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ABSTRACT

The effect of chemogenetic modulation of the medial prefrontal cortex to the nucleus accumbens circuit on decision-making toward risk preference

Decision-making necessitates the estimation of various options' potential costs and benefits, a process that is notably impaired in individuals with psychiatric conditions such as gambling disorder. This impairment is further exacerbated by the abuse of substances like cocaine, leading to significant disruptions in cognitive functions related to decision-making abilities. These disruptions are believed to be associated with deficiencies in top-down executive control primarily regulated by medial prefrontal cortex (mPFC) and its interconnected fronto-striatal neural network to the nucleus accumbens (NAc). Specifically, the prelimbic (PrL), a sub-region of the mPFC, is more involved in executive functions like estimating the value of rewards and punishments. The NAc core, a subregion of the NAc, which receives glutamatergic input from PrL, plays an essential role in reward related motivation. However, it remains unclear whether directly manipulating the PrL to NAc core neural circuits' activity can alter risky choices. In the present study, we adopted a sophisticated chemogenetic technique using Designer Receptor Exclusively Activated by Designer Drug (DREADD) to examine the role of this circuit in risky decision-making in rat gambling task (rGT). Viruses encoding Cre-dependent hM4Di or hM3Dq that expresses artificial Gi or Gq protein, and Cre recombinase are injected into the prelimbic cortex (PrL) and the NAc core, respectively. Then, rats were trained in 30-minute daily sessions to freely choose one of the four windows with different probabilities of reward/punishment contingencies until a stable preference pattern was observed. Once they were categorized as risk-averse or risk-seeking, rats were examined their preference shift in two separate environments. One was exposed to cocaine sensitization regime and was examined how their risky decision-making changes under the modulation of neuronal activity with JHU37160, a specific synthetic ligand to DREADD. The other was exposed to DREADD mediated neuronal activity modulation without cocaine administration. As expected, risk-averse rats shifted their preference toward risk-seeking by chronic cocaine. Interestingly, however, this effect was significantly attenuated by Gi activation of PrL-NAc core circuit. These effects were not observed

in risk-averse rats with Gq DREADD expression and the risk-seeking. These results indicate that the PrL-NAc core circuit is one of the major target areas exacerbated by chronic cocaine leading to risky decision-making and further suggest that this effect can be controlled by neuronal activity modulation to this circuit. Moreover, through western blot analysis, the study revealed molecular mechanisms underpinning these behavioral changes, particularly focusing on DARPP32 phosphorylation and L-type calcium channel (LTCC) expression, crucial elements in cocaine-induced behavioral alterations. Phosphorylation of DARPP32 at the serine 97 site was found to be closely associated with risky decision-making behaviors, while the CaV1.2 channel identified as a potential molecular target for interventions aimed at mitigating cocaine-induced shifts towards riskier preferences. These results advance our understanding of the neurobiological underpinnings of decision-making toward risky choice in the context of substance use and gambling disorders, suggesting that targeted modulation of specific neural circuits may offer novel therapeutic strategies.

Key words: rat gambling task, cocaine sensitization, prelimbic cortex, nucleus accumbens core, designer receptor exclusively activated by designer drug

1. Introduction

Decision-making is an intricate behavior that requires the integration of numerous brain regions and circuits. This complex process involves anticipating reward uncertainty, assessing the likelihood of punishment, and evaluating the costs and benefits of various options[1]. Under typical conditions, individuals are capable of weighing these factors to make rational decisions. However, certain psychiatric conditions, notably gambling disorder, are characterized by impaired decision-making, causing individuals to make risky choices[1,2]. This tendency is exacerbated by exposure to highly addictive substances, such as cocaine[3].

To facilitate a systematic examination of decision-making, the Iowa Gambling Task (IGT) has been developed[4]. This neuropsychological tool aims to assess human decision-making capabilities by considering elements such as risk evaluation, outcome uncertainty, immediate rewards, and the deferment of punishments[5]. Building on the basic concept of the IGT, a rodent version of the gambling task (rGT) has been developed for translational research on decision-making using animal models[6]. During the IGT, participants are presented with four decks of options, each containing advantageous and disadvantageous choices. The probability of risk, represented by the potential loss of money, and reward, represented by the potential gain of money, varies across the decks[5]. The rGT task shares many characteristics with the IGT, as it requires animals to make choices among a set of options with varying probabilities of rewards but differs in the use of sucrose pellets as a reward and time-out as a punishment (risk)[7]. The rodent version of the IGT enables researchers not only to assess choice preference under conditions of uncertainty involving risk and rewards but also to study more detailed mechanisms such as the effect of direct manipulation of neuronal circuit activity, which is not available with human subjects.

Considering drug addiction as a chronic brain disease is crucial, as the inability to control drug use is intricately linked to disruptions in various cognitive functions, particularly decision-making[8]. Such disruptions are believed to be associated with deficiencies in top-down executive control, which is regulated by the dorsolateral prefrontal cortex (dlPFC) and its interconnected neural networks. The dlPFC plays a pivotal role in modulating cognitive processes that are adversely affected by substance use disorders[9-10]. Clinical evidence suggests that damage or dysfunction within

the dlPFC and drug addiction can lead to a preference for risky decision-making tasks such as the IGT[11], highlighting the critical role of this brain region in addiction and decision-making.

Expanding upon this groundwork, research utilizing rodent models has elucidated the mechanisms behind maladaptive decision-making, revealing abnormal neuronal activity in crucial brain regions such as the medial prefrontal cortex (mPFC) and the nucleus accumbens (NAc)[12-14]. The mPFC plays a crucial role in the regulation of decision-making behaviors including working memory, attention, planning, impulse control, action/outcome monitoring, behavioral flexibility, and motivation.[1,15,16] Pharmacological lesion studies indicate that, among the two sub-regions of the mPFC, the prelimbic (PrL), which is functionally homologous to the dlPFC in humans[17], is more involved in executive functions like evaluating the value of rewards and punishments in decision-making compared to the infralimbic (IL)[17,18]. The NAc, known as a hub for reward-related motivation, receives glutamatergic input from the mPFC and plays an important role in reward-related motivation in decision-making.[19] Increased neuronal activity in this region has been linked to making risky choices[20], while inactivation has been shown to reduce preference for risky options in decision-making tasks.[21] The core, a subregion of the NAc, which receives glutamatergic input from the prelimbic cortex, plays an essential role in determining the value of rewards based on effort or delay, which is related to motivation.[21]

Designer Receptor Exclusively Activated by Designer Drug (DREADD) is a sophisticated chemogenetic technology used for circuit studies. It is based on genetically engineered artificial G-protein coupled receptors (GPCRs) that can only be activated by artificial ligands such as clozapine-N-oxide (CNO) and JHU37160 (J60).[22,23] Compared to traditional pharmacological studies, DREADDs allow for precise targeting of specific neurons and their projections in a non-invasive manner. By incorporating the coding sequence of DREADDs into a viral vector in an inverted orientation, functional expression of DREADDs in target cells can only occur in the presence of Cre recombinase, which is introduced through surgical injection of an additional retrograde viral vector encoding the gene into the terminal area of the target brain region.[24]

DREADDs are divided into three main categories: Gi, Gq, and Gs, based on their interaction with different types of G proteins in the G protein signaling cascade. Each type of DREADD plays a distinct role in modulating neural activity and can affect various aspects of brain function,

including behavior, cognition, and emotion. Gi-DREADD (hM4Di) inhibits neuronal activity and synaptic silencing. When Gi-DREADD is activated, the Gi α-subunit inhibits adenylyl cyclase, resulting in a decrease in cAMP levels and protein kinase A (PKA) activity (synaptic silencing). Gq-DREADD (hM3Dq), on the other hand, enhances neuronal activity through the activation of phospholipase C, which hydrolyzes phosphatidylinositol 4,5-biphosphate (PIP_2). This causes the potassium channel (KCNQ) to close,[22,25] resulting in membrane depolarization and an increase in neuronal excitability. These DREADDs are designed to interact exclusively with synthetic ligands, such as CNO and J60, and not with endogenous ligands. Furthermore, it has been shown that even high levels of virally mediated expression of various DREADDs do not affect basal phenotypes.[24] Compared to CNO, which has poor brain penetration and lower potency *in vivo*, J60 has higher potency and occupancy *in vivo*.[26]

It has not yet been determined whether direct modulation of the prelimbic cortex (PrL) to nucleus accumbens core (NAc) circuit would lead to changes in decision-making in gambling behavior, in the presence of normal or psychiatric disorder states induced by cocaine, despite the importance of this circuit in decision-making. Thus, this study aims to examine this relationship by utilizing the rGT to examine changes in risky choice behavior following modulation of neuronal activity in this pathway using DREADD technology. Furthermore, the study investigates the underlying molecular mechanisms through western blot analysis.

2. Methods

2.1 Subjects

Male Sprague-Dawley rats (6 weeks old) were obtained from Orient Bio Inc. (Seongnam-si, Korea). They were housed two per cage in a 12-hour light/dark cycle room (lights out at 8:00 pm), and all experiments were conducted during the daytime. To increase their motivation for the task, rats received a restricted diet that lowered their body weight to 85% of normal levels, which started 2 days before the pre-training experiments and was maintained until the end of the experimentation. All animal use procedures were conducted according to an approved Institutional Animal Care and Use Committee protocol of Yonsei University College of Medicine.

