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c-fos 발현에 관한 연구

Studies on c-fos activity of VTA dopaminergic neurons

and NAc D2 receptor-expressing neurons

during reward associated learning

2023 년 2월

서울대학교 대학원

뇌과학 전공

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이 논문을 이학석사 학위논문으로 제출함
2023 년 2 월

서울대학교 대학원
뇌과학 전공
이 해 영

이 해 영의 이학석사 학위논문을 인준함
2023 년 2 월

위 원 장 —————— 이 용 석 —————— (인)

부위원장 —————— 강 봉 균 —————— (인)

위   원 —————— 김   형 —————— (인)

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Abstract

Studies on c-fos activity of VTA dopaminergic neurons and NAc D2 receptor-expressing neurons during reward associated learning

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Ventral tegmental area (VTA) is well known for the source of reward-associated dopamine (DA). VTA dopaminergic neurons are strongly connected to the nucleus accumbens (NAc) dopaminergic receptor-expressing neurons. NAc dopamine receptor-expressing neurons are composed of dopamine D1 receptors-medium spiny neurons (D1R-MSNs) and D2R-MSNs. In the classical view, these two populations of neurons have shown opposite functions. D1R-MSNs process rewarding behavior, whereas D2R-MSNs mediate aversive responses. However, recent studies show that D2R-MSNs also contribute to rewarding behaviors. This study

focuses on neuronal activity in VTA dopaminergic neurons and NAc D2R-MSNs and aims to provide information on the IEG activity dynamics of these neurons during reward-associated learning. The relationship between dopamine release and IEG activity remains elusive so this study will help understand the relationship between dopamine and reward-associated learning in the cellular level. As a behavioral experiment, we chose an autoshaping task in which rewards were delivered only after exposure to a specific visual cue. We investigated the IEG activity of VTA TH neurons and NAc D2R-MSNs at 3 time points during the learning task. There are 1) the last training day, 2) the day after the training is completed, and 3) the early learning stage in which the mice have the propensity to approach a specific visual cue. Trained mice showed increased fos activity in the VTA and NAc regions compared to the control mice. TH neurons in the VTA are highly activated during the early learning stage. In contrast, NAc D2R neurons in the trained group showed increased activity when reward-associated memory was formed. In conclusion, this result suggests that VTA TH and NAc D2R neurons participate in reward-associated learning and have different activity patterns during reward-associated memory formation.

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Student Number : 2021-20341

Introduction

Memory association between positive valence and specific stimulus is important ability to select profitable decisions for survival. Previous studies had focused on the dopaminergic system in the mammalian brain as key mechanism of reward−associated memory (Spanagel and Weiss 1999). Although the importance of dopamine during reward associated learning has been well known, what dopamine signals are exactly encoded is still controversial. Here, I suggest how activities of dopaminergic cells and medium spiny neurons are changed according to the reward learning by immediate early gene (IEG)−based−regional study.

Reward associated learning

Reward-related learning is divided into stimulus-outcome and action-outcome. Stimulus-outcome is Pavlovian learning that makes specific memory by pairing repeatedly conditioned stimulus (CS) with the unconditioned stimulus (US). The procedure in which a particular cue is repeatedly paired with a reward is autoshaping, which is known as 'sign-tracking'(Vanover and Barrett 1998). This conditioned response (CR) in autoshaping is characterized by approach behavior directed towards either the cue itself or the location of reward delivery (Bussey, Everitt et al. 1997,

Robbins and Everitt 2002). To confirm memory formation related reward, I measure approach behavior toward cues.

Dopamine and reward associated learning

Dopamine-dependent reward signals are positive value that make animal approach specific object encoded future reward. Biological roles of dopamine in reward-related behavior have been debated (Bromberg-Martin, Matsumoto et al. 2010). There have been two theories that dopamine release reflects motivation and reward prediction error (RPE), respectively. The first theory suggests that dopamine induces Pavlovian motivation. Researchers find that increased artificially dopamine release make mice learn to perform action and describe dopamine dynamics as a continuous motivation (Mohebi, Pettibone et al. 2019). Another theory suggests that dopamine reflects reward prediction errors (Bayer and Glimcher 2005, Schultz 2016). When a mouse gets more reward than predicted, dopaminergic neurons show sharp firing. However, when they get less reward than expected, dopamine response becomes below the basal level. Thus, they suggest the dopaminergic neurons are activated when a mouse get more reward than predicted (Mohebi, Pettibone et al. 2019).

