Pharmacological Interventions on The Dopamine Motivation System Effectively Disturbed Cue-Induced Memory Reconsolidation Cocaine-Seeking Behavior for Rats

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Research Article

Keywords: Dopamine motivation system, Cue-induced, Self-administration, Memory reconsolidation, Behavior, Cocaine

Posted Date: April 30th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-426726/v1>

Abstract

Drug addiction is a disorder related to dysfunction in the neural reward memory circuits, and it is characterized by compulsive drug use despite terrible negative consequences. Memory reconsolidation, during which aroused memory is easy to strengthening, weakening or updating, plays an extremely important role in drug addiction. Effectively interfering with the drug memory reconsolidation process would be key in treating drug addiction, but this intervention currently remains impossible. The dopamine motivation system has been widely recognized as an important system for reward, but whether the dopamine motivation system participates in drug memory reconsolidation is unclear. We aimed to explore the role of the dopamine motivation system during the cue-induced cocaine memory reconsolidation process by examining the effect of different pharmacological interventions on the dopamine motivation system during cue-induced cocaine self-administration-related memory reconsolidation drug-seeking behavior. Using a combined behavioral and biological method, our results showed that high concentrations of SCH 23390 and raclopride, or VTA lesions, could effectively disturb subsequent cue-induced cocaine self-administration-related memory reconsolidation drug-seeking behavior in rats. However, low concentrations of SCH 23390 and raclopride could not block this behavior. In summary, only a high dose of dopamine D₁ and D₂ receptor antagonists, or VTA lesions, could effectively disturb subsequent cue-induced cocaine self-administration-related memory reconsolidation drug-seeking behavior. These findings indicated that pharmacological interventions in the dopamine motivation system could effectively disturb subsequent cue-induced drug memory reconsolidation. Thus, pharmacological interventions on the dopamine motivation system might have therapeutic potential for drug addiction.

Introduction

Drug addiction is a disorder related to dysfunction in the neural reward memory loop that is characterized by compulsive drug use despite terrible negative consequences (Hyman, Malenka, & Nestler, 2006; Y. Li et al., 2016). Through associative learning, drug-paired environmental stimuli could have very powerful influence on drug-seeking behavior; therefore, drug memory reconsolidation critically underlies persistent drug-seeking behavior. Drug-related memory could be reactivated after re-exposure to drug-paired environmental stimuli (Namely, cues) or the drug itself (Everitt, Giuliano, & Belin, 2018; Gauthier et al., 2017; Torregrossa & Taylor, 2016), and a relapse in drug-seeking behavior could occur (Childress et al., 1999). Further studies will need to be powerful enough to diminish drug-seeking behavior under drug-paired environmental stimuli. Therefore, extinguishing the impact of drug-paired environmental stimuli is an extremely important goal for the valid treatment of drug addiction (Gauthier et al., 2017).

Environmental stimuli can improve learning and memory and induce neuroplasticity in the brain (Sale, Berardi, & Maffei, 2014; van Praag, Kempermann, & Gage, 2000). In many previous studies, environmental stimuli included a lot of condition in which experimental animals had access to cognitive stimulation, social and physical (Thiel et al., 2012; Thiel, Sanabria, Pentkowski, & Neisewander, 2009; van Praag et al., 2000). In rodent drug addiction experiments, there are two main classic animal models: the conditioned

place preference (CPP) model and the self-administration (SA) model. Most studies examining the effects of environmental stimuli on experimental animals have used relatively long-term enriched housing conditions over several days (Chauvet, Lardeux, Goldberg, Jaber, & Solinas, 2009).

Many studies demonstrated that pharmacological manipulations of several neurotransmitters and relevant receptors, mostly including glutamatergic, dopaminergic and β -adrenergic receptors, could have an important effect on memory reconsolidation (Diergaarde, Schoffelmeer, & De Vries, 2008; Y. Li et al., 2016) and could also lead to an absence of the subsequent CPP memory (Brown, Lee, & Sorg, 2008; Otis & Mueller, 2011; Spina, Fenu, Longoni, Rivas, & Di Chiara, 2006). Dopamine (DA) is a catecholamine neurotransmitter in the mesocorticolimbic system (Chen et al., 2017). DA plays important roles in many aspects of cognition, including reward, punishment, attention, learning and memory (Smythies, 2005). Dysfunction in DA and DA neurons could lead to neurological and psychiatric diseases, such as drug addiction, Parkinson's disease, schizophrenia, hyperprolactinemia and Tourette's syndrome (Bellioti et al., 1999; Zhang et al., 2007). After chronic exposure to several kinds of addictive substances, many molecular and cellular adaptive changes that occur in the DA mesocorticolimbic system may contribute to drug addiction. Neuroadaptations in the dopamine motivation system could mediate enhanced motivation towards related reinforced predictors. Cocaine inhibited the clearance of dopamine from the synaptic cleft by blocking plasma membrane monoamine transporters.

The role of dopamine (DA) in motivated behaviors and cognitive processes has been well established. The DA motivation system mainly includes DA neurons in the ventral tegmental area (VTA) and the substantia nigra (SN). On the one hand, limbic structures, including the hippocampus and nucleus accumbens, which have traditionally been associated with motivation and reinforcement learning, receive dopaminergic innervation from the VTA, which is known to be a key dopaminergic center in the brain (Gasbarri, Sulli, & Packard, 1997; Wahlstrom, White, & Luciana, 2010). On the other hand, DA neurons in the SN mainly project to the dorsal striatum, which has traditionally been associated with action selection, goal-directed behavior and the emergence of habits (Howard, Li, Geddes, & Jin, 2017; Wise, 2009; Yager, Garcia, Wunsch, & Ferguson, 2015). More importantly, the dopamine motivation system, as a driver of reinforcement, acts as the neurobiological basis of drug addiction (Drugs & Crime, 2010; Koob & Volkow, 2010; Volkow, Wise, & Baler, 2017). Several studies have demonstrated a critical role for dopamine in the reward circuit. The pharmacological agents that were previously used to target the dopamine system in animal models mainly acted on dopamine D₁ and D₂ receptors. Several recent studies have highlighted a role for other subfamilies of receptors (Fraser, Haight, Gardner, & Flagel, 2016).

