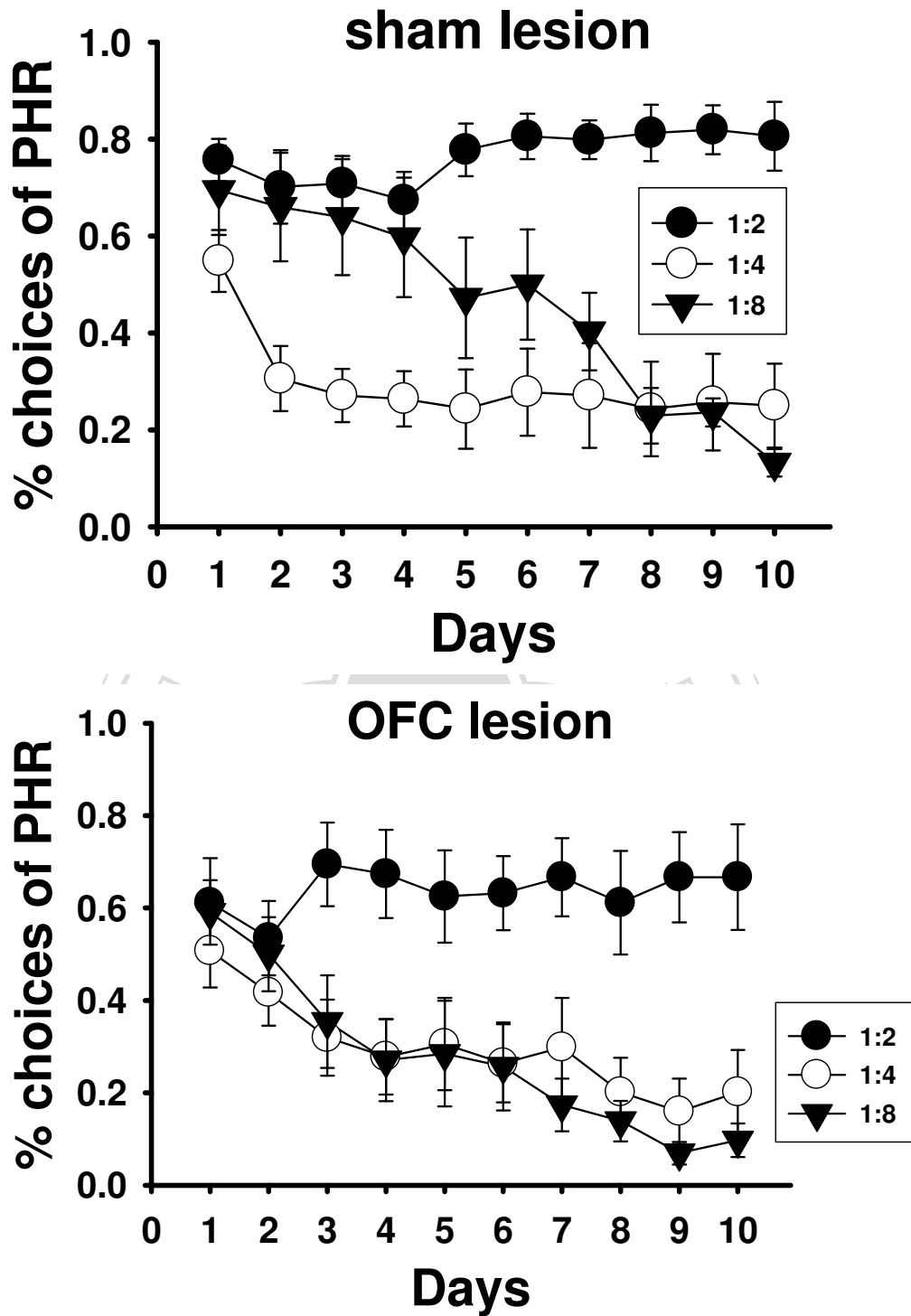
Figure 21 Lesion effects of orbitofrontal cortex (OFC) on percentage of choosing

probabilistic high reward (PHR) on three conditions of reward ratio in the

probabilistic risky choice task over a ten-day post-lesion test (bottom panel).   The

data for the sham lesion control group is presented in the top panel.