2.2 Drugs

Cocaine hydrochloride was purchased from Belgopia (Louvain-La-Neuve, Belgium). It was dissolved in sterile 0.9% saline to a final concentration of 15 mg/kg and injected intraperitoneally (IP) during cocaine sensitization. JHU37160 (Hellobio, Bristol, UK) was dissolved in 0.9% saline to a final concentration of 0.5mg/kg, respectively.

2.3 Stereotaxic virus infusion surgery

Rats were anesthetized with IP ketamine (100 mg/kg) and xylazine (6 mg/kg) and placed in a stereotaxic instrument with the incisor bar at 5.0 mm above the interaural line. After making an incision on the skin, the skull surface was exposed and infusion cannulas (28 gauge, Plastics One, Roanoke, VA, USA) connected to 1μl Hamilton syringes (Reno, NV, USA) via PE-20 tubing were angled at 10º to the vertical line and aimed at the PL (A/P, +3.2; L, +1.3; D/V, -4.0 mm from bregma and skull) and the NAc core (A/P, +3.2; L, +2.8; D/V, -7.1 mm from bregma and skull). AAVs (0.5 μl; 7 × 10^12 vg/ml) encoding an inhibitory Cre-dependent hM4Di (pAAV5-hSyn-DIO-hM4D(Gi)-mCherry, Addgene, Watertown, MA, USA) or Cre-dependent hM3Dq (pAAV5-hSyn-DIO-hM3D(Gq)-mCherry, Addgene, Watertown, MA, USA) were infused bilaterally into the PL for 5 minutes. For circuit experiments, AAV (0.5 μl; 7 × 10^12 vg/ml) encoding retrograde Cre recombinase (AAVrg-hSyn-Cre, Addgene, Watertown, MA, USA) was additionally infused

bilaterally in the NAc core. Infusions were conducted by an infusion pump at a rate of 0.1 μl/min. The infusion cannulas were slowly removed after an additional 5 minutes to allow for viral diffusion. After viral injection, rats were returned to their home cages for a 2-week recovery period.

2.4 Tissue preparation and immunostaining for cFos cell-counting

Double immunofluorescence labeling of c-Fos and a neuronal marker, NeuN, was performed, and c-Fos expression within neurons was counted23 to verify whether the DREADD was operating efficiently. A separate group of rats, which did not undergo rGT and cocaine sensitization, received viral injections of Cre-dependent Gi or Gq DREADD on the PL and retrograde Cre recombinase on the NAc core. After 6 weeks of Cre-dependent Gi or Gq DREADD expression, perfusion was conducted 90 minutes after saline or cocaine injection to analyze c-Fos expression in the NAc core and the PL. J60 (0.5 mg/kg) or saline injection was held 45 minutes after the cocaine/saline injection, which coincided with the challenge session timeline. The rats were deeply anesthetized with ketamine (100 mg/kg) and xylazine (6 mg/kg) and then perfused transcardially with 10 mM PBS (pH 7.4) followed by 4% paraformaldehyde solution. Brains were removed and post-fixed in ice-cold 4% paraformaldehyde solution for 24 hours, followed by cryoprotection in 30% sucrose solution at 4 °C for 3 days, and stored at -80 °C. Free-floating coronal sections (50 μm) from frozen tissue blocks were prepared on a freezing microtome (HM 525, Fisher Scientific, Waltham, MA, USA). Sections were blocked for 1 hour in 10 mM PBS containing 5% normal goat serum (Jackson Immunoresearch Laboratories, West Grove, PA, USA) and 0.3% triton X-100. Then they were incubated overnight at 4 °C with anti-c-Fos (1:2000) (Cell Signaling, Beverly, MA, USA) and anti-NeuN antibodies (Abcam, Cambridge, UK) diluted in 10 mM PBS containing 2% normal goat serum and 0.1% triton X-100. Following overnight incubation, the sections were rinsed with 0.1% triton X-100 three times, and then they were incubated with anti-rabbit secondary antibody conjugated with Alexa 488 (1:2000; Invitrogen, Waltham, MA, USA) and anti-mouse secondary antibody conjugated with Alexa 405 (1:2000; Invitrogen, Waltham, MA, USA) for 2 hours at room temperature. Again, rinsed with 0.1% triton X-100 three times, the sections were transferred onto slide glasses with Vectashield mounting medium (H1400; Vector Laboratories, Peterborough, UK).

2.5 c-Fos cell-counting analysis and histology

About 10 images co-labeled with c-Fos and NeuN were acquired each from the PL and the NAc core. The images that had inappropriate signals or damaged regions were excluded from the final data. All images were acquired under the LSM710 confocal laser scanning microscope (Carl Zeiss, Oberkochen, Germany) with a 405 nm laser diode for NeuN, and a 488 nm argon laser for c-Fos. The thickness of the images was 6 μm and obtained using a 20x objective (numerical aperture 0.8) with 0.6x digital zoom. All images were taken with a resolution of 1024 pixels in x-y dimensions. Cell-counting was performed using Zen desk software (Carl Zeiss), which semi-automatically counted the number of NeuN cells and a portion of them including c-Fos expression. Minimum areas of the cells containing c-Fos or NeuN signals were optimized according to the size of the cells or signals in the PL and NAc core and fixed during all analyses. Smooth and Sharpen settings were also optimized and fixed. Exceptionally, a threshold of each image was manually set up depending on the quality of the image. The level of NeuN or c-Fos expression was manually assessed. All analysis was conducted by an experimenter blind to the experimental groups.

2.6 Behavioral experiment

2.6.1 Apparatus

The rGT was conducted in a set of eight identical touchscreen-based automated operant chambers housed in dense sound- and light-attenuating boxes measuring 68.6 cm high, 60.7 cm long, and 53.5 cm wide (Campden Instruments Ltd., Leics, UK). Each chamber was equipped with a house light (light-emitting diode), touch-sensitive liquid crystal display monitor (touchscreen; 15.0 inches, screen resolution 1,024×768), pellet dispenser, and food magazine unit (with light and infrared beam to detect entries) facing the touchscreen. Whisker Standard Software (Campden Instruments, Ltd., Leics, UK) was used as the controlling software, and two computers were used to control the four chambers each.

2.6.2 Pre-training

Animals were trained once daily in a 30-minute session, five days per week. Sucrose pellets (45 mg) (Bio-Serv, Flemington, NJ, USA) were used as a reward. In stage 1, animals were first habituated to the touchscreen chamber for one session. In stages 2 and 3, which lasted over five daily sessions, animals were trained to learn the relationship between the light stimulus on the screen and the reward pellet, and to touch the screen to receive a pellet as a reward. In this stage, the inter-trial interval (ITI) of the 5-second rule was first applied, such that animals had to wait for 5 seconds after pushing their noses into the food magazine to start a new trial. In stage 4, which lasted over 16 to 18 daily sessions, animals serially learned to touch one of the four windows which were randomly lit, within different stimulus durations (starting from 40 seconds, then serially reduced to 20, and finally, 10 seconds), to receive one pellet. Sessions were completed within 100 trials or 30 minutes, whichever came first. In this stage, animals learned for the first time that they were punished with a time-out (i.e., the white house light was lit for 5 seconds) if they touched the screen without waiting during ITI (premature response) or if they did not touch the screen within the stimulus duration (omission). They were also punished if they touched other windows which were not lit (incorrect). When the accuracy was greater than 80% and omissions were fewer than 20%, the animals were considered to have acquired the task successfully.

2.6.3 Rat Gambling task

Essentially, During the rat gambling task, animals were confronted with four choices differing in their probability and magnitude of reward (food) and punishment (time-out), and they had to learn an optimal strategy to determine the choice that provided the most reward per session. In stage 5, which lasted over 7 daily sessions, animals learned for the first time the relationship between each window and the reward/punishment ratio assigned to that window. Touching the first window (P1) produced 1 pellet (90%) or a 5-second time-out (10%); the second window (P2) yielded 2 pellets (80%) or a 10-second time-out (20%); the third window (P3) resulted in 3 pellets (50%) or a 30-second time-out (50%); and the fourth window (P4) provided 4 pellets (30%) or a 40-second time-out (70%). In this stage, one of the four windows was randomly lit for 10 seconds, and animals were punished (i.e., the white house light was lit for 5 seconds) for a premature response. Additionally, for the first time in this stage, animals were punished (time-out, i.e., the white house light was lit,

and all the windows on the screen simultaneously flashed for 5 to 40 seconds) even on correctly touching the screen according to the pre-designated schedule for each window.

From stages 1 to 5, only one of the four windows on the screen was randomly lit. However, in stage 6, four windows were simultaneously lit, and animals were allowed to choose one of the four windows. The reward and punishment settings designated for each window were the same as those introduced in stage 5, as well as the ITI and stimulus duration time. Depending on which window animals chose, they received either a reward (pellet) or punishment (time-out) with differently programmed probabilities. Once a trial was finished, regardless of the outcome, they encountered four different choices in the next trial, and this process was repeated for 30 minutes. Hypothetically, if one window was chosen exclusively, the amount of reward pellets per session that an animal could obtain would have been as follows: P1, 295; P2, 411; P3, 135; and P4, 99 pellets. The P1 and P2 options were called 'advantageous choices' because they had lower punishment probabilities despite a smaller gain of pellets at once, which eventually maximized rewards per session. On the other hand, the P3 and P4 options, despite the larger number of pellets provided at once, were called 'disadvantageous choices' because of the high probability of punishment, resulting in less overall gain of rewards per session. The percentage of choices ([number of choices for a specific window divided by the total number of choices made] ×100) was used to measure the animals' preferences for the 4 different windows. The baseline score for rGT was calculated by averaging the preference score from the last three days of the free-choice task. Rats were divided into two groups based on their score of the most optimal choice, P2. Then, rats were classified as a risk-averse if their baseline P2 choice preference was equal to or greater than 60%, and as a risk-seeking if their baseline P2 choice preference was less than 60%.