In this study, to test the hypothesis that interventions targeting on the dopamine motivation system could affect drug memory reconsolidation, we employed a cue-induced cocaine self-administration paradigm to explore the effect of pharmacological interventions on rebalancing the dopamine motivation system and on cue-induced cocaine SA-related memory reconsolidation drug-seeking behavior. These results might help us to elucidate the neuropharmacological mechanism underlying the function of the dopamine motivation system during drug memory reconsolidation.

Materials And Methods

Animals

All experimental procedures in our study were consistent with the guidelines of the Committee for Animal Care and Use (No. TDLL2018-03-180, Tangdu Hospital, the Fourth Military Medical University, Xi'an, Shaanxi, China). And the experimental protocols were approved by the Committee for Animal Care and Use of Tangdu Hospital, the Fourth Military Medical University. Sprague-Dawley rats (Male; 300 ~ 350 g) were individually housed in our animal center under controlled temperature ($21 \pm 2^{\circ}\text{C}$) and humidity (40%~60%) on a 12-hour light-dark cycle (lights on at 7:00 a.m.), and they were provided with food and water *ad libitum*.

Drug preparation

Cocaine hydrochloride (Qinghai Pharmaceutical Co. Ltd., Xining, Qinghai, China), which was dissolved in saline to a final concentration of 8 mg/mL, was stored at room temperature away from light.(Y. Li et al., 2016) 6-hydroxydopamine (6-OHDA, 10 $\mu\text{g}/\mu\text{L}$, H4381-100MG, Sigma-Aldrich. Co. USA) was dissolved in 0.2 mg/mL ascorbic acid and 0.9% w/v saline solution and stored at -20°C away from light. SCH 23390 (dopamine D_1 receptor antagonist, 2 $\mu\text{g}/\mu\text{L}$, D054-5 MG, Sigma-Aldrich. Co. USA) was dissolved in 0.9% w/v saline solution and stored at -20°C away from light. Raclopride (dopamine D_2 receptor antagonist, 10 $\mu\text{g}/\mu\text{L}$, R121-25 MG, Sigma-Aldrich. Co. USA) was dissolved in 0.9% w/v saline solution and stored at -20°C away from light.

Surgical procedure

Jugular vein catheterization surgery

Rats were anesthetized with sodium pentobarbital (50 mg/kg body weight, i.p.) and fixed in the prone position. A skin opening approximately 0.8 cm in length was cut longitudinally in the right neck to fully expose the right jugular vein. A sterile special silicone catheter was inserted into the right jugular vein. Following surgery, the jugular vein catheter was rinsed daily with saline containing heparin (10 U/mL) and penicillin (200,000 IU/rat) to prevent catheter blockage and infection, respectively.

VTA lesion surgery

After anesthetization, the rats in the VTA-lesioned group were fixed in a stereotaxic apparatus (68025, RWD Life Science Co., Ltd, Shenzhen, Guangdong, China) in the prone position(Y. Li et al., 2016). 6-OHDA (10 $\mu\text{g}/\mu\text{L}$, 0.8 μL) was intracranially infused into the VTA (according to the rat stereotaxic atlas (Paxinos and Watson, 2005): -5.20 mm anterior to bregma, 0.80 mm lateral to the midline (left side), and 8.80 mm ventral to the brain surface) using 1 μL Hamilton syringes (Plastics One) at a rate of 0.1 $\mu\text{L}/\text{min}$ for every rat. The injection needle was held in place for an additional 15 min so that 6-OHDA could completely diffuse from the needle tip.

Drug self-administration apparatus

The self-administration (SA) apparatus (40 cm × 40 cm × 50 cm; AniLab Co., Ltd., Ningbo, Zhejiang, China) received an input signal once the rat made a valid nose poke into the port, and then, it could produce as many output signals as we required (for example, for the pump, signal lights, nose poke light, or sound). According to the experimental requirements, we first needed to edit an appropriate experimental method (fixed ratio, FR = 1). Specifically, when a rat finished a valid nose poke, the system would pump one bolus of drug (T = 1.25 s, v = 1.60 mL/min), accompanied by several changes in environmental cues such as the signal lights (green light is off, and red light is on for 20 s), valid nose poke light (T = 20 s), and sound (buzzer, T = 1.25 s). Every time a valid nose poke was completed, the system would immediately enter into a 20-s refractory period. Then, the system would enter into the next cycle (green light would remain on until the next valid nose poke).

Drug self-administration training procedure and cue-induced drug addictive memory reconsolidation procedure

After 3 d of recovery following jugular vein catheterization surgery, all rats underwent a drug self-administration training procedure on a 14-day training schedule (Fig. 1). Training lasted 2 h per day, and the number of nose pokes (valid nose poke and invalid nose poke) and the number of pumps (with drug) were recorded.

In our study, when the number of valid nose pokes increased significantly and reached a relatively stable level at the end of the training period (varying less than 3 times or 15% for three consecutive days), the drug SA model for rats was considered basically successful.

After the successful establishment of the SA model, the rats began the memory reconsolidation procedure. Experimental rats were again placed into the SA apparatus for 2 h. When rats finished a “valid” nose poke, the system would only provide the related environmental cues without pumping cocaine.