Figure 22 Mean percentage of choosing probabilistic high reward (PHR) on three conditions of reward ratio in the probabilistic risky choice task in the orbitofrontal cortex lesion and sham lesion control groups as behavioral data are separately analyzed in the first three days (top panel), the intermediate four days (intermediate panel), and the last three days (bottom panel) of the ten-day post-lesion test.
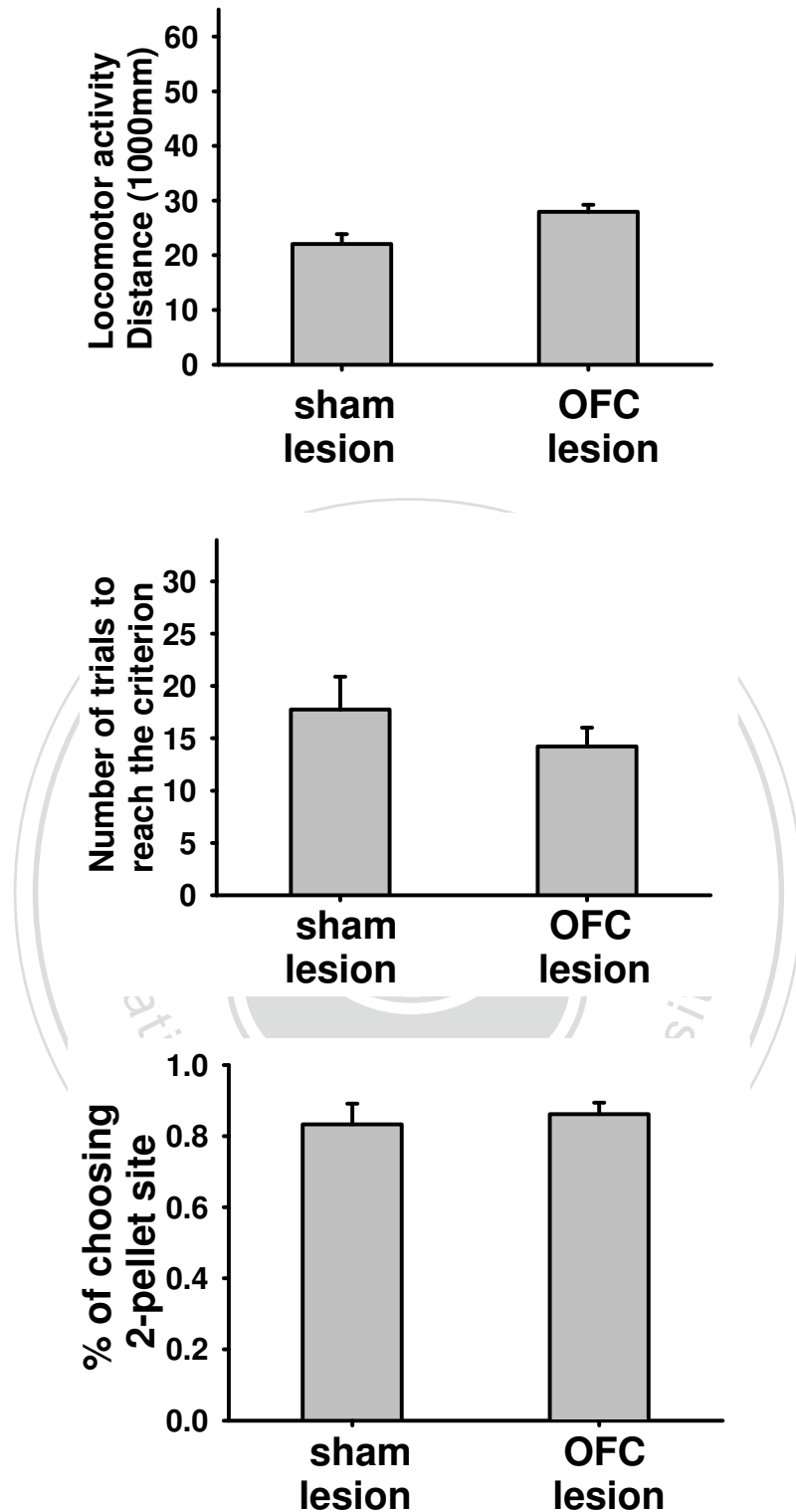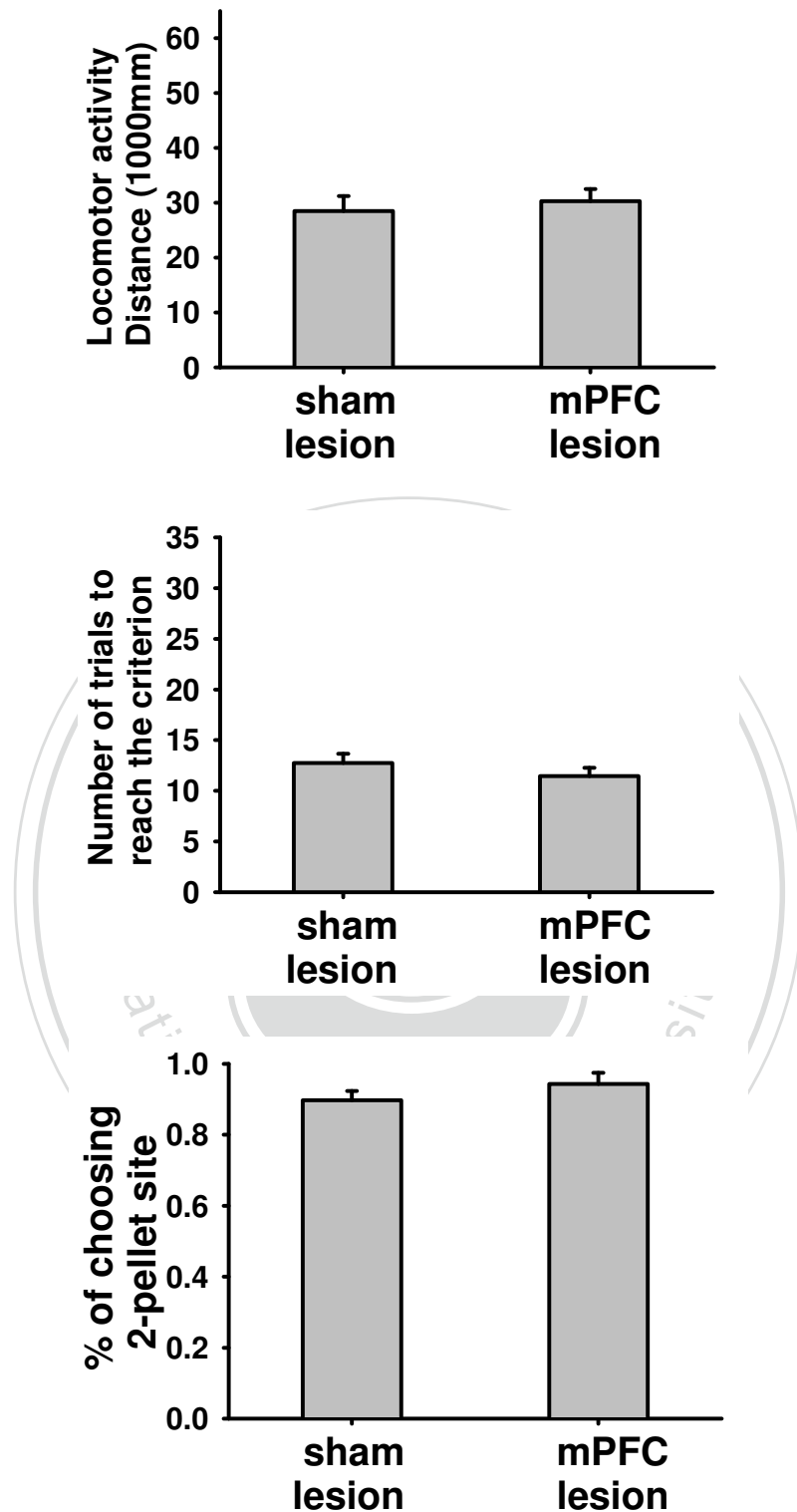
Figure 23   Post-lesion tests of locomotor activity (top panel) and the discrimination