Choice-related behavioral parameters were also measured during the rGT session. The percentage of premature responses, which indicated impulsivity, was calculated as [premature response/total initiated trials (omission + premature + choice response)] × 100. Omission percentage, which indicated attention or motivation to the task, was calculated as [omission / (omission + choice response)] × 100. Perseverative response ratio, which measured repeated screen touch during punishment, was calculated as (total number of screen touches during punishment / the total number of punishment trials). Feed-tray entry ratio, which measured repeated entry of the food magazine during ITI, was calculated as [the number of feed-tray entries during ITI / total initiated trials

(omission + premature + choice response)]. Reward latency (the time required for animals to enter the food magazine to obtain the reward after a screen touch when it was rewarded) and correct latency (the time required for animals to touch one of the four illuminated screens, after the end of the ITI) were also analyzed.

2.6.4 Neuronal activity manipulation in Cocaine sensitization with rGT (experiment 1)

After the rGT stage 6 sessions were completed, the cocaine pre-exposure period was initiated. In this phase, adhering to a well-established cocaine sensitization protocol, rats were administered cocaine (15mg/kg, IP) once daily for seven consecutive days. On Day 1 and Day 7, they received a cocaine injection followed by an rGT session 60 minutes later, whereas on the intervening days, they only received cocaine injections in their home cage. Upon completing the cocaine pre-exposure, five to seven days of reminder rGT sessions were performed throughout the seven-day withdrawal phase. After the withdrawal period ended, a challenge rGT session was conducted. During this session, cocaine was administered 60 minutes before the rGT, followed by a DREADD agonist J60 (0.5mg/kg, IP) or saline injection 15 minutes prior to the rGT. Seven days later, a session of the counterbalanced challenge was held in the same manner.

2.6.5 Neuronal activity manipulation during rGT without cocaine (experiment 2)

After completing stage 6 of the rGT task, the J60 pre-exposure period began. This period followed a cocaine sensitization schedule, where the rats received a daily injection of J60 (0.5 mg/kg, IP) for seven consecutive days. On Days 1 and 7, the rats received a J60 injection 15 minutes before the rGT session, while on the remaining days, they only received J60 injections in their home cages. Once the J60 pre-exposure period was finished, five to seven days of remind rGT sessions were conducted during the seven-day withdrawal phase. After the withdrawal period ended, a challenge rGT was administered. During this session, the rats received a J60 injection or a saline injection 15 minutes before the rGT task. Three days later, a session of the counterbalanced challenge was held in a similar manner.

2.7 Tissue preparation and western blotting

Rats were decapitated right after last counterbalanced rGT was completed. Brains were rapidly removed, and coronal sections were obtained with an ice-cold brain slicer (1 mm thick extending 1.60–2.60 mm from bregma). The Dual Fluorescent Protein (DFP) flashlight from NIGHTSEA (1560 Industry Rd, Hatfield, PA 19440, USA) was used for histological analysis detecting mCherry red fluorescent expressed rats in PrL region. Bilateral PL and NAc core tissues were obtained using a surgical blade. In the western blotting procedure, tissues were homogenized in lysis buffer (pH 7.4) containing 0.32 M sucrose, 20 mM Tris, 2 mM EDTA, 1% SDS with protease, and phosphatase inhibitor. The concentration of protein was determined by using the Pierce BCA Protein Assay Kit (Thermo Scientific). Samples were then boiled for 10 minutes and subjected to SDS-polyacrylamide gel electrophoresis. Proteins were transferred electrophoretically to nitrocellulose membranes at 180 mA for 100 min. Membranes were blocked with 5% bovine serum albumin (BSA) in PBS-T buffer (10 mM phosphate-buffered saline plus 0.05% Tween-20) and were incubated overnight at 4 ℃ with primary antibodies [against total DARPP-32, phospho DARPP-32 Thr 34 (1:1000; Abcam), phospho DARPP-32 Ser97 (1:1000; Abcam)] diluted in PBS-T with 5% BSA. Primary antibodies were detected with peroxidase-conjugated secondary antibodies, anti-rabbit IgG (1:2000; KOMA Biotech, Seoul, Korea), anti-mouse IgG (1:5000; Cell Signaling), diluted in PBS-T with 5% skim milk. Membranes were developed using enhanced chemiluminescence (ECL) reagents (Amersham Biosciences) followed by exposure to X-ray film. Band intensities were quantified based on densitometric values using Fujifilm Science Lab 97 Image Gauge software (version 2.54).

2.8 Statistical analysis

Data were shown as mean + standard error of the mean (SEM), and they were analyzed using SigmaPlot (version 12.5), or GraphPad Prism (version 10.2). T-test was used to compare the mean basal level expression of risk-averse and risk-seeking rats in western blot analysis. One-way analysis of variance (ANOVA) was used to compare the c-Fos+ neurons in cell counting or molecular expressions following cocaine and J60 administration in western blot. Two-way ANOVA analysis or two-way repeated ANOVA measures were used to compare the preference scores following cocaine and subsequent DREADD agonist (J60) injection. ANOVAs were followed by *post hoc* Tukey or Bonferroni t-test. Differences between experimental conditions were considered statistically significant when $p < 0.05$.

3. Result

3.1 DREADDs effectively modulate neuronal activity

It is well known that c-Fos serves as an indicator of neuronal activation, and cocaine increases neuronal activity, thereby enhancing c-Fos expression. Therefore, before proceeding to full behavioral experiments, a series of tests were conducted to assess the functional effects of DREADD mediated modulation of neuronal activity by comparing the c-Fos expression per NeuN (Figure 1A).

Firstly, among rats expressing Gi DREADDs, c-Fos expression levels were compared between those that received saline (COC-sal) or JHU37160 (COC-J60) 45minutes after the cocaine injection, and a control group (Sal-sal) that received neither cocaine nor the agonist (Sal-sal). The one-way ANOVA conducted on the result of c-Fos expression in PrL region by drug treatment [$F_{2, 23} = 6.549$, P=0.006] (Figure 1B) showed significant differences among three groups. Post hoc Tukey's multiple comparison test revealed that a group of rats treated saline after cocaine injection (COC-sal), exhibited a significant increase in c-Fos expression compared to the control group ($p < 0.05$) indicating an increase of neuronal activity due to cocaine. whereas another group of rats treated JHU37160(J60) after cocaine injection (COC-J60) showed effectively reduced c-Fos expression compared to COC-sal group ($p < 0.01$) and there was no significant difference between COC-J60 and control group, suggesting that Gi DREADD activation successfully moderated neuronal activity in the PL region expressing the DREADD receptor. There were no significant differences between groups in the NAc-core region (Figure 1C).

Secondly, I conducted similar experiments with rats expressing Gq DREADDs. There were significant differences between groups in PrL region as revealed in one-way ANOVA following drug treatment [$F_{2, 21} = 29.56$, $P < 0.001$] (Figure 1D). Post hoc analysis of Tukey's multiple comparison revealed that rats treated with J60 after cocaine showed an increase in c-Fos expression than those induced by cocaine alone ($P < 0.001$), which is higher than control group ($P < 0.001$). Furthermore, the J60 treatment after cocaine injection also significantly increased c-Fos expression in the NAc region [$F_{2, 21} = 18.16$, $P < 0.001$] compared to the control group ($P < 0.001$) (Figure 1E). But it didn't bolster the effect of cocaine in NAc core region. These results confirmed that both Gi and Gq DREADDs were working to significantly modulate neuronal activity in the opposite directions.

Additionally, sacrificing the rats 45 minutes after the administration of saline or J60, following cocaine treatment, were well matched with the timeline for the upcoming behavioral experiments, demonstrating the effectiveness of DREADD-mediated neuronal modulation under similar conditions (Figure 1A).