Tissue preparation

After deep anesthetization, rats were transcardially perfused with 200 mL of ice-cold 0.1 M sodium phosphate-buffered saline (PBS; pH 7.4), followed by 400 mL of 4% (w/v) paraformaldehyde in 0.1 M PB. The brains were removed and postfixed at 4°C overnight in 4% (w/v) paraformaldehyde in 0.1 M PB. After cryoprotection with 30% (w/v) sucrose in 0.1 M PB, the brain was sliced into 30 µm-thick transverse sections using a cryostat (-20°C). The brain sections including the VTA were stored in freeze protection solution (Ethylene glycol: Glycerol: PBS = 3:3:4).

Immunohistochemical staining

The sections were first incubated at room temperature in 0.3% Triton X-100 for 20 min, 3% hydrogen peroxide for 15 min and normal goat serum for 30 min. Then, the sections were incubated sequentially at room temperature as follows: Firstly, anti-tyrosine hydroxylase (TH) mouse Ig G (1 µg/mL, ab129991, Abcam, Cambridge, UK) for 24 h at 4°C. Secondly, a biotinylated goat antibody against mouse Ig G (SP-9002, ZSGB-BIO, Beijing, China) at room temperature for 30 min (Y. Li et al., 2016). Thirdly, avidin-

conjugated horseradish peroxidase at room temperature for 30 min, and then peroxidase activity was visualized after an approximately 3-min reaction with DAB Kit (ZLI-9018, ZSGB-BIO, Beijing, China). Finally, the sections were mounted on gelatin-coated slides, dried, dehydrated in increasing concentrations of ethanol, cleared with xylene, and cover-slipped with neutral balsam(Y. Li et al., 2016). Next, digital images were captured with an ordinary optical microscope (Eclipse E100, Nikon, Nikon Instruments (Shanghai) Co., Ltd., China), modified (15 ~ 20% contrast enhancement) with Photoshop CS6 (Adobe Systems, San Jose, CA, USA), and saved as TIFF files(Li et al., 2020).

Statistical analysis

All results in this experiment were expressed as the means $\pm$ SEM. The data were statistically assessed via Student's two-tailed t-test and one-way ANOVA using SPSS 18.0 (SPSS Inc., USA). *P* values of less than 0.05 were accepted as statistically significant. All group data in our study are reported in the table or figure legends.

Ethics

All experimental procedures in our study were consistent with the guidelines of the Committee for Animal Care and Use (No. TDLL2018-03-180, Tangdu Hospital, the Fourth Military Medical University, Xi'an, Shaanxi, China). And the study procedures were carried out in accordance with the Declaration of Helsinki.

Results

1. Establishment of cocaine self-administration (SA) model for rats

In this experiment, all rats ($n = 70$) were divided into two groups: the Saline group ($n = 8$) and the Cocaine-addicted group ($n = 62$). During cocaine SA training, many rats can be removed due to a number of complications, for example, failed jugular vein catheterization surgery (e.g., leaking, blockage, serious infection, etc.). Overall, 7 of 70 rats in the Saline group (1/8) and the Cocaine-addicted group (6/62) were removed (see Table 1).

Table 1
Design and grouping in our experiment

	Saline group	Cocaine-addictive group			
		Control group	VTA- lesioned group	SCH 23390 group	Raclopride group
Total	n = 8	n = 62			
SA training	-1 ^α	-6 ^α			
Successful SA model	n = 7	n = 56			
Grouping	n = 7	n = 6	n = 8	n = 21	n = 21
Sub-grouping	n = 7	n = 6	n = 8	0.02 mg/kg ^β (n = 7)	0.02 mg/kg ^β (n = 7)
				0.20 mg/kg ^β (n = 7)	0.10 mg/kg ^β (n = 7)
				0.50 mg/kg ^β (n = 7)	0.20 mg/kg ^β (n = 7)
-n (1/6) ^α presented that n (1/6) rat/rats was/were removed for specific reasons described in Results.					

(0.02, 0.20, 0.50, 0.10) mg/kg^β presented a certain corresponding concentration of SCH 23390/raclopride.

SA: Self-administration.

Figure 2A and 2D showed the cocaine self-administration training procedure. Then, we performed statistical analysis on the rats' behavior according to the number of valid nose pokes in the first three days and the last three days in the Saline group (n = 7) and the Cocaine-addicted group (n = 56). Compared to the first three days, we found that the number of valid nose pokes in the last three days in the Cocaine-addicted group increased significantly ($t_{\text{Cocaine-addicted group}}=30.76$, $P<0.01$; Fig. 2F) during the cocaine SA training period and remained relatively stable during the last three days of the training period ($F_{\text{Cocaine-addicted group}}=0.1244$, $P=0.8831$; Fig. 2E). However, there was a slight decrease in the number of nose pokes in the Saline group ($t_{\text{Saline group}}=2.977$, $P<0.01$; Fig. 2C), and the number of valid nose pokes reached a relatively stable level during the last three days of the training period ($F_{\text{Saline group}}=1.028$, $P=0.3777$; Fig. 2B).

In addition, the number of valid nose pokes in the last three days between the Cocaine-addicted group and the Saline group was significantly different ($t_{\text{Cocaine-addicted group, Saline group}}=23.17$, $P<0.01$; Fig. 3A). Figure 3B showed several original recordings of the experimental rat's behavioral events. Figure 3C and

3D Showed the change for cues before and after valid nose poke. The above results indicated that the Cocaine-addicted group had effectively established a cocaine self-administration model.