task of one vs. two chocolate pellets (lower two panel) for the rats with lesion of the

medial frontal cortex (mPFC) and sham lesion control.   See text for the details

regarding the discrimination task.
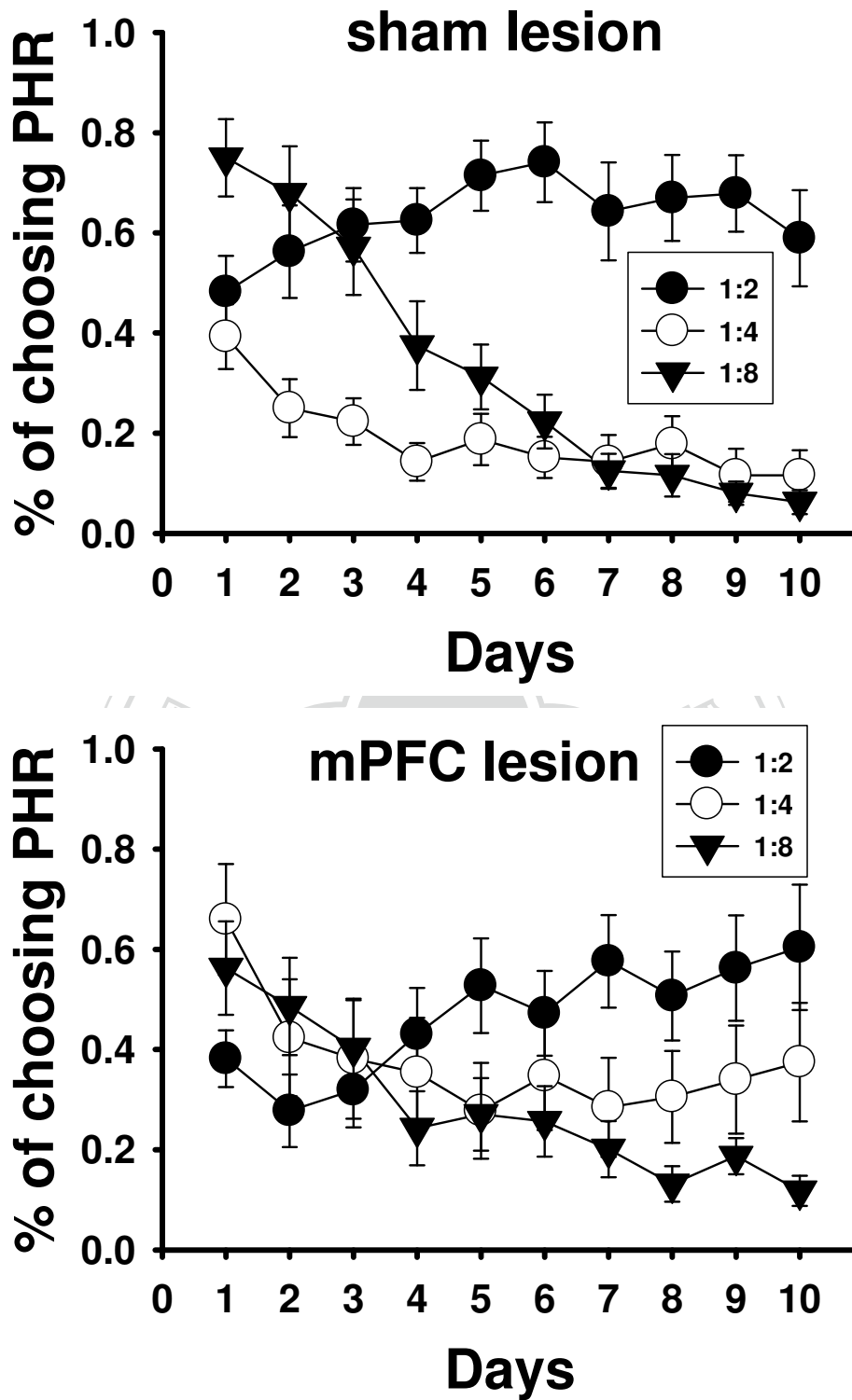
Figure 24   Lesion effects of medial prefrontal cortex (mPFC) on percentage of choosing probabilistic high reward (PHR) on three conditions of reward ratio in the probabilistic risky choice task over a ten-day post-lesion test (bottom panel).   