In the mammalian brain, there are four distinct dopamine pathways, which is the mesolimbic, mesocortical, nigrostriatal, and tuberoinfundibular

pathways. The mesolimbic pathway contains dopaminergic projection from ventral tegmental area (VTA) to the nucleus accumbens (NAc), which participates reward-associated memory and reinforcement behavior (Spanagel and Weiss 1999). The mesocortical pathway is projection from the VTA to the PFC, which involves in the formation of stimulus-response association and behavioral adaptation to rule changes (Balleine and O'Doherty 2010). The nigrostriatal pathway represents dopaminergic projection from substantia (SN) to the striatum or the basal ganglia, which controls motor coordination and motor skill behavior (Quessy, Bittar et al. 2021). The tuberoinfundibular pathway is DA projection from the hypothalamus to the anterior pituitary, which may provide a suppressive signal on pituitary lactotrope cells (Habibi 2010). This study focuses on the dopaminergic projection from the VTA to the NAc.

VTA dopaminergic neurons to the medium spiny neurons (MSNs) of the NAc

In VTA, dopamine neurons are a major cell type that accounts for more than 70% of the total population (Yamaguchi, Sheen et al. 2007). These neurons are strongly connected to the nucleus accumbens (NAc). The DA projection is well known to mediate motivated behavior. Previous studies show that activation of this circuit evokes greater dopaminergic

activity that subsequently promotes the acquisition of goal-directed action (Owesson-White, Belle et al. 2016). Activation of mesolimbic DA neurons is thought to transfer this reward-associated information to the preceding cue.

NAc neurons are mainly composed of GABAergic medium spiny neurons (MSNs) expressing either dopamine D1 or D2 receptor-expressing neurons, but few contain both (Soares-Cunha, de Vasconcelos et al. 2020). The classical view on the field proposes that two subtypes have distinct role for D1R-expressing neurons and D2R-expressing neurons in encoding reward and aversion (Hikida, Kimura et al. 2010, Lobo, Covington et al. 2010, Kravitz, Tye et al. 2012, Soares-Cunha, de Vasconcelos et al. 2020). Yet, recent studies suggest these two subgroups can involve simultaneously in reward-associated behavior. They postulated that D2-MSNs also contribute to the reward- associated behavior depending on the neural stimulation pattern (Soares-Cunha, Coimbra et al. 2018, Soares-Cunha, Domingues et al. 2022). They used various optogenetic stimulation protocol to manipulate NAc-MSNs activity. The result showed that brief optogenetic stimulation (1 s stimulus—40 pulses of 12.5 ms at 40 Hz) of D2-MSNs enhanced cocaine conditioning, but prolonged activation (60 s, 12.5 ms pulses at 40 Hz) reduced cocaine conditioning (Soares-Cunha, de Vasconcelos et al. 2020). These findings support D2R neurons are also

involved in mediating reward-associated behavior. There are controversial ideas about the role of D2R neurons in reward-seeking behavior. From these data, cell-type-specific and subregion-specific research is required for the NAc study.

Immediate early gene (IEG)

IEGs are the first identified genes for which the activation of transcription is fast within minutes and transient after stimuli. Thus, the activation of IEGs has been used as markers of neural activity and a powerful tool to identify neural circuits involved in the learning and memory process (Maleeva, Ivolgina et al. 1989, Hoffman, Smith et al. 1993, Minatohara, Akiyoshi et al. 2016). Surprisingly, research on dopamine release dynamics and firing patterns depending on cue or unconditioned stimulus has been actively discovered, but IEG studies still need to be better understood. I try to find the neural activity dynamics of VTA DA neurons and NAc-MSNs in motivated behavior by IEG study and help understand reward-associated learning and memory.

Materials and methods

Mice

All experiments were performed on 10~20-week-old male D2-EGFP (Fig.1,2,3,5,6) or D2-cre mice (Fig.4) provided by Ja Wook Koo in DGST. Mice were raised in 12-hr light/dark cycle in standard laboratory cages and given ad libitum access to food and water. Before task training, mice were subject to mild food restriction and continued during training. All procedures are animal care were follwed the regulation and guidelines of the Institutional Animal Care and Use Committees (IACUC) of Seoul National University.

Autoshaping task

Autoshaping were followed as previously described (Horner, Heath et al. 2013, Boulos, Nasseef et al. 2019). Mice were tested in the active period of circadian cycle. After a variable ITI, a trial is initiated when the animal breaks the IR beam at the rear of the chamber and a stimulus is displayed (CS+ or CS−). Regardless of the animal's behavior, stimulus offset occurs after a prespecified display time (standard: 10 s). Upon CS+ offset, a reward is delivered, and when the animal enters the magazine to collect it another variable ITI begins. Upon CS− offset, reward is not

delivered and another variable ITI begins. The house light is off throughout the task. Each pair of trials comprises one CS+ trial and one CS− trial, such that each 50-trial session includes 25 presentations of each type.