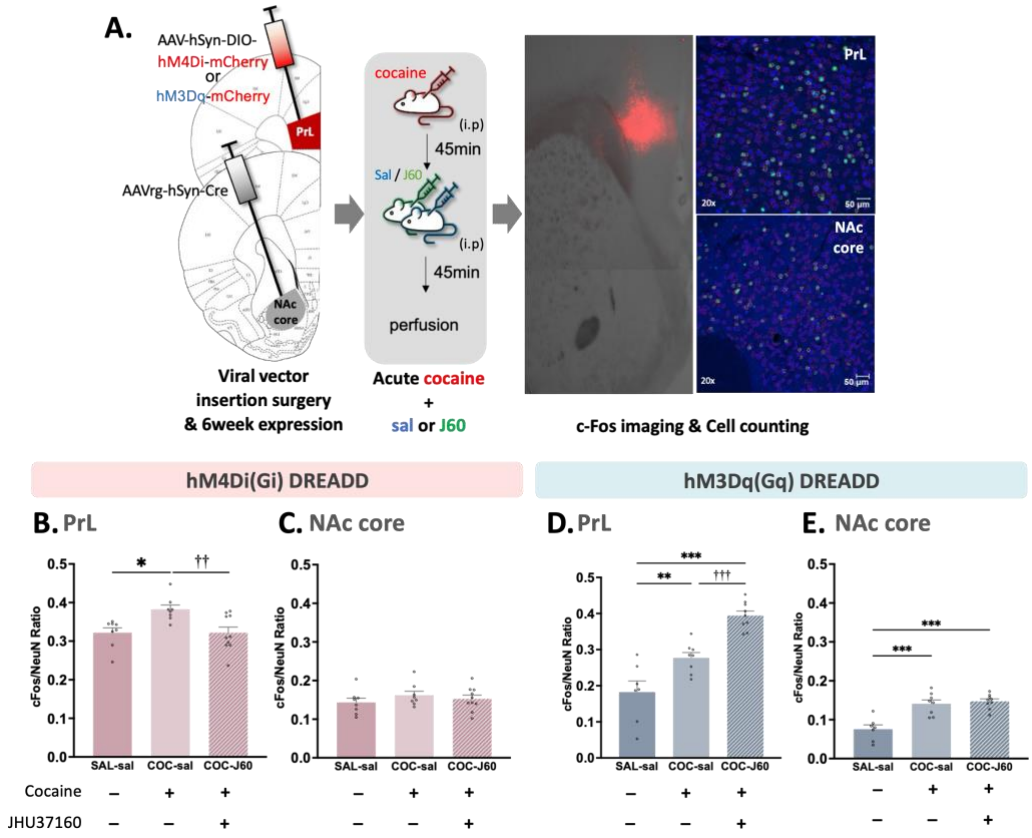

Figure 1. **Functional test of DREADD by comparing c-Fos expression.** The illustration represents the strategy for delivering DREADD vectors into the rat brain and the procedures for comparing cFos expression. **A.** It shows the injection of an hM4Di(Gi)-mCherry or hM3Dq(Gq)-mCherry DREADD virus into the prelimbic (PrL) region and retrograde Cre recombinase into the nucleus accumbens (NAc) core. The use of Cre recombinase ensures that the expression of Gi or Gq-DREADD is restricted to neurons that project from the PL to the NAc. The experimental protocol shows that rats received cocaine injections, followed by either saline or J60 (a DREADD agonist) 45 minutes later, and were then perfused to prepare for brain analysis. The fluorescent image of brain slices likely shows the localized red fluorescence indicating mCherry-tagged Gi or Gq-DREADD expression on PL region. And the confocal images show counting of expressed c-Fos on NeuN-tagged neurons. **B-C.** Cocaine significantly increased the c-Fos level ($P < 0.05$), and this

phenomenon was significantly attenuated by Gi DREADD activation using J60 ($P < 0.01$) in the PrL region. But there were no significant changes in the NAc core. **D-E.** Gq DREADD virus-expressing rats show a significant increase of c-Fos expression compared to cocaine-treated rats by injection of J60 ($p < 0.01$) in the PrL region. And there was also significant increase of c-Fos expression comparing control groups in both PrL ($p < 0.001$) and NAc core regions ($p < 0.01$). The numbers of rats for each group are as follows: [Gi]Sal-sal (8), [Gi]Coc-sal (7), [Gi]Coc-J60 (10), [Gq]Sal-sal (7), [Gq]COC-sal (8), [Gq]COC-J60 (9).

3.2 Classification into risk-averse and risk-seeking after rGT

Figure 2 shows the whole process of experiments. Rats that had learned the probabilities associated with four different choices in stage 5, following the surgical implantation and expression of DREADD receptors in stage 4, successfully proceeded to stage 6 (rGT) designed to allow them to make free choices based on their preferences. They were allowed to perform for 18 days until their preferences were stabled. Subsequently, rats were divided into two groups based on their preference for the P2 choice, which represents the most optimal choice, on average of the last three days. Rats that chose the P2 option more than 60% among the four options were categorized as risk-averse, demonstrating a tendency to avoid risk, while those selecting P2 less than 60% were classified as risk-seeking, indicating a propensity to pursue riskier outcomes (bottom row in Fig.2). The rats were then transitioned to the next phase of the study, where they were placed in two distinct environments. One group of rats was exposed to cocaine-sensitization to mimic the psychiatric disorder state (Exp. 1), while the other group remained unexposed to cocaine (Exp. 2) (top row in Fig.2).

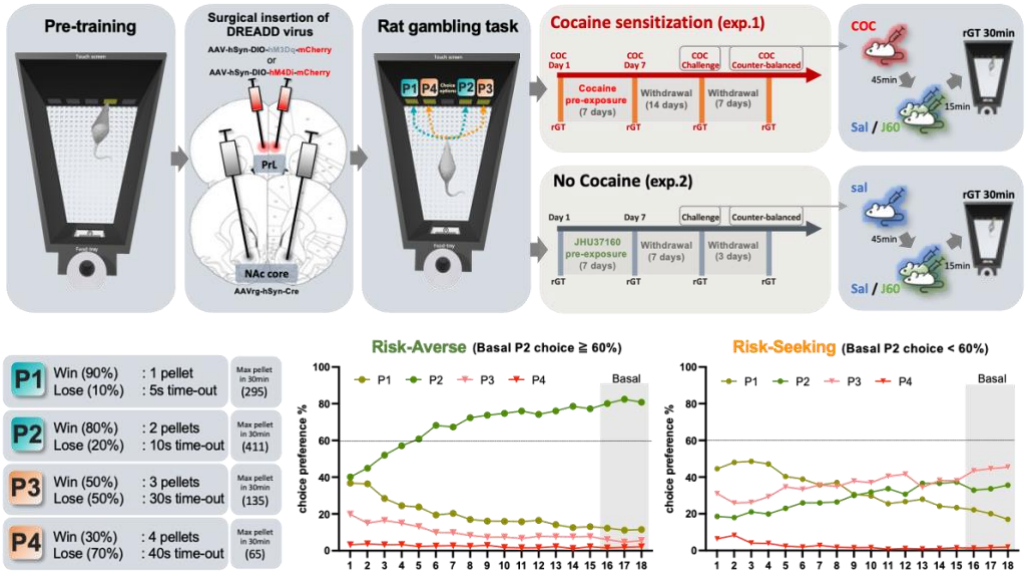

Figure 2. Schematic diagram for the whole behavioral experiment of rGT with Gi or Gq DREADD. From left to right, the rats underwent a series of steps in the experimental protocol. Initially, they were pre-trained in the touch-screen chamber, followed by the surgical insertion of the DREADD virus, which is exclusively expressed by cre-recombinase inserted in the NAc core. Subsequently, they underwent training to learn and choose among four options (P1, P2, P3, P4) with varying probabilities and numbers of reward and punishment as a time-out (bottom left) until their preference choices stabilized. Next, they were divided into two separate groups based on their performance in the last three days of rGT stage 6: risk-averse and risk-seeking. (bottom right). Experiments were then conducted in two different environments. One involved cocaine sensitization (Exp. 1), while the other represented the normal state without cocaine administration (Exp. 2). In Exp. 1, on the first challenge day, rats were treated with saline or J60 (I.P.) 45 minutes after cocaine administration and 15minutes later, they underwent the rat gambling task for 30 minutes. On the second challenge day in a counter-balanced manner, the saline or J60 administration was conducted. In Exp. 2, the same protocol was conducted, with cocaine being replaced by saline. (Top right)

3.3 The effect of neuronal activity modulation in PrL to NAc circuit on risk choice behavior in psychiatric condition (Exp.1)

To investigate how drug abuse affects decision-making and whether activating Gi or Gq DREADD in the PrL-NAc core circuit can modify preferences towards risky options, cocaine sensitization was conducted combined with rGT. Consistent with previous results,[9] cocaine induced a significant increase in risky choices in risk-averse rats, while this effect was not observed in risk-seeking rats, suggesting a potential floor effect in these rats. Administration of J60, aimed at reducing neuronal activity by activating Gi DREADD on the PrL-NAc core circuit, selectively attenuated the cocaine-induced preference for risky choices in the risk-averse rats (Figure 3A). The Two-way RM ANOVA conducted on the preference choice [$F_{3, 136} = 318.2$, $P < 0.001$] and choice x drug interaction for Gi DREADD rGT risk-averse rats [$F_{6, 272} = 16.07$, $P < 0.001$] showed significant differences among three groups. Post hoc Tukey's multiple comparison test revealed a significant reduction in P2 choice following cocaine administration ($P < 0.001$) and an increase in risky options (P3, P4) ($P < 0.001$) compared to basal. This effect was notably alleviated by Gi DREADD activation with injection of DREADD agonist(J60). Specifically, the decrease in P2 choice was attenuated ($P < 0.01$), and the increase in P3 choice induced by cocaine was effectively blocked by J60 administration ($P < 0.001$). Contrary to Gi DREADD activation, enhancing neuronal activity of PrL-NAc core circuit by activation of Gq DREADD did not yield significant alterations against cocaine effect on risk-averse rats (Figure 3C). The Two-way RM ANOVA conducted on the preference choice [$F_{3, 168} = 318.2$, $P < 0.001$] and choice x drug interaction for Gq DREADD rGT risk-averse rats [$F_{6, 336} = 16.07$, $P < 0.001$] showed significant differences for only cocaine administration. Post hoc Tukey's multiple comparison test revealed a significant reduction in P2 choice following cocaine administration ($P < 0.001$) and an increase in P3 choice ($P < 0.001$), P4 choice ($P<0.05$) compared to basal. This effect wasn't affected by J60 administration. These observations suggest that the cocaine-induced propensity for risky choices could be counteracted by reducing the neuronal activity within the PrL-NAc core circuit in risk-averse rats.