The data for the sham lesion control group is presented in the top panel.
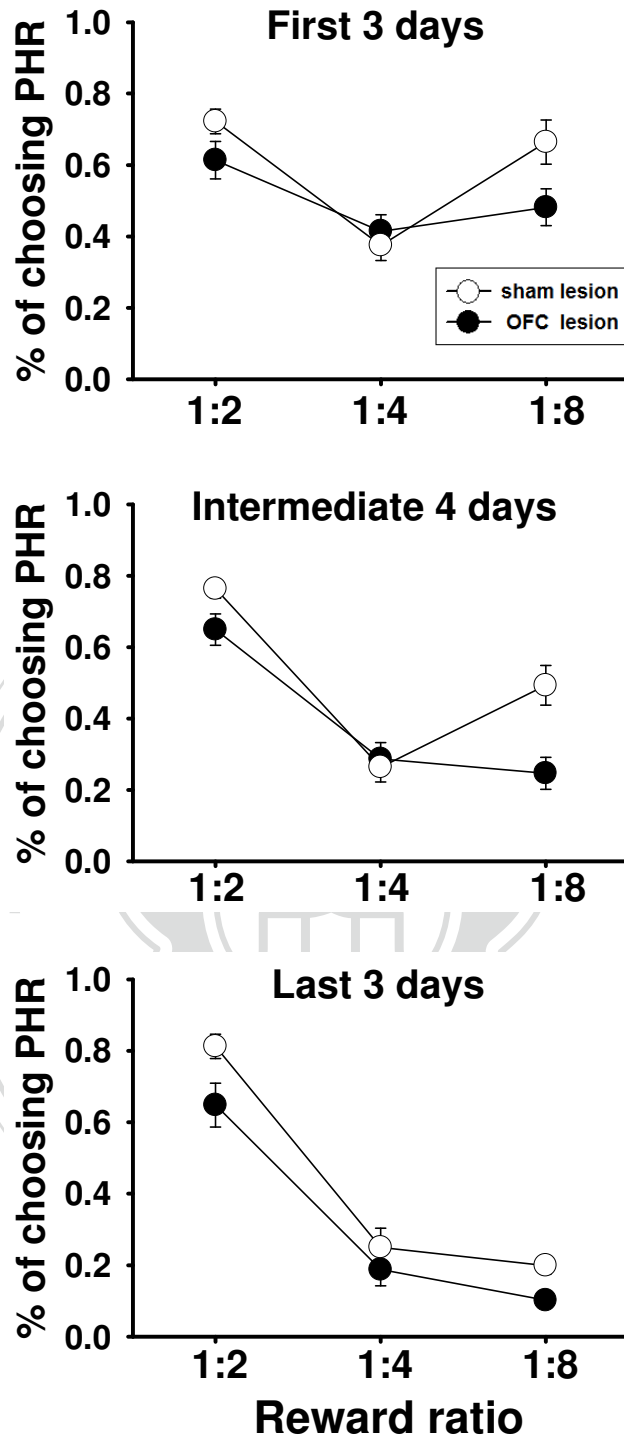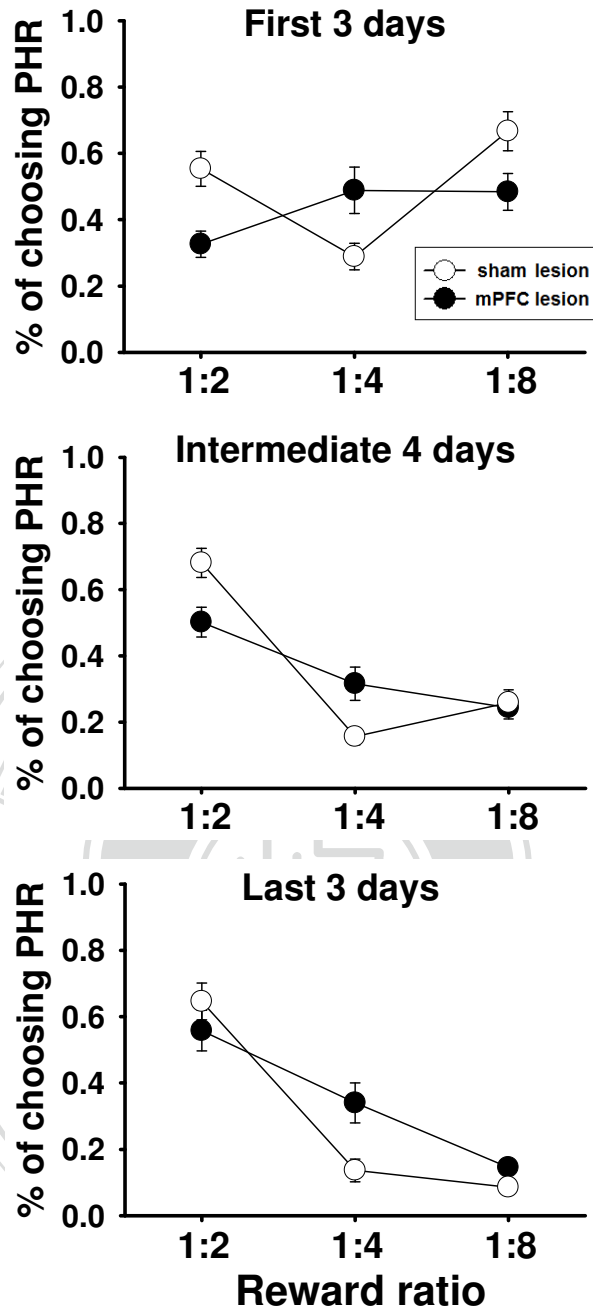
Figure 25 Mean percentage of choosing probabilistic high reward (PHR) on three conditions of reward ratio in the probabilistic risky choice task in the medial prefrontal cortex lesion and sham lesion control groups as behavioral data are separately analyzed in the first three days (top panel), the