2. Behavioral changes in the self-administration (SA) model for rats after different pharmacological manipulations in the dopamine pathway during cue-induced cocaine memory reconsolidation

During the following 14-day withdrawal period, 56 rats in the Cocaine-addicted group remained randomly divided into several subgroups as follows: the Control group (n = 6), VTA-lesioned group (n = 8), SCH 23390 group (n = 21, 0.02 mg/kg (n = 7); 0.20 mg/kg (n = 7); or 0.50 mg/kg (n = 7)), and Raclopride group (n = 21, 0.02 mg/kg (n = 7); 0.10 mg/kg (n = 7); or 0.20 mg/kg (n = 7)) (Table 1).

After different pharmacological manipulations (intravenous injection, iv) in the dopamine pathway, the rats were placed in the SA apparatus for 2 h. After our study, many coronal brain sections (30 μ m) in the target region in the VTA-lesioned group were processed using TH-immunohistochemical staining to confirm the location and range of VTA lesions (Fig. 4). Compare to the unlesioned side, the number of VTA dopamine neurons in the lesioned side reduced significantly ($n_{\text{lesioned}} = 21.67 \pm 3.077$, $n_{\text{unlesioned}} = 107.67 \pm 9.688$, $t = 20.72$, $P < 0.001$. not seen in Tables or Figures).

Step 1: We analyzed the rats' behavior during drug self-administration training procedure in every group.

Firstly, we found the number of valid nose poke in every Cocaine-addicted group increased significantly during cocaine SA training period and it reached a relatively stable level during the last three days ($F_{\text{Control group}} = 0.3360$, $P = 0.7199$; $F_{\text{VTA-lesioned group}} = 0.4505$, $P = 0.6433$; $F_{0.02 \text{ mg/kg SCH23390 group}} = 0.5892$, $P = 0.5651$; $F_{0.20 \text{ mg/kg SCH23390 group}} = 0.03359$, $P = 0.9670$; $F_{0.50 \text{ mg/kg SCH23390 group}} = 0.01210$, $P = 0.9880$; $F_{0.02 \text{ mg/kg Raclopride group}} = 0.2750$, $P = 0.7627$; $F_{0.10 \text{ mg/kg Raclopride group}} = 0.03628$, $P = 0.9644$; $F_{0.20 \text{ mg/kg Raclopride group}} = 0.02997$, $P = 0.9705$; Fig. 5 and Fig. 6).

Secondly, we analyzed the rats' behavior in the same group. Compared to the number of valid nose pokes for the first three days, the number for the last three days showed a significant increase in every Cocaine-addicted group ($t_{\text{Control group}} = 18.46$, $P < 0.01$; $t_{\text{VTA-lesioned group}} = 12.79$, $P < 0.01$; $t_{0.02 \text{ mg/kg SCH23390 group}} = 16.48$, $P < 0.01$; $t_{0.20 \text{ mg/kg SCH23390 group}} = 6.228$, $P < 0.01$; $t_{0.50 \text{ mg/kg SCH23390 group}} = 10.24$, $P < 0.01$; $t_{0.02 \text{ mg/kg Raclopride group}} = 13.91$, $P < 0.01$; $t_{0.10 \text{ mg/kg Raclopride group}} = 9.565$, $P < 0.01$; $t_{0.20 \text{ mg/kg Raclopride group}} = 14.55$, $P < 0.01$; Table 2 and Fig. 7).

However, compared to the number of valid nose pokes in the first three days, there was a slight decrease in the last three days in the Saline group ($t_{\text{Saline group}} = 2.977$, $P < 0.01$; Table 2 and Fig. 5), and that in the Saline group also remained relatively stable during the last three days of the training period ($F_{\text{Saline group}} = 1.028$, $P = 0.3777$; Table 2 and Fig. 7).

Table 2
The change for valid nose pokes before and after drug self-administration training procedure in every group.

Group	Day _{1~3}	Day _{12~14}	t	P
Saline group	4.095 ± 0.5385	2.095 ± 0.4018	2.977	< 0.01
Control group	6.389 ± 0.9293	29.06 ± 0.8023	18.46	< 0.01
VTA-lesioned group	7.833 ± 1.185	26.67 ± 0.8737	12.79	< 0.01
0.02 mg/kg SCH23390 group	7.429 ± 0.9400	27.81 ± 0.8036	16.48	< 0.01
0.20 mg/kg SCH23390 group	15.29 ± 1.804	29.90 ± 1.502	6.228	< 0.01
0.50 mg/kg SCH23390 group	9.238 ± 1.555	31.24 ± 1.481	10.24	< 0.01
0.02 mg/kg Raclopride group	8.905 ± 1.005	27.90 ± 0.9256	13.91	< 0.01
0.10 mg/kg Raclopride group	10.90 ± 1.499	28.33 ± 1.036	9.565	< 0.01
0.20 mg/kg Raclopride group	5.762 ± 0.9333	27.86 ± 1.198	14.55	< 0.01

In addition, we analyzed the rats' behavior between every Cocaine-addicted group and the Saline group. The number of valid nose pokes in the last three days in every Cocaine-addicted group increased compared to that in the Saline group ($t_{\text{Control group, Saline group}}=31.38$, $P<0.01$; $t_{\text{VTA-lesioned group, Saline group}}=24.38$, $P<0.01$; $t_{\text{0.02 mg/kg SCH23390 group, Saline group}}=28.62$, $P<0.01$; $t_{\text{0.20 mg/kg SCH23390 group, Saline group}}=17.88$, $P<0.01$; $t_{\text{0.50 mg/kg SCH23390 group, Saline group}}=18.99$, $P<0.01$; $t_{\text{0.02 mg/kg Raclopride group, Saline group}}=25.58$, $P<0.01$; $t_{\text{0.10 mg/kg Raclopride group, Saline group}}=23.62$, $P<0.01$; and $t_{\text{Saline group}}=20.39$, $P<0.01$; Table 2 and Fig. 8). And there was no significant difference in the number of valid nose pokes in the last three days among every Cocaine-addicted group ($F = 1.706$, $P = 0.1111$), which indicates that every Cocaine-addicted group had effectively established a cocaine self-administration model.