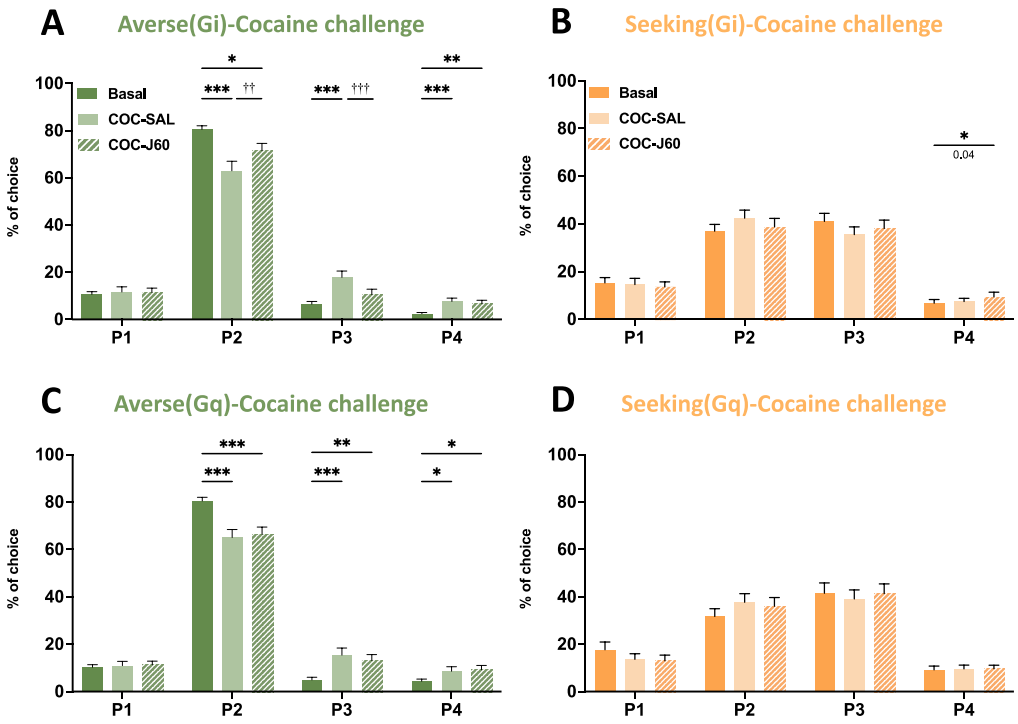

Figure 3. Cocaine increased the preference for risky options, and it was significantly attenuated by Gi DREADD activation in PrL to NAc circuit. The preference scores for each window (P1, P2, P3, P4) are shown at the Y-axis. The green and orange colors of each bar represent risk-Averse and risk-Seeking, respectively. **A-B.** Compared to the average score of the last three days in rGT stage 6 (Basal), cocaine significantly decreased P2 choice ($P < 0.001$) and increased P3 and P4 choice ($P < 0.001$) in risk-averse rats expressing Gi DREADD, but this effect was attenuated on P2 and P3 choices ($P < 0.01$, $P < 0.001$ respectively) by subsequent administration of J60. There were no significant changes in risk-seeking rats. **C-D.** In Gq DREADD-expressing rats, there was only the effect of cocaine in risk-averse rats on P2, P3 ($P < 0.001$, respectively) and P4 choices ($P < 0.05$), compared to the basal group ($P < 0.001$). The numbers of rats for each group are as follows: Gi-averse (35), Gi-Seeking (37), Gq-Averse (43), Gq-seeking (30).

3.4 The effect of neuronal activity modulation in PrL to NAc circuit on risk choice behavior in normal condition (Exp.2)

To measure the effects of modulating neural activity on decision making in normal condition, similar scheme of cocaine sensitization was conducted without cocaine administration. Interestingly, modulating neuronal activity in normal condition either attenuation or enhancement has no effects on choice preferences, implying that the observed effects of Gi activation on choice preference in Exp.1 are specific to the cocaine-modified neural and behavioral state, and direct manipulation of neural activity alone, without any influence of cocaine, is not sufficient to drive changes in decision-making processes.

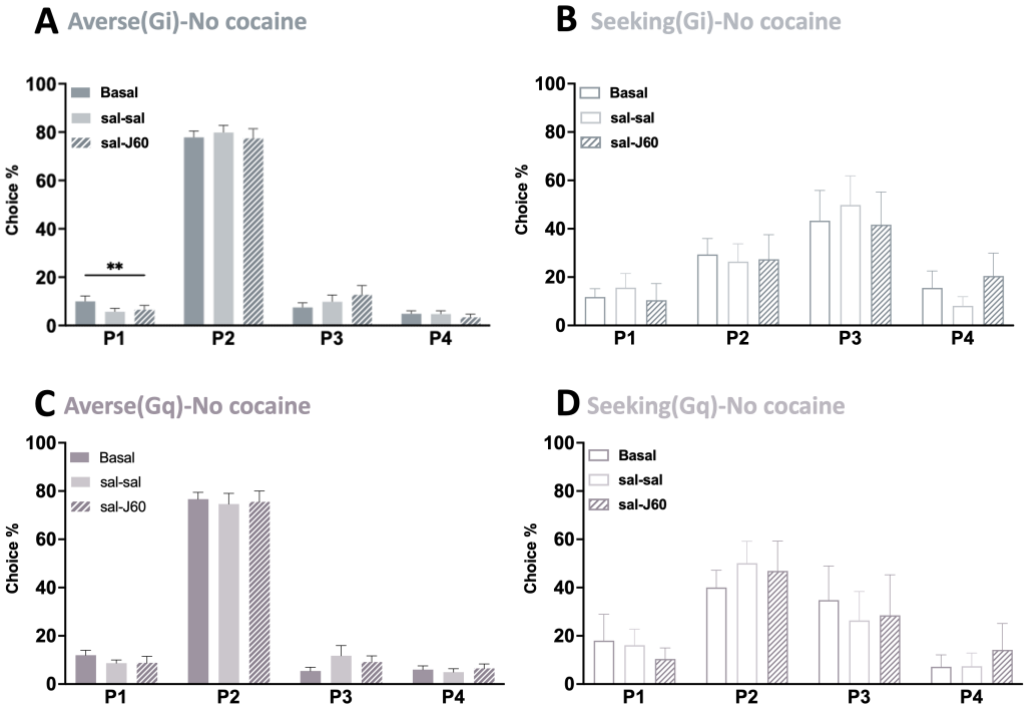

Figure 4. Neuronal activity modulation in PrL to NAc circuit no effect on preference choice in normal condition. The dark blue and purple colors of each bar represent Gi and Gq DREADD expressed rats, respectively. No significant changes were observed in all groups except for the P1 choice between sal-J60 and basal groups ($P < 0.01$) in the risk-averse rats with Gi DREADD. The numbers of rats for each group are as follows: Gi-averse (11), Gi-Seeking (5), Gq-Averse (12), Gq-seeking (4).

3.5 Cocaine and activation of Gi DREADD has no effect on the expression levels of PKAc proteins in the PrL

To investigate the underlying molecular mechanisms mediating the effects of Gi DREADD activation on choice preference in psychiatric condition, western blot analysis was conducted. As it is known that Gi DREADDs modulate neuronal activity by hyperpolarizing neurons through the inactivation of G-protein-mediated cyclic adenosine monophosphate (cAMP)[22,27], the expression levels of its downstream effector protein kinase A (PKA) were measured. It turns out that there were no significant changes observed for these proteins between all groups (Figure.5). Rats in Basal group were sacrificed on the last day of stage 6. Rats in Coc-sal and COC-J60 group were sacrificed right after the end of counter-balanced rGT session in Exp.1.

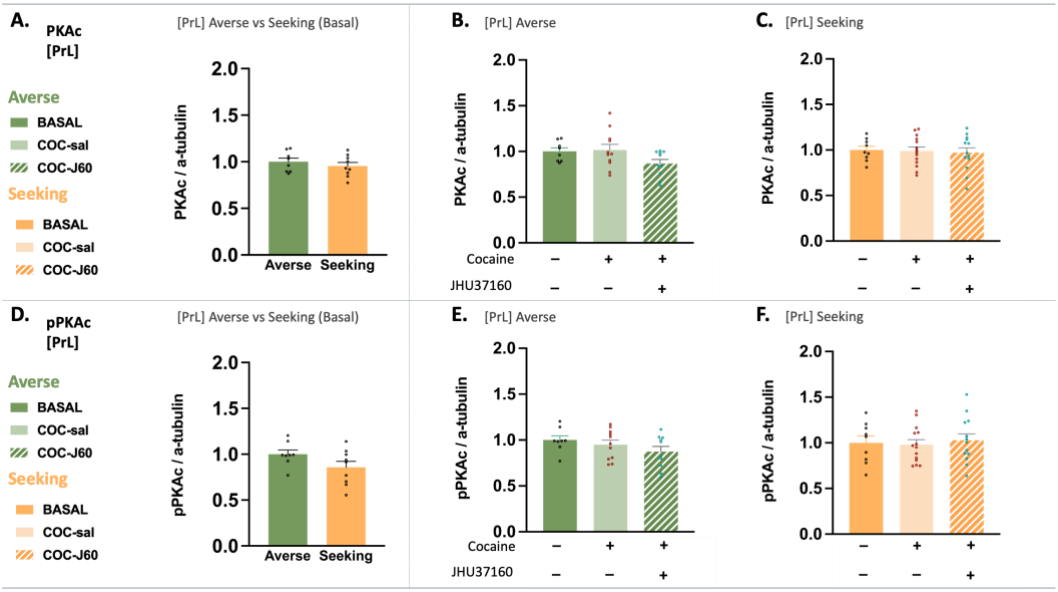

Figure 5. No significant changes were observed in total and phosphorylated PKAc proteins. The basal expression levels of total and phosphorylated PKAc were not different between risk-averse and risk-seeking rats. By cocaine and J60 administration, it remains not significantly changed. The numbers of rats for each group are as follows: Averse-Basal (8), Averse COC-sal (11), Averse COC-J60 (10), Seeking-Basal (9), Seeking COC-sal (14), Seeking COC-J60 (13).