intermediate four days (intermediate panel), and the last three days (bottom panel) of the ten-day post-lesion test.
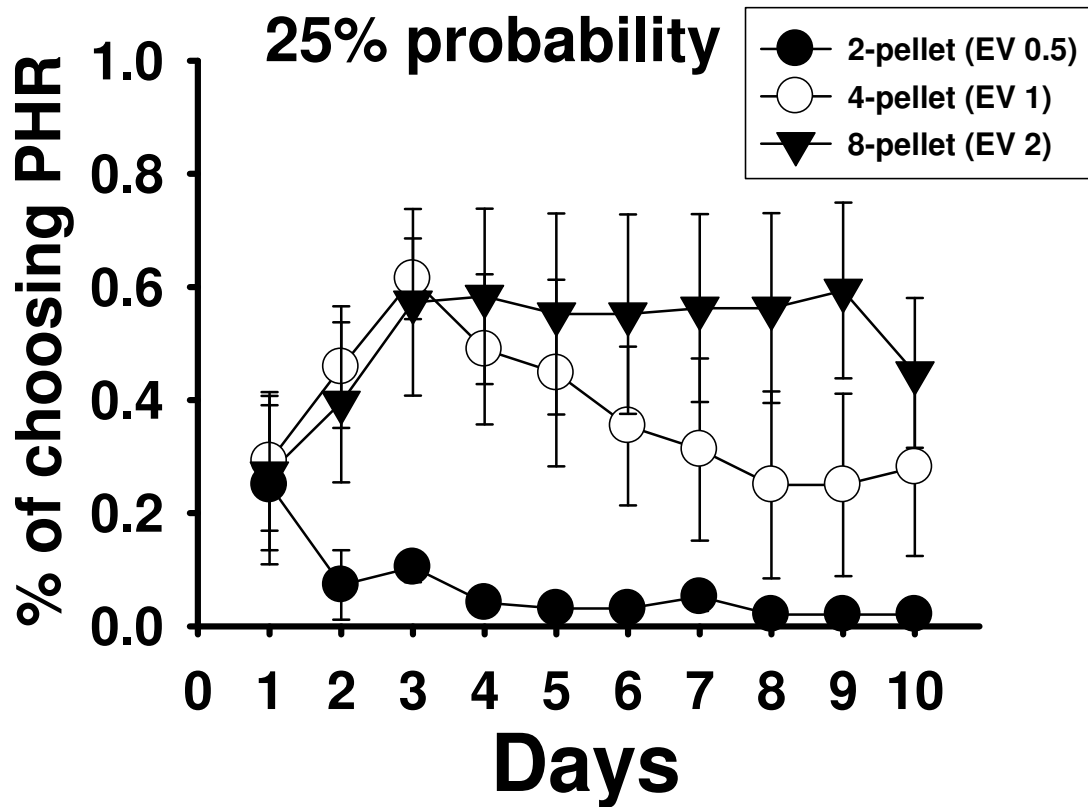
Figure 26   Percentage of choosing probabilistic high reward (PHR) on each of three different reward magnitudes (2, 4, and 8 pellets) in the probabilistic risky choice task with the reward probability of 25% set in PHR arm but 100% in CLR arm with 1 pellet.   Each of reward magnitudes was tested over ten daily sessions.