Step 2: We analyzed the rats' behavior during cue-induced cocaine memory reconsolidation in every group.

After statistical analysis from the same subgroup, the number of "valid" nose pokes showed a significant decrease before and after cue-induced cocaine memory reconsolidation in the following groups ($t'_{\text{VTA-lesioned group}}=12.79$, $P<0.01$; $t'_{\text{0.20 mg/kg SCH23390 group}}=8.978$, $P<0.01$; $t'_{\text{0.50 mg/kg SCH23390 group}}=11.66$, $P<0.01$; $t'_{\text{0.10 mg/kg Raclopride group}}=10.39$, $P<0.01$; $t'_{\text{0.20 mg/kg Raclopride group}}=12.52$, $P<0.01$; Table 3 and Fig. 7), while there was no significant change in the number of valid nose pokes in Saline, low SCH 23390 and low Raclopride groups ($t'_{\text{Saline group}}=1.090$, $P = 0.2855$; $t'_{\text{0.02 mg/kg SCH23390 group}}=1.863$, $P = 0.0738$; $t'_{\text{0.02 mg/kg Raclopride group}}=1.758$, $P = 0.0904$; Table 3 and Fig. 7). Interestingly, the control group showed a significant increase in the number of valid nose pokes ($t'_{\text{Control group}}=4.221$, $P<0.01$; Table 3 and Fig. 7).

Table 3
The change for “valid” nose pokes during cue-induced cocaine memory reconsolidation in every group.

Group	Day _{12~14}	Day ₂₉	t'	P
Saline group	2.095 ± 0.4018	1.286 ± 0.4206	1.090	0.2855
Control group	29.06 ± 0.8023	36.33 ± 1.801	4.221	< 0.01
VTA-lesioned group	26.67 ± 0.8737	6.375 ± 0.7545	12.79	< 0.01
0.02 mg/kg SCH23390 group	27.81 ± 0.8036	32.43 ± 3.677	1.863	0.0738
0.20 mg/kg SCH23390 group	29.90 ± 1.502	6.000 ± 0.6901	8.978	< 0.01
0.50 mg/kg SCH23390 group	31.24 ± 1.481	0.8571 ± 0.3401	11.66	< 0.01
0.02 mg/kg Raclopride group	27.90 ± 0.9256	31.57 ± 2.359	1.758	0.0904
0.10 mg/kg Raclopride group	28.33 ± 1.036	9.143 ± 0.5948	10.39	< 0.01
0.20 mg/kg Raclopride group	27.86 ± 1.198	1.429 ± 0.3689	12.52	< 0.01

Next, we analyzed the rats' behavior between every experimental group and the Control group.

At the beginning, Our experimental results showed that the number of “valid” nose pokes before and after cue-induced cocaine memory reconsolidation showed a significant increase in the Control group compared with the Saline group ($t'_{\text{Control group, Saline group}}=20.41$, $P<0.01$; Fig. 9).

And further, we found the number of “valid” nose pokes between the VTA-lesioned/high SCH 23390/high raclopride group and the Control group all showed a significant decrease during cue-induced cocaine memory reconsolidation ($t'_{\text{VTA-lesioned group, Control group}}=16.91$, $P<0.01$; $t'_{\text{0.20 mg/kg SCH23390 group, Control group}}=16.69$, $P<0.01$; $t'_{\text{0.50 mg/kg SCH23390 group, Control group}}=20.92$, $P<0.01$; $t'_{\text{0.10 mg/kg Raclopride group, Control group}}=15.30$, $P<0.01$; $t'_{\text{0.20 mg/kg Raclopride group, Control group}}=20.50$, $P<0.01$; Fig. 9).

However, there was no significant difference between the low SCH 23390/low raclopride group and the Control group during cue-induced cocaine memory reconsolidation ($t'_{\text{0.02 mg/kg SCH23390 group, Control group}}=0.9026$, $P=0.3861$; $t'_{\text{0.02 mg/kg Raclopride group, Control group}}=1.560$, $P=0.1470$; Fig. 9).

These data demonstrated that only a certain high concentration of dopamine D₁ and D₂ receptor antagonists, or VTA lesions, could effectively disturb subsequent cue-induced cocaine SA-related memory reconsolidation drug-seeking behavior in rats. These results indicate that pharmacological interventions for the dopamine motivation system could effectively disturb subsequent cue-induced drug memory reconsolidation.

Discussion

In our study, a close correlation between drug (cocaine) and valid nose poke in the presence of drug-paired environmental stimuli was effectively established using the classic drug self-administration model in rats, and drug memory reconsolidation could be strongly reactivated by the drug-paired environmental stimuli alone. Using a biological behavior method, we explored the role of the dopamine system in the cue-induced cocaine memory reconsolidation process. The main results in our study were as follows. First, dopamine played an important role in cue-induced cocaine SA-related memory reconsolidation. Second, pharmacological interventions on the dopamine motivation system could effectively disturb subsequent cue-induced cocaine SA-related memory reconsolidation drug-seeking behavior after re-exposure to drug-paired environmental stimuli. Third, only a certain high dose of dopamine D₁ and D₂ receptor antagonists, or VTA lesions, could effectively disturb subsequent cue-induced cocaine SA-related memory reconsolidation behavior in rats. The above results strongly indicated that pharmacological interventions on the dopamine motivation system could effectively disturb subsequent cue-induced drug memory reconsolidation.