3.6 Cocaine and activation of Gi DREADD in the PrL affect the expression levels of total and phosphorylated DARPP32 in the risk-averse rats

DARPP-32 (Dopamine and cAMP-regulated phosphoprotein, 32 kDa) serves as a pivotal integrator of dopaminergic signaling, particularly in response to cocaine, which elevates synaptic dopamine levels, thereby influencing various downstream pathways.[28] Cocaine's effect was hypothesized to induce a significant alteration in DARPP-32 phosphorylation patterns, especially at the T34 and S97 sites, which are critical for its regulatory functions. Phosphorylation at T34 by PKA enhances DARPP-32's ability to inhibit protein phosphatase-1 (PP1), amplifying dopaminergic and cAMP signaling[29]. This mechanism operates fully through the dephosphorylation of S97, which facilitates the nuclear translocation of DARPP-32 and enables phosphorylated DARPP-32 at T34 to function effectively[28,29]. In consideration of the importance of DARPP-32, its expression level was analyzed in cocaine and J60 treated brain samples. The one-way ANOVA conducted on the drug treatment showed that there were significant changes in pDARPP32-S97 expression levels [$F_{2, 26} = 7.174$, $P = 0.003$], but not in pDARPP32-T34 (Figure 6E, H). Post hoc Tukey's multiple comparison test revealed a significant reduction in the phosphorylation at the S97 site in cocaine relative to basal groups ($P < 0.01$). These effects were significantly blocked by administration of J60 ($P < 0.05$).

There were also significant changes in total DARPP-32 following cocaine administration and J60. The one-way ANOVA conducted on the drug treatment [$F_{2, 26} = 7.323$, $P = 0.003$] revealed significant differences in DARPP-32 among three groups in risk-Averse (Figure 6B). Post hoc Tukey's multiple comparison test showed a significant increase in the total DARPP-32 following cocaine administration ($P < 0.01$), and it is effectively blocked by administration of J60 ($P < 0.05$). These results were observed exclusively in the PrL region of the risk-averse rats, with no significant alterations in the risk-seeking rats or the NAc core region.

Additionally, the unpaired T-test conducted for pDARPP32(S97) level between the basal risk-Averse and risk-seeking rats showed significant differences ($t = 2.617$, $P < 0.05$) (Figure 6G). These results suggest that there might be inherent differences in the regulation of dopamine and

cAMP/PKA signaling pathways between risk-Averse and risk-seeking rats mediating choice differences between two groups.

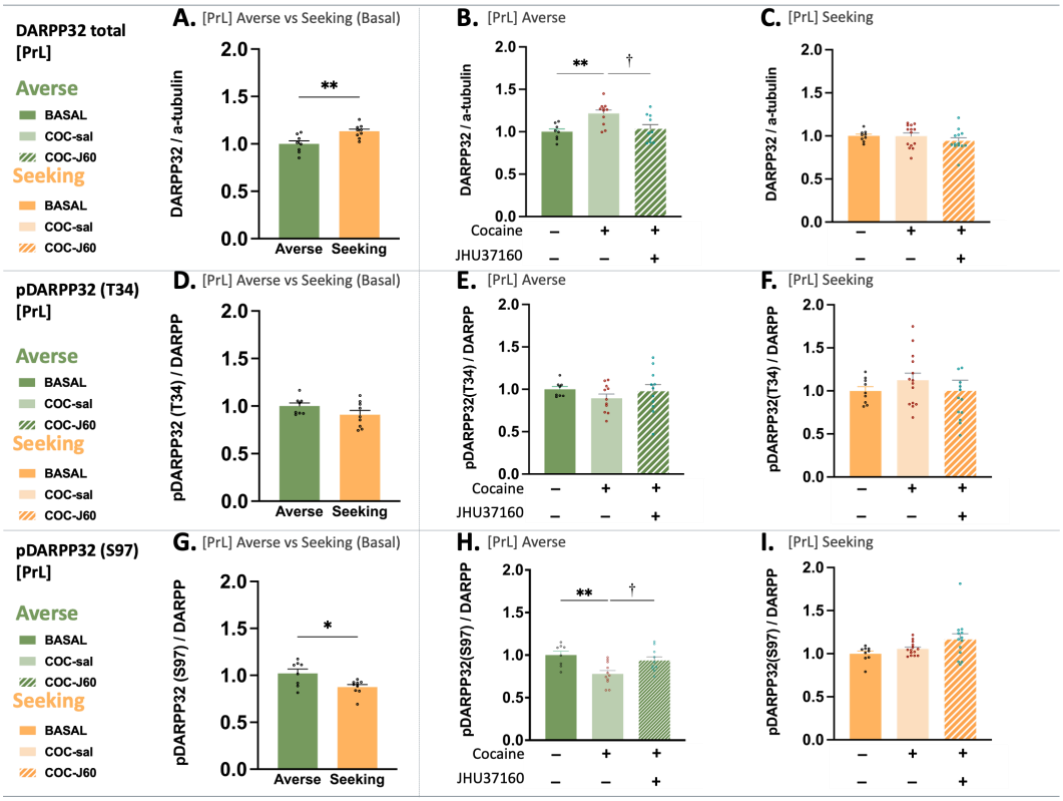

Figure 6. Significant changes of total and phosphorylated DARPP32 were observed by cocaine and J60 in risk-averse rats. Rats in Basal group were sacrificed on the last day of stage 6. Rats in Coc-sal and COC-J60 group were sacrificed right after the end of counter-balanced rGT session in Exp.1. **A, D, G** The basal expression levels of DARPP32 were observed significantly different for total ($t=3.311$, $P<0.01$) and the phosphorylated DARPP32 on S97 site ($t=2.617$, $P < 0.05$) in the PrL. **B, E, H** Significant changes were observed for total DARPP32 and pDARPP32(S97) by cocaine and J60 in risk-Averse rats. **C, F, I** There was no significant differences for DARPP32 in risk-Seeking rats. The numbers of rats for each group are as follows: Averse-Basal (8), Averse COC-sal (11), Averse COC-J60 (10), Seeking-Basal (9), Seeking COC-sal (14), Seeking COC-J60 (13).

3.7 Cocaine and activation of Gi DREADD in the PrL affect the expression levels of CaV1.2 in the risk-averse rats

Acute cocaine exposure causes a notable surge in Ca^{2+} influx within cortical neurons, primarily mediated by L-type calcium channels (LTCC), emphasizing the rapid effect of cocaine on cellular calcium dynamics[30]. Moreover, chronic cocaine use induces an increase in the expression of LTCC and neuronal response in pyramidal cortical neurons[31,32]. Given the significance of calcium channels, particularly LTCC, in cognitive function and decision-making processes in PFC[33], their expression levels were examined in this study. Among three different subtypes of calcium channels examined, CaV1.2 was found to be significantly different in its expression levels by cocaine and J60 in risk-averse rats, as shown by the one-way ANOVA conducted on the drug treatment [$F_{2, 26} = 6.454$, $P = 0.005$] (Figure 7B). Post hoc Tukey's multiple comparison test revealed a significant upregulated CaV1.2 following cocaine administration ($P < 0.05$), and these effects were significantly reduced by J60 ($P < 0.05$). These effects were not observed in risk-seeking and NAc core region. These findings suggest that acute downregulation of neuronal activity via Gi DREADD can counteract cocaine's upregulation of CaV1.2 in the PrL region in the risk-averse rats.