Appendix

Table 1   The results of a two-way ANOVA of amphetamine treatment on probabilistic risky choice behavior (* $p < 0.05$).

ANOVA summary table for top panel of Figure 8:

Source of variance	SS	df	MS	F	p
EV	.057	2	.028	5.798	.021 *
Reward ratio	3.795	2	1.898	6.883	.013 *
EV-by-reward-ratio	.039	4	.010	1.101	.383
Error (EV)	.049	10	.005		
Error (reward ratio)	2.757	10	.276		
Error (EV-by-reward ratio)	.176	20	.009		

ANOVA summary table for intermediate panel of Figure 8:

Source of variance	SS	df	MS	F	p
EV	.061	2	.030	1.848	.207
Reward ratio	.000	2	7.23E-005	.014	.986
EV-by-reward-ratio	.023	4	.006	2.365	.088
Error (EV)	.165	10	.016		
Error (reward ratio)	.051	10	.005		
Error (EV-by-reward ratio)	.048	20	.002		

ANOVA summary table for bottom panel of Figure 8:

Source of variance	SS	df	MS	F	p
EV	1.689	2	.845	2.594	.124
Reward ratio	.036	2	.018	2.343	.146
EV-by-reward-ratio	.032	4	.008	.469	.758
Error (EV)	3.256	10	.326		
Error (reward ratio)	.076	10	.008		
Error (EV-by-reward ratio)	.345	20	.017		

Table 2 The results of a two-way ANOVA of effects of NAC lesion on probabilistic risky choice behavior in the first 3 days, the intermediate 4 days, and the last 3 days of ten-day free choice phase (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

ANOVA summary table for top panel of Figure 16:

Source of variance	SS	df	MS	F	p
Lesion	.005	1	.005	.200	.661
Reward ratio	.911	2	.455	7.384	.002 **
Lesion-by-reward-ratio	.042	2	.021	.340	.714
Error (lesion)	.370	16	.023		
Error (reward ratio)	1.974	32	.062		

ANOVA summary table for intermediate panel of Figure 16:

Source of variance	SS	df	MS	F	p
Lesion	.330	1	.330	8.792	.009 **
Reward ratio	2.664	2	1.332	18.809	.000 ***
Lesion-by-reward-ratio	.166	2	.083	1.172	.323
Error (lesion)	.600	16	.037		
Error (reward ratio)	2.266	32	.071		

ANOVA summary table for bottom panel of Figure 16:

Source of variance	SS	df	MS	F	p
Lesion	.306	1	.306	6.939	.018 *
Reward ratio	2.973	2	1.486	32.539	.000 ***
Lesion-by-reward-ratio	.126	2	.063	1.379	.266
Error (lesion)	.705	16	.044		
Error (reward ratio)	1.462	32	.046		

Table 3   The results of a two-way ANOVA of effects of DLS lesion on probabilistic risky choice behavior in the first 3 days, the intermediate 4 days, and the last 3 days of ten-day free choice phase (** *p* < 0.01, *** *p* < 0.001).