AAV production

Adeno-Associated Viruses serotype 1/2 (AAV1/2; AAV particle that contains both serotype 1 and 2 capsids) were used in all the experiments. AAV1/2s were purified from HEK293T cells that were transfected with plasmids containing each expression cassette flanked by AAV2 ITRs, p5E18, p5E18-RXC1 and pAd-ΔF6 and cultured in 18 ml or 8 ml Opti-MEM (Gibco-BRL/Invitrogen, cat# 31985070) in a 150-mm or 100-mm culture dish, respectively. Four days after transfection, the medium containing AAV1/2 particles was collected and centrifuged at 3,000 rpm for 10 min. After 1 ml of heparin-agarose suspension (Sigma, cat# H6508) was loaded onto a poly-prep chromatography column (Bio-Rad Laboratories, Inc. cat# 731-1550), the supernatant was loaded onto the column carefully. The column was washed by 4 ml of Buffer 4-150 (150 mM NaCl, pH4 10 mM citrate buffer) and 12 ml of Buffer 4-400 (400 mM NaCl, pH4 10 mM citrate buffer). The virus particles were eluted by 4 ml of Buffer 4-1200 (1.2 M NaCl, pH4 10 mM citrate buffer). The eluted solution was exchanged with PBS and concentrated using an Amicon Ultra-15 centrifugal filter unit

(Millipore, cat# UFC910024). The titer was measured using quantitative RT-PCR.

Stereotaxic surgery

Mice (8~10 weeks) were anesthetized with a ketamine/xylazine solution and positioned in a stereotaxic apparatus (Stoelting Co.). The viruses were injected using 33 gauge needle with Hamilton syringe at a 0.1 μl/min rate into target regions. At all injected points, the tip of the needle was positioned 0.05mm below the target coordinate and returned to the target site after 2min. After injection, the needle stayed in place for an additional 7 mins and was withdrawn slowly. Stereotaxic coordinates for each target sites are ventral tegmental area (AP:-2.9,ML:±0.7,DV:-4.6),

nucleus accumbens (AP:+1.5,ML:±0.9,DV:-4.15).

Immunohistochemistry

Brains were postfixed in 4% PFA at 4°C overnight and transferred to 30% sucrose for 2 days at 4°C. Brains were embedded in optimal cutting temperature mounting medium, and 40-μm sections were cut using a cryostat (Leica). For immunohistochemistry, primary antibodies rabbit anti-fos (#226003; Sysy), chicken anti-TH (ab76442; Abcam), were diluted to

1:1000 to 1:300 in blocking solution and incubated for 24 hours at 4°C. Secondary antibodies in the blocking solution were incubated for 2 hours at room temperature, and sections were mounted with Vectashield. Images were viewed under Zeiss LSM 700.

Statistical analysis

All data were analyzed by Prism 8 software. Unpaired t-test was conducted to compare two different groups. In all statistics, significance was shown with n.s ($p > 0.05$), * ($p < 0.05$), ** ($p<0.01$), and *** ($p<0.001$). Data is represented with mean ± SEM. Two-way ANOVA was used to analyzed the mean differences between groups. In all statistcal analyses, significnace was shown as *p <0.0332, **p<0.0021, ***p<0.0002, and ****p<0.0001. Data are presented as the mean±SEM.

Results

Experimental design and validation of autoshaping protocol

Autoshaping behavior is one of the used tasks to form the reward associated memory and quantify the reward−related performance of animal. This behavior task consists of two parts, which are food restriction and autoshaping training. Mice restrict food for 3 days, habituation for 1 day, and autoshaping training for 9 days (Horner, Heath et al. 2013, Boulos, Nasseef et al. 2019) (Fig. 1A). Visual stimuli appear randomly in either left or right position on the touchscreen. Visual stimulus in the right position is CS+ delivered reward, and the left position is CS- in the trained group (Fig. 1D). The Control group is no-reward group that isn't given reward when any stimulus is exposed (Fig. 1C). CS+ and CS- appear 25 times in a session, respectively. Mice conduct two sessions per day for 9 days. To quantify the reward-related memory performance, I measured the approach to the stimulus when each visual stimulus was exposed. The trained group has the propensity to approach to the CS+ location through autoshaping training (Fig. 1F). There is not this propensity in the control group (Fig. 1E).