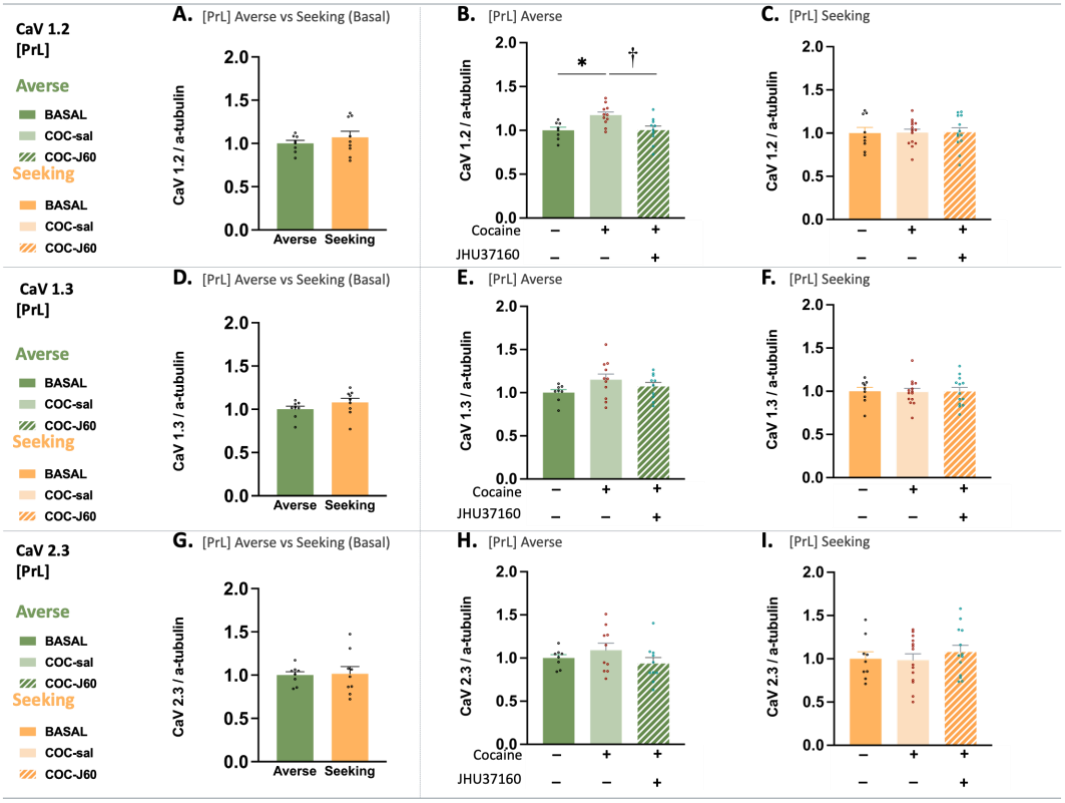

Figure 7. Significant changes of CaV1.2 were observed by cocaine and J60 in risk-averse rats. Rats in Basal group were sacrificed on the last day of stage 6. Rats in Coc-sal and COC-J60 group were sacrificed right after the end of counter-balanced rGT session in Exp.1. **A, D, G** There was no significant changes for the basal levels between risk-averse and risk-seeking rats. **B.** There was a significant upregulation of the expression levels of CaV1.2 in cocaine compared to the basal group in the risk-averse rats ($P < 0.05$), which was significantly attenuated by artificial downregulation of neuronal activity using J60 ($P < 0.05$). This change in CaV1.2 expression was the only significant alteration observed among the calcium channels examined. The numbers of rats for each group are as follows: Averse-Basal (8), Averse COC-sal (11), Averse COC-J60 (10), Seeking-Basal (9), Seeking COC-sal (14), Seeking COC-J60 (13).

3.8 Confirmation of significant changes of preference choices by cocaine and Gi DREADD activation in rats used for western analysis

In order to confirm whether behavioral data obtained from rats used for western experiments showed same pattern of significant differences in their preference choice, analysis of those data were separately conducted. The Two-way ANOVA conducted on choice x drug interaction for Gi DREADD rGT risk-averse rats [$F_{6, 104} = 3.685$, $P = 0.002$] showed significant differences. Cocaine-administered rats displayed a significantly lower choice of P2 ($P < 0.05$) and a higher choice of P3 ($P < 0.05$) compared to the basal group indicating that cocaine contributed to increase risky choices. However, rats injected with J60, 45 minutes after cocaine administration, showed a significant recovery in choice responses towards the normal state for both P2 and P3 choice ($P < 0.05$), confirming that behavioral data obtained from rats used for western experiments showed same pattern of significant differences in their preference choice. The two-way ANOVA conducted on the choice x drug interaction for risk-seeking rats [$F_{6, 132} = 2.110$, $P = 0.06$] (Figure 8B) showed no significant effects, except a minor effect of lowered P3 choice ($P < 0.05$) in COC-J60 group compared to basal.

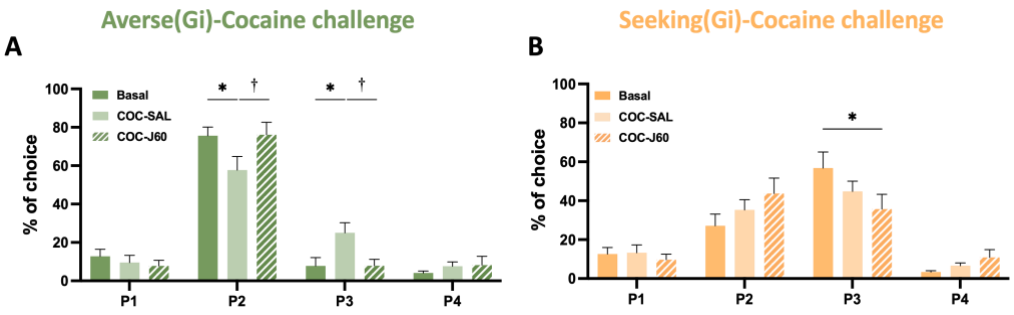

Figure 8. Confirmation of significant changes of preference choices by cocaine and Gi DREADD activation in rats used for western analysis. A. Cocaine-administered risk-averse rats displayed a significantly lower choice of P2 ($P < 0.05$) and a higher choice of P3 ($P < 0.05$) compared to the basal group, indicative of increased risky choices. Rats injected with J60, 45 minutes after cocaine administration, showed a significant recovery in choice responses towards the normal state for both P2 and P3 choice ($P < 0.05$). **B.** In risk-seeking rats, COC-J60 group compared to basal showed significant lowered P3 choice ($P < 0.05$).

4. Discussion

Individuals with Substance Use Disorders (SUDs) typically prefer immediate large rewards with high risk in the Iowa Gambling Task (IGT), rather than immediate small rewards with low risk, despite the potential for greater long-term rewards[34,35]. The top-down executive control of cognitive function by the dorsolateral prefrontal cortex (dlPFC), which is homologous to the prelimbic cortex (PrL) in rodents, is crucial for evaluating the expected value of choices, but this function disrupted in individuals with SUDs[9,17,36-38].

Expanding on this foundation, this study aimed to investigate the impact of the PrL to NAc core circuit on decision-making behaviors using animal models, particularly risk-taking preference, under normal conditions and following cocaine sensitization. Using an advanced noninvasive chemogenetic tool called DREADD, I manipulated this circuit in rats that were performing the rat Gambling Task (rGT), a behavioral paradigm similar to the human Iowa Gambling Task designed to evaluate decision-making under uncertainty.

Cocaine sensitization is characterized by a gradual increase in the locomotor and rewarding effects of the drug after repeated exposure, and the development of cravings afterwards. This phenomenon is not merely a pharmacological effect but rather reflects the neuroadaptive changes that occur during the transition from drug use to addiction[39,40]. In humans, these neuroadaptive changes manifest as increased propensity to engage in risky behaviors, a hallmark of addiction[41,42]. This study demonstrates that rats, following cocaine sensitization, exhibit a pronounced shift towards riskier choices in the risk-averse rats (Figure 3A, 3C, and 8A). This shift highlights the effect of cocaine on the brain's reward system, particularly on the dopaminergic pathways within the PrL-NAc core circuit, which play a crucial role in evaluating preferences for risky choices[43].

In the context of cocaine-sensitized rats, the activation of inhibitory Gi-DREADDs was found to significantly mitigate the cocaine-induced preference for riskier choices in the risk-averse rats (Figure 3A, 8A). This effect emphasizes the critical role of PrL-NAc core circuit in mediating cocaine's influence on decision-making. Although there was a boosted expression of cFos with Gq-DREADD activation over cocaine (Fig. 1D), no behavioral changes were observed (Fig. 3C), suggesting a ceiling effect where additional activation of this already stimulated circuit by cocaine

does not further impact on decision-making behaviors. These finding sheds light on the nuanced balance of neuronal activity required for optimal decision-making and how substances like cocaine can disrupt this balance.

Under normal circumstances, neuronal activity downregulation by Gi-DREADD activation on this circuit does not influence choice preferences (Figure 4A, B). Additionally, contrary to expectation, increasing neuronal activity via Gq-DREADD activation did not induce a preference shift towards risky options (Figure 4C, D). Considering the mechanism of neuronal activity upregulation by Gq-DREADD, which induce neuronal activation mainly through calcium influx[22], these results suggest that manipulating neuronal activity of the PrL-NAc core may not be solely responsible for changes in decision-making. Instead, it appears that dopaminergic signaling with cocaine administration plays a crucial role in the shift towards risky preferences, as indicated by the research on dopaminergic neuronal modulation of the PFC to NAc circuit, suggesting that D1 signaling is deeply involved in decision-making. This research demonstrates that inhibiting D1 activity with antagonists in this circuit shifts choice preferences away from risky choices, suggesting that D1 dopamine activity predisposes individuals to riskier choices.[43]

To understand how cocaine and subsequent Gi-DREADD activation alter preference in decision-making, this study investigated the changes of specific molecular mechanisms. Gi-DREADD downregulates neuronal activity by inhibiting the G protein-mediated cAMP-PKA-DARPP32 signaling pathway, while cocaine administration is known to activate this pathway through the D1 dopaminergic receptor. Considering that the dephosphorylation of the S97 site of DARPP32 is essential for successful transmission of dopaminergic signaling, by mediating the internalization of DARPP32 into the nucleus for gene transcription, it was hypothesized that the Gi-DREADD-mediated mitigation of risky choices in decision-making would be associated with a higher expression of phosphorylated DARPP32 on the S97 site compared to the effects of cocaine.