ANOVA summary table for top panel of Figure 19:

Source of variance	SS	*df*	MS	*F*	*p*
Lesion	.021	1	.021	.563	.464
Reward ratio	1.201	2	.601	6.999	.003 **
Lesion-by-reward-ratio	.101	2	.050	.588	.561
Error (lesion)	.594	16	.037		
Error (reward ratio)	2.745	32	.086		

ANOVA summary table for intermediate panel of Figure 19:

Source of variance	SS	*df*	MS	*F*	*p*
Lesion	.002	1	.002	.093	.764
Reward ratio	3.533	2	1.767	26.241	.000 ***
Lesion-by-reward-ratio	.104	2	.052	.771	.471
Error (lesion)	.281	16	.018		
Error (reward ratio)	2.154	32	.067		

ANOVA summary table for bottom panel of Figure 19:

Source of variance	SS	*df*	MS	*F*	*p*
Lesion	.026	1	.026	2.935	.106
Reward ratio	5.724	2	2.862	48.346	.000 ***
Lesion-by-reward-ratio	.233	2	.116	1.968	.156
Error (lesion)	.142	16	.009		
Error (reward ratio)	1.894	32	.059		

Table 4   The results of a two-way ANOVA of effects of OFC lesion on probabilistic

risky choice behavior in the first 3 days, the intermediate 4 days, and the last 3 days of

ten-day free choice phase (* *p* < 0.05, ** *p* < 0.01, *** *p* < 0.001).

ANOVA summary table for top panel of Figure 22:

Source of variance	SS	*df*	MS	*F*	*p*
Lesion	.095	1	.095	2.695	.120
Reward ratio	.692	2	.346	8.338	.001 **
Lesion-by-reward-ratio	.115	2	.058	1.388	.264
Error (lesion)	.567	16	.035		
Error (reward ratio)	1.328	32	.042		

ANOVA summary table for intermediate panel of Figure 22:

Source of variance	SS	*df*	MS	*F*	*p*
Lesion	.172	1	.172	5.592	.031 *
Reward ratio	1.851	2	.926	12.235	.000 ***
Lesion-by-reward-ratio	.163	2	.081	1.077	.353
Error (lesion)	.492	16	.031		
Error (reward ratio)	2.421	32	.076		

ANOVA summary table for bottom panel of Figure 22:

Source of variance	SS	*df*	MS	*F*	*p*
Lesion	.158	1	.158	4.194	.057
Reward ratio	3.616	2	1.808	35.494	.000 ***
Lesion-by-reward-ratio	.024	2	.012	.237	.790
Error (lesion)	.601	16	.038		
Error (reward ratio)	1.630	32	.051		

Table 5 The results of a two-way ANOVA of effects of mPFC lesion on probabilistic risky choice behavior in the first 3 days, the intermediate 4 days, and the last 3 days of ten-day free choice phase (* $p < 0.05$, *** $p < 0.001$).

ANOVA summary table for top panel of Figure 25:

Source of variance	SS	df	MS	F	p
Lesion	.058	1	.058	1.356	.264
Reward ratio	.293	2	.146	2.535	.097
Lesion-by-reward-ratio	.434	2	.217	3.755	.036 *
Error (lesion)	.599	14	.043		
Error (reward ratio)	1.618	28	.058		

ANOVA summary table for intermediate panel of Figure 25:

Source of variance	SS	df	MS	F	p
Lesion	.002	1	.002	.061	.809
Reward ratio	1.271	2	.636	11.509	.000 ***
Lesion-by-reward-ratio	.226	2	.113	2.047	.148
Error (lesion)	.375	14	.027		
Error (reward ratio)	1.546	28	.055		

ANOVA summary table for bottom panel of Figure 25:

Source of variance	SS	df	MS	F	p
Lesion	.040	1	.040	1.084	.315
Reward ratio	2.010	2	1.005	17.693	.000 ***
Lesion-by-reward-ratio	.167	2	.084	1.471	.247
Error (lesion)	.519	14	.037		
Error (reward ratio)	1.591	28	.057		