Dopamine D₁ and D₂ receptors are critical for learning and memory, as well as reward and reinforcement (Fraser et al., 2016; Goldman-Rakic, Castner, Svensson, Siever, & Williams, 2004; Xu et al., 2009). A related study reported that 6-hydroxydopamine (6-OHDA) lesions to cells in the ventral tegmental area (VTA) could affect cocaine intake and disrupt cocaine self-administration (Roberts & Koob, 1982). This result is consistent with our results. Above all, in our experimental findings, dopamine played an important role in cue-induced cocaine SA-related memory reconsolidation. In the CPP (conditioned place preference) model, interventions targeting certain kinds of receptors, such as glutamatergic and dopaminergic receptors, could lead to the absence of late CPP memory (Brown et al., 2008; Y. Li et al., 2016; Otis & Mueller, 2011; Spina et al., 2006). Many related experiments showed that the repeated systemic administration of SCH23390 could prevent cocaine CPP (Baker, Fuchs, Specio, Khroyan, & Neisewander, 1998; Cervo & Samanin, 1995). Our previous findings confirmed that bilateral NAc-shell infusion of SCH 23390 (dopamine D₁ receptor antagonist) could disturb CPP related behavior during cocaine memory reconsolidation (Y Li et al., 2016). Many previous studies showed that dopamine D₁ receptors can modulate IEG-encoded protein expression via the cyclic adenosine monophosphate (cAMP)-protein kinase A (PKA)-ERK-CREB pathway (Goto, Otani, & Grace, 2007; Y. Li et al., 2016; David W Self, 2004). Additionally, many studies demonstrated that dopamine D₁ receptors are necessary for the induction of long-term potentiation (LTP) and long-term depression (LTD) both *in vitro* and *in vivo* (Centonze et al., 2003; Kerr & Wickens, 2001; Shen, Flajolet, Greengard, & Surmeier, 2008). The activation of dopamine D₁ receptors has been shown to enhance cocaine CPP (Kreitzer & Berke, 2011; Lobo & Nestler, 2011). As a result, dopamine D₁ receptor antagonists could effectively disturb subsequent cue-induced cocaine SA-related memory reconsolidation drug-seeking behavior after re-exposure to drug-paired environmental stimuli.

Numerous studies have verified that cocaine-seeking behavior can be reinstated following the administration of dopamine D₂ receptor agonists (De Vries, 2002; D. W. Self, Barnhart, Lehman, & Nestler, 1996), while cocaine priming-induced drug-seeking behavior was attenuated following the administration

of a dopamine D₂ receptor antagonist (Khroyan, Barrett-Larimore, Rowlett, & Spealman, 2000). Previous studies showed that raclopride (dopamine D₂ antagonist, s.c.) could completely prevent drug-seeking behavior induced by the reintroduction of cocaine-paired stimuli (Cervo, Carnovali, Stark, & Mennini, 2003; Froger-Colleaux & Castagne, 2016). This finding is the same as our results. The above results showed that dopamine D₂ receptors could affect subsequent cue-induced cocaine SA-related memory reconsolidation drug-seeking behavior after re-exposure to drug-paired environmental stimuli.

However, conditioned place preference for psychostimulants was reduced by the activation of D₂ receptors (Kreitzer & Berke, 2011; Lobo & Nestler, 2011) and increased by their inactivation (Ferguson et al., 2011). Moreover, D₂ inhibition increased motivation for cocaine, whereas the activation of D₂ receptors reduced cocaine self-administration (Bock et al., 2013). Dopamine mainly includes D₁ and D₂ two subtype receptors, both of which are slow metabotropic receptors coupled with G-proteins. D₁ receptors activation increases intracellular cAMP, while D₂ receptors activation decreases intracellular cAMP (Goto et al., 2007). Therefore, it is theoretically possible that two dopamine receptors may have the opposite effect by inducing different forms of neuronal plasticity, leading to subsequent disruption of learning and memory functions (Floresco & Phillips, 2001; Nasehi et al., 2010). However, in our experiment, we did not find this phenomenon. We previously demonstrated that a certain concentration of SCH 23390 (dopamine D₁ receptor antagonist) could disrupt cue-induced cocaine memory reconsolidation after re-exposure to cocaine-associated environmental cues in a cocaine-induced CPP model for rats but that raclopride (dopamine D₂ receptor antagonist) could not (Y Li et al., 2016). It is possible that there are different mechanisms for CPP and SA animal models of drug addiction. Several previous studies have shown that MK-801 (an NMDA receptor antagonist) can disrupt the reconsolidation of cocaine-related memory in the CPP model but not in the SA model in rats (Brown et al., 2008). Thus, further investigation is needed to explore the mechanism to clarify the molecular network involved in addictive memory reconsolidation and the specific molecular mechanism underlying drug addiction (Y. Li et al., 2016).

In summary, according to our data, the dopamine system could be the main site of reward circuit activation in the process of cue-induced drug memory reconsolidation, and this result may provide a theoretical basis for the clinical development of interventions focused on a critical target-dopamine system for the purpose of treating drug addiction. Furthermore, our results showed that dopamine D₁ and D₂ receptors are key regulators of dopamine system function during the reconsolidation addictive memory process. Memory reconsolidation theory suggests that it may be a key stage in the treatment of pathological memory function. Importantly, dopamine D₁ and D₂ receptors may represent pharmacological targets for the treatment of drug addiction with therapies that interfere with drug memory reconsolidation.

Declarations

Funding sources

This project was supported by the National Natural Science Foundation of China (No. 81671366 and 81971244, awarded to Xue-lian Wang) and Science and Technology Innovation Foundation of Tangdu Hospital, the Fourth Military Medical University (No. 2017LCYJ002, awarded to Xue-lian Wang).