The trained mice increased c-fos expression in the VTA and NAc regions compared to the no-reward control mice

To compare fos activity between the no-reward control and trained groups, I perfuse mice with 4% PFA after 90min from second session of day 9 of training. And then, the c-fos is detected by immunohistochemical approach (IHC) (Fig. 2A). Trained mice show the propensity of approach to the CS+ over the training day (Fig. 2C). No-reward group doesn't show this propensity. No-reward group have no significant difference in response to visual stimuli over the training day (Fig. 2B). Trained group increase c-fos expression in the VTA and the NAc regions relative to no-reward group (Fig 2D,2E).

c-fos activity of VTA dopaminergic neurons and NAc D2R neurons in response to the conditioned stimulus after reward associated memory formation.

D2-EGFP mice conducted autoshaping task for 9 days. To test response to cue itself, I exposed the mice with only right visual stimulus, or CS+ for 3 minutes a day after reward-associated learning (Fig. 3A). I confirmed that dopamine level of the trained mice was sustained within 3 minutes of visual stimulus. It is the reason to decide CS+ exposure for 3 minutes. If a stimulus is exposed longer than 3 minutes, the animal could be

habituated to the task. I analyzed approach trials of stimuli and percent of fos expression. The fos activity of D2R neurons included fos expression of $D2R^+ D1R^+$ neurons and $D2R^+ D1R^-$ neurons. No significant difference between control and trained group was showed in NAc, medial NAc shell, and VTA regions (Fig. 3D,3E,3F). Trained group showed that percent of fos activity was increased in NAc and VTA, not in NAc shell. But it was no significant. To test fos expression in cell-type specific and activity-dependent manner, I analyzed cell type specific fos expression. NAc D2 receptor - expressing cells and VTA dopaminergic neurons increased fos protein than control, but not significant.

Overlapping population between the activated population during training and the activated population in response to the conditioned stimulus after training

To compare the activated population between day 9 of training and day 10 ended autoshaping learning, each population should be distinguished. To distinguish the population, I used fos-rtTA and dox system. To label activated cells on day 9 of training, I expressed TRE3G-mCherrynuc with FAH-rtTA3G in the VTA and NAc regions (Fig. 4B). I injected doxycycline 2 hrs before autoshaping learning to label cells that activated during these events. To label activated cells by cue, the mice was perfused 90 mins after

the right visual stimuli exposure and then detected fos expression by immunohistochemical (Fig. 4A).

I confirmed whether the trained group has reward-associated memory by measuring the approach trials toward the visual cue (Fig. 4C). I estimated NAc cells expressing mcherrynuc with fos antibody to be 46.4(%) in no-reward group and 43.3(%) in the trained group, based on the percentage of overlapping fluorescence (Fig. 4D). In VTA, the percentage of mcherrynuc signal that contains fos labeling A488 is 75.9(%) in the control mice and 66.9(%) in the trained mice (Fig. 4E). In total populations, no significant difference in fos expression between the control and the trained group was showed in the VTA and the NAc regions.

NAc D2R neurons are highly activated compared to the random-reward group when the reward-associated memory is formed.

To experiment whether increased fos expression represents reward-associated learning of a particular cue, I compared cell activity between a control group that obtained a reward randomly after a stimulus and a trained group that delivered a reward only after a specific visual cue was presented (Fig. 5A-5C). In the trained group, fos expression of NAc neurons was

increased and activity of D2R neurons was increased compared to those given reward randomly (Fig. 5E,5F). Interestingly, activation of total neurons in the VTA was increased, but the fos expression of TH neurons was similar to those of the random-reward control group (Fig. 5G,5H).

VTA dopaminergic neurons are highly activated compared to the random reward group during early learning stage.

In previous studies, the pattern of cue- and reward-evoked dopamine signal was different across learning. As learning progresses, the dopamine release for a cue increases, and the dopamine release for a reward gradually decreases (Stelly, Girven et al. 2021). Thus, in the well-trained mice, the dopamine release for a cue is larger than the response for a reward (Sun, Zeng et al. 2018). Based on this photometry data, in this IEG study, I also tried to compare the cell activity in the early stages of learning as well as the stage of learning completed (Fig. 6A-6C). I tried to compare the cell activity when learning was not completed but discrimination of cues behaviorally. I defined the learning stage at this time as an early learning stage. In other words, day 7, when the approach to CS+ begins to increase, was defined as the early learning stage (Fig. 5D) (Stelly, Girven et al. 2021). There was no significant difference in approach trials between groups (Fig. 6D). I compared fos expression in the random-reward control group and the

trained group (Fig. 6E-6H). Specifically, only the fos level of the dopaminergic neurons of the VTA significantly increased compared to the random-reward control group (Fig. 6G)