Through western blot analysis to quantify protein expression in neuronal pathways of rat brains with Gi-DREADD activation revealed significant alterations in DARPP32 levels. Intriguingly, cocaine administration led to a notable decrease in phosphorylation at the S97 site

of DARPP-32 within the PrL region of risk-averse rats compared to the basal levels. As hypothesized, this decrease was effectively mitigated by Gi-DREADD activation (Fig. 6H). When comparing the basal states of risk-averse and risk-seeking rats, a marked lower pDARPP-32(S97) expression was observed in the risk-seeking rats (Fig. 6G), similar to cocaine-treated risk-averse rats. This suggests a potential link between DARPP32 mediated dopaminergic signaling and risk preference behavior. Given the 90-minute interval between post-cocaine administration and tissue sampling, the focus was primarily on detecting changes in phosphorylation at the S97 site of DARPP32 over other molecules in the PKA signaling pathway because the sustained nucleus accumulation of DARPP32 has been known to last up to three hours[29,44].

L-type calcium channels (LTCCs), particularly CaV1.2, are pivotal in mediating the sensitized effects induced by cocaine exposure, reducing the threshold for Spike-timing-dependent LTP (t-LTP)[45]. Cocaine triggers a marked increase in Ca^{2+} influx within cortical neurons, primarily through LTCC activation,[46] a process that is significantly potentiated by PKA.[47] This acute response to cocaine underscores the profound influence of the drug on cellular calcium dynamics. Furthermore, chronic cocaine use increases the expression of LTCCs and amplifies neuronal responsiveness in both the NAc and the mPFC, resulting in a sensitized response. Specifically, CaV1.2 plays an essential role in the expression of cocaine-induced sensitization, while CaV1.3 is implicated in the acute neuronal response to cocaine and the development of sensitization.[47,48.]

The findings in this study indicate a significant upregulation of CaV1.2 expression following cocaine administration in risk-Averse rats, an effect that was substantially mitigated by the activation of inhibitory Gi-DREADD with J60 (Fig. 7B). In contrast, CaV1.3 expression remained unchanged after cocaine exposure and subsequent Gi-DREADD activation (Fig. 7E). These observations underscore CaV1.2's critical contribution in modulating the effects of cocaine-induced sensitization on decision-making processes. Through the regulation of CaV1.2, repeated cocaine exposure can alter neuronal excitability and plasticity in key brain regions involved in decision-making[45-47], suggesting that targeting CaV1.2 could be a promising approach for modulating the enhanced risk preferences associated with cocaine sensitization. While significant changes were observed in the PrL region following cocaine and J60 administration, no significant changes were detected in the NAc core.

Risk-seeking rats did not show significant changes in preference choices when subjected to cocaine sensitization and subsequent J60 administration in Gi-DREADD and Gq-DREADD expressing rats, which might be due to a floor effect. There were also no notable alterations observed in molecules related to dopaminergic signaling and calcium channels in this group, differently from the risk-averse rats. Interestingly, it was observed that there was significantly lower basal expression of pDARPP32(S97) in the risk-seeking rats compared to the risk-averse rats, suggesting that there were inherent differences between the two groups. Supporting these findings, previous research revealed that there are intrinsic disparities in transcriptome expressions between risk-averse and risk-seeking rats [49]. This is further corroborated by human studies which emphasize the role of genetic contributions to personality traits in the susceptibility to gambling disorders[50]. For instance, genome-wide association studies (GWAS), particularly with larger sample sizes like the Vietnam Era Twin Registry (VET-R), suggest that about 35–54% of the genetic component of gambling disorder is inherited[51,52], and there are shared genetic factors with other psychiatric conditions such as major depression, anxiety disorder, substance use disorder, and alcohol use disorder[53-56].

Recent clinical studies employing noninvasive techniques such as repeated Transcranial Magnetic Stimulation (rTMS) and Transcranial Direct Current Stimulation (tDCS) have shown promise in modulating PFC activities as a therapeutic approach for gambling disorder (GD) and SUDs. Some of these studies have specifically targeted the dorsolateral PFC (dlPFC) in patients with SUDs or GD, leading to a reduction in risky choices[57-59]. However, due to the broad stimulation area, results have varied, with some studies reporting no significant changes in behavior[60,61]. This study, as translational research, highlights the PrL-NAc core circuit as a critical neural pathway in the context of cocaine-induced maladaptive decision-making. Downregulation of this specific circuit significantly reduced the tendency towards riskier choices. Regarding its molecular mechanisms, the phosphorylation of DARPP32 at the S97 site was found to be closely associated with risky decision-making behaviors, with the CaV1.2 channel identified as a potential molecular target for interventions aimed at mitigating cocaine-induced shifts towards riskier preferences. These findings may suggest a novel treatment strategy for managing risky decision-making behavior in GD and SUDs, pointing towards more targeted and effective therapeutic interventions in the future.

5. Conclusion

This research has advanced our understanding of the neurobiological mechanisms that influence decision-making in the context of substance use and gambling disorders. By investigating the circuitry from the PrL to the NAc core and utilizing chemogenetic tools, this study has revealed how cocaine sensitization can lead to a preference shift towards riskier choices. These findings underscore the important roles of dopaminergic signaling and the L-type calcium channel CaV1.2 in driving such behavioral changes. Importantly, this study suggests that targeted interventions within specific neuronal circuits could provide new avenues for treating these disorders. Future efforts should focus on refining these therapeutic strategies, potentially revolutionizing the management of substance use and gambling disorders by tailoring interventions to the underlying neurobiological and genetic susceptibilities.

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Abstract In Korean

전전두엽-중격측좌핵 회로의 화학유전학적 조절이 위험 선호도 의사결정에 미치는 효과

의사 결정은 다양한 선택지의 잠재적 비용과 이익을 추정하는 과정을 필요로 하며, 이러한 과정은 도박중독과 같은 정신적인 문제를 가진 사람에게서 현저히 손상되어 있다. 이러한 손상은 코카인과 같은 약물의 남용으로 인해 더욱 악화되어, 의사 결정 능력과 관련된 인지 기능에 상당한 장애를 초래한다. 이러한 장애는 주로 내측전전두피질(mPFC)과 그것과 연결된 전두-선조 신경회로에 의해 조절되는 상의하달식 집행제어의 결핍과 관련이 있다고 한다. 구체적으로, mPFC 의 하위 영역인 전변연피질(PrL)은 보상과 처벌의 가치를 추정하는 것과 같은 집행 기능에 더 많이 관여하며, PrL 로부터 글루타메이트 입력을 받는 NAc 의 하위 영역인 중격측좌핵 중심측(NAc core)은 보상 관련 동기 형성에 중요한 역할을 한다. 현재까지 밝혀진 바로는 PrL 에서 NAc core 로의 신경 회로 활동을 직접 조작하는 것이 위험추구 성향을 바꿀 수 있는지 아직 명확하게 알려지지 않았다. 따라서 본 연구에서는 위험추구 성향의 의사 결정에 대한 이 회로의 역할을 조사하기 위해 인공적으로 만들어진 약물에 의해서만 활성화되는 수용체(DREADD)를 사용하는 최신의 화학유전학 기술을 사용했다. Cre 의존적인 hM4Di 나 hM3Dq 를 표현하는 바이러스, 그리고 Cre 재조합효소는 각각 PrL 과 NAc core 에 주입되었다. 이후, 쥐들은 보상/처벌 확률이 다른 네 개의 선택지 중에서 자유롭게 선택할 수 있도록 하루 30 분간의 훈련을 받았고, 안정적인 선호도 패턴이 관찰될 때까지 훈련을 지속하였다. 마지막 3 일 평균을 기준으로 이들을 위험 회피형이나 위험 추구형으로 분류하고 난 후, 두 개의 별도 환경에서 그들의 선호도 변화를 측정했다. 하나는 코카인 민감화 체제에 노출하여 DREADD 에 대한 인공 합성 리간드인 JHU37160 을 사용한 신경 활동 조절하에 그들의 의사결정 변화를 관찰하였고, 다른 하나는 코카인 투여 없이 DREADD 매개 신경 활동 조절에 의한 의사결정 변화를 관찰하였다.

예상대로, 위험 회피형 그룹은 만성 코카인에 의해 위험 추구형으로 선택지에 대한 선호도가 바뀌었는데, 흥미롭게도, 이 효과는 PrL-NAc core 회로의 Gi DREADD 활성화에 의해 현저히 완화되었다. 이러한 효과는 Gq DREADD 가 발현된 위험 회피형 쥐에선 발견되지 않았고, 위험 추구형 쥐 그룹에서는 두 경우 모두 관찰되지 않았다. 이 결과들은 PrL-NAc core 회로가 만성적인 코카인 사용으로 유도되는 위험추구 의사 결정의 주요 대상 영역 중 하나임을 나타내며, 또한 그 활성을 조절함으로써 제어할 수 있음을 시사한다. 더해서, 본 연구는 코카인으로 유발된 행동 변화에서 중요한 요소인 DARPP32 인산화와 L-형 칼슘 채널(LTCC) 발현에 중점을 두어 웨스턴블롯 분석을 통해 분자적인 메커니즘을 조사하였다. 그 결과 DARPP32 의 S97 위치에서의 인산화는 위험추구성향의 의사 결정과 관련되어 있음을 밝혀냈으며, CaV1.2 채널은 코카인으로 유발된 위험추구성향으로의 변화를 완화하는 데 목표로 할 수 있는 잠재적 대상으로 확인할 수 있었다. 위 결과들은 약물중독 또는 도박중독으로 인한 위험추구 성향의 의사 결정에 대해 신경생물학적인 이해를 확장하였으며, 특정 신경 회로를 대상으로 한 활성 조절이 새로운 치료법에 대한 전략을 제공할 수 있음을 제안한다.

핵심되는 말 : 도박성 게임 동물모델, 코카인 민감화, 화학유전학적 활성 조절, 전변연피질-중격측좌핵 중심층 신경회로