Authors' contribution

Yang Li, Nan Li and Liang Qu equally contributed to this work including animal surgeries, drug self-administration training, data collection, immunohistochemical staining, manuscript draft and so on.

Xue-lian Wang was involved in study concept and design, and also provided funding.

Shun-nan Ge helped design the primary study, provided advice on the data analysis, and edited the manuscript.

Xin Wang and Ping Wang processed data and conducted literature searches.

Jian Fu, Yu-kun Chen and Jian-cai Wang provided advice on the data analysis and implemented statistical analysis.

All authors read and approved the final manuscript.

Conflicts of interest

The authors have no conflicts of interest to declare.

Statement

All animal procedures in our experiments were consistent with the guidelines of the Committee for Animal Care and Use (No. TDLL2018-03-180, Tangdu Hospital, the Fourth Military Medical University, Xi'an, Shaanxi, China). And the experimental protocols were approved by the Committee for Animal Care and Use of Tangdu Hospital, the Fourth Military Medical University. In addition, all methods in our experiment are reported in accordance with ARRIVE guidelines.

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Figures

Figure 1

The experimental process.

Figure 2

The cocaine self-administration training procedure (A and D). Compared to the first three days, the number of valid nose poke for the last three days in Cocaine-addicted group increased significantly (Day1~3: 8.994 ± 0.4945 , Day12~14: 28.55 ± 0.3998 , $t_{\text{Cocaine-addicted group}}=30.76$, $P<0.01$. (F)) during cocaine SA training period and kept relatively stable during the last three days of training period ($F_{\text{Cocaine-addicted group}}=0.1244$, $P=0.8831$. (E)). However, there was a little decreasing for that in Saline group (Day1~3: 4.095 ± 0.5385 , Day12~14: 2.095 ± 0.4018 , $t_{\text{Saline group}}=2.977$, $P<0.01$. (C)), and the

number of valid nose pokes reached a relatively stable level during the last three days of training period (FSaline group=1.028, $P=0.3777$. (B)).

Figure 3

A. The number of valid nose pokes for the last three days between Cocaine-addicted group and Saline group was significant difference (Day12~14 in Saline group: 2.095 ± 0.4018 , Day12~14 in Cocaine-addicted group: 28.55 ± 0.3998 , $t_{\text{Cocaine-addicted group, Saline group}}=23.17$, $P<0.01$). B. The original recording of the experimental rat's behavioral events. The drug self-administration training lasted 2 h per day, and the number of nose pokes (valid nose poke and invalid nose poke) and the number of pump were all recorded. C and D. The comparison before and after valid nose poke for the experimental rat. Before valid nose poke, only green light is on (C). When the experimental rat finished a valid nose poke, the system will pump drug once ($T=1.25$ s, $v=1.60$ mL/min), accompanied with several changes in environmental cues such as signal lights (Green light is off, and red light is on for 20 s), valid nose poke light ($T=20$ s), and sound (buzzer, $T=1.25$ s) (D). SA: Self-administration. Data were presented as means $\pm$ SEM, ** $P<0.01$.

Figure 4

The location of VTA lesion. The figure "A" and "B" were coronal schematic diagrams in the VTA level (Bregma -5.20 mm). The figure "D" was a coronal real diagram in the VTA level. The figure "C" was the coronal real diagram in the VTA level by TH-immunohistochemical staining. The two framed areas in "C" were magnified in "E" and "F", respectively. The yellow arrows showed the injection site of 6-OHDA by a 1 μ L syringe. "*" presented the tip of syringe. The Green arrows showed many dopamine neurons labelled by TH. There was almost no dopamine neuron labelled by TH on the left side, although a lot of dopamine neurons labelled by TH existed in the right side. 6-OHDA: 6-hydroxydopamine; TH: tyrosine hydroxylase; VTA: ventral tegmental area; MT: medial terminal nucleus of the accessory optic tract; fr: fasciculus retroflexus. Scale bar could be seen in the corresponding figures, respectively.

Figure 5

The cocaine self-administration training procedure in every subgroup. The number of valid nose poke in every Cocaine-addicted group (Control group, VTA-lesioned group, 0.02 mg/kg SCH 23390 group, 0.20 mg/kg SCH 23390 group, 0.50 mg/kg SCH 23390 group, 0.02 mg/kg Raclopride group, 0.10 mg/kg Raclopride group and 0.20 mg/kg Raclopride group) increased significantly during cocaine SA training period and kept relatively stable during the last three days of training period. However, there was a little decreasing for that in Saline group and the number of valid nose poke reached a relatively stable level during the last three days of training period. Data were presented as means $\pm$ SEM.

Figure 6

The number of valid nose poke for the last three days in every Cocaine-addicted group reached a relatively stable level during the last three days of training period (FControl group=0.3360, $P=0.7199$; FVTA-lesioned group=0.4505, $P=0.6433$; F0.02 mg/kg SCH23390 group=0.5892, $P=0.5651$; F0.20 mg/kg SCH23390 group=0.03359, $P=0.9670$; F0.50 mg/kg SCH23390 group=0.01210, $P=0.9880$; F0.02 mg/kg Raclopride group=0.2750, $P=0.7627$; F0.10 mg/kg Raclopride group=0.03628, $P=0.9644$; F0.20 mg/kg Raclopride group=0.02997, $P=0.9705$). And that in Saline group also kept relatively stable during the last three days of training period (FSaline group=1.028, $P=0.3777$). Data were presented as means $\pm$ SEM, $**P<0.01$.