Figures

A

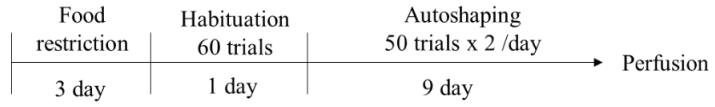|	Food restriction	Habituation 60 trials	Autoshaping 50 trials x 2 /day
	3 day	1 day	9 day

B
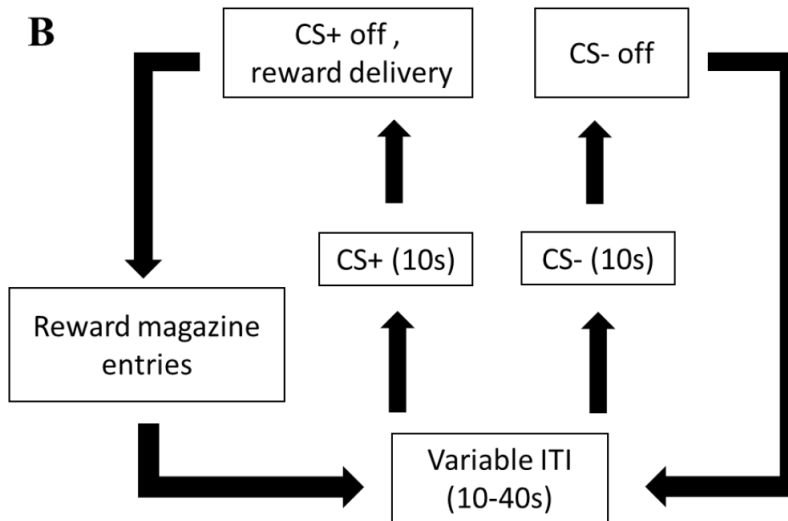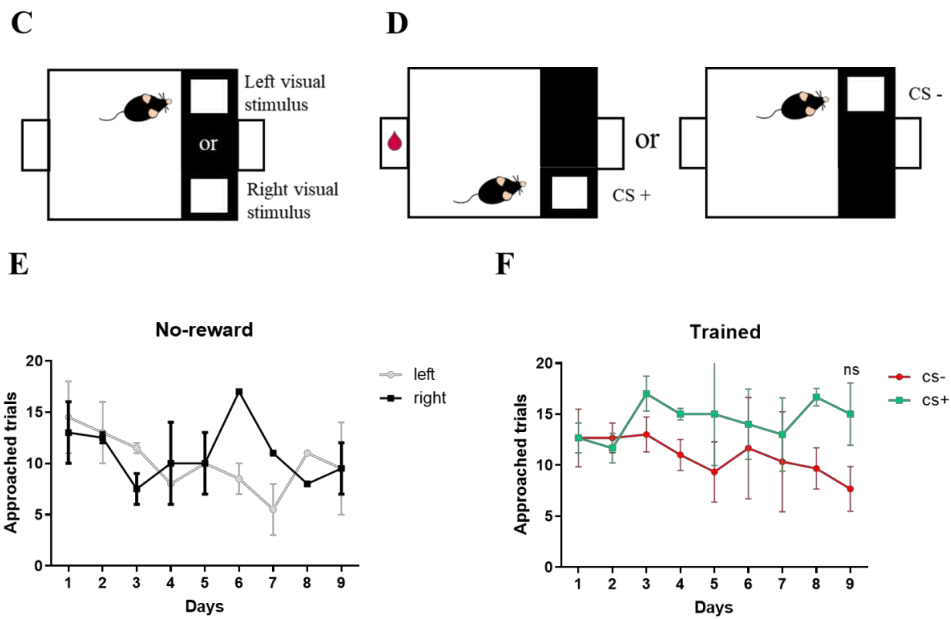
Figure 1. Experimental design and validation of autoshaping protocol.

(A)Behavioral scheme. (B) Flowchart overview of the autoshaping task. (C, E) Approached trials in no-reward group (n=2). (D,F) Approached trials in trained group (n=3). Two way ANOVA was applied for the trained mice; n.s (p=0.7145).

A

Food restriction	Habituation 60 trials	Autoshaping 50 trials x 2 /day	→ Perfusion
3 day	1 day	9 day	

B

No-reward

C

Trained

D

NAc

E

VTA

F