Figure 7

Compared to the number of valid nose poke for the first three days, that for the last three days showed a significant increasing in every Cocaine-addicted group (tControl group=18.46, $P<0.01$; tVTA-lesioned group=12.79, $P<0.01$; t0.02 mg/kg SCH23390 group=16.48, $P<0.01$; t0.20 mg/kg SCH23390 group=6.228, $P<0.01$; t0.50 mg/kg SCH23390 group=10.24, $P<0.01$; t0.02 mg/kg Raclopride group=13.91, $P<0.01$; t0.10 mg/kg Raclopride group=9.565, $P<0.01$; t0.20 mg/kg Raclopride group=14.55, $P<0.01$). In contrast, there was a little decreasing for that in Saline group (tSaline group=2.977, $P<0.01$). Next, the number of “valid” nose poke showed a significant decreasing before and after cue-induced cocaine memory reconsolidation in the following groups (tVTA-lesioned group=12.79, $P<0.01$; t0.20 mg/kg SCH23390 group=8.978, $P<0.01$; t0.50 mg/kg SCH23390 group=11.66, $P<0.01$; t0.10 mg/kg Raclopride group=10.39, $P<0.01$; t0.20 mg/kg Raclopride group=12.52, $P<0.01$). While there were no significant change for that in Saline, low SCH 23390 and low raclopride group (tSaline group=1.090, $P=0.2855$; t0.02 mg/kg SCH23390 group=1.863, $P=0.0738$; t0.02 mg/kg Raclopride group=1.758, $P=0.0904$). Interestingly, that in Control group showed a significant increasing (tControl group=4.221, $P<0.01$). Data were presented as means $\pm$ SEM, $**P<0.01$.

Figure 8

The number of valid nose poke for the last three days between every Cocaine-addicted group and Saline group all showed a significant increasing (Day12~14 in Saline group: 2.095 ± 0.4018 ; Day12~14 in Control group: 29.06 ± 0.8023 ; Day12~14 in VTA-lesioned group: 26.67 ± 0.8737 ; Day12~14 in 0.02 mg/kg SCH23390 group: 27.81 ± 0.8036 ; Day12~14 in 0.20 mg/kg SCH23390 group: 29.90 ± 1.502 ; Day12~14 in 0.50 mg/kg SCH23390 group: 31.24 ± 1.481 ; Day12~14 in 0.02 mg/kg Raclopride group: 27.90 ± 0.9256 ; Day12~14 in 0.10 mg/kg Raclopride group: 28.33 ± 1.036 ; Day12~14 in 0.20 mg/kg Raclopride group: 27.86 ± 1.198 . $t_{\text{Control group, Saline group}}=31.38$, $P<0.01$; $t_{\text{VTA-lesioned group, Saline group}}=24.38$, $P<0.01$; $t_{\text{0.02 mg/kg SCH23390 group, Saline group}}=28.62$, $P<0.01$; $t_{\text{0.20 mg/kg SCH23390 group, Saline group}}=17.88$, $P<0.01$; $t_{\text{0.50 mg/kg SCH23390 group, Saline group}}=18.99$, $P<0.01$; $t_{\text{0.02 mg/kg Raclopride group, Saline group}}=25.58$, $P<0.01$; $t_{\text{0.10 mg/kg Raclopride group, Saline group}}=23.62$, $P<0.01$; $t_{\text{0.20 mg/kg Raclopride group, Saline group}}=20.39$, $P<0.01$). In addition, there was no significant difference for the number of valid nose poke for the last three days among every Cocaine-addicted group ($F=1.706$, $P=0.1111$). Data were presented as means $\pm$ SEM, ** $P<0.01$.

Figure 9

The number of “valid” nose poke before and after cue-induced cocaine memory reconsolidation between Control group and Saline group showed a significant increasing (Day29 in Saline group: 1.286 ± 0.4206 , Day29 in Control group: 36.33 ± 1.801 , $t_{\text{Control group, Saline group}}=20.41$, $P<0.01$). The number of “valid” nose poke between VTA-lesioned/high SCH 23390/high raclopride group and Control group all showed a significant decreasing during cue-induced cocaine addictive memory reconsolidation (Day29 in Control group: 36.33 ± 1.801 ; Day29 in VTA-lesioned group: 6.375 ± 0.7545 ; Day29 in 0.20 mg/kg SCH23390 group: 6.000 ± 0.6901 ; Day29 in 0.50 mg/kg SCH23390 group: 0.8571 ± 0.3401 ; Day29 in 0.10 mg/kg Raclopride group: 9.143 ± 0.5948 ; Day29 in 0.20 mg/kg Raclopride group: 1.429 ± 0.3689 . $t_{\text{VTA-lesioned group, Control group}}=16.91$, $P<0.01$; $t_{\text{0.20 mg/kg SCH23390 group, Control group}}=16.69$, $P<0.01$; $t_{\text{0.50 mg/kg SCH23390 group, Control group}}=20.92$, $P<0.01$; $t_{\text{0.10 mg/kg Raclopride group, Control group}}=15.30$, $P<0.01$; $t_{\text{0.20 mg/kg Raclopride group, Control group}}=20.50$, $P<0.01$). However, there was no significant difference for that between low SCH 23390/low raclopride group and Control group during cue-induced cocaine memory reconsolidation (Day29 in Control group: 36.33 ± 1.801 ; Day29 in 0.02 mg/kg SCH23390 group: 32.43 ± 3.677 ; Day29 in 0.02 mg/kg Raclopride group: 31.57 ± 2.359 . $t_{\text{0.02 mg/kg SCH23390 group, Control group}}=0.9026$, $P=0.3861$; $t_{\text{0.02 mg/kg Raclopride group, Control group}}=1.560$, $P=0.1470$). Data were presented as means $\pm$ SEM, $**P<0.01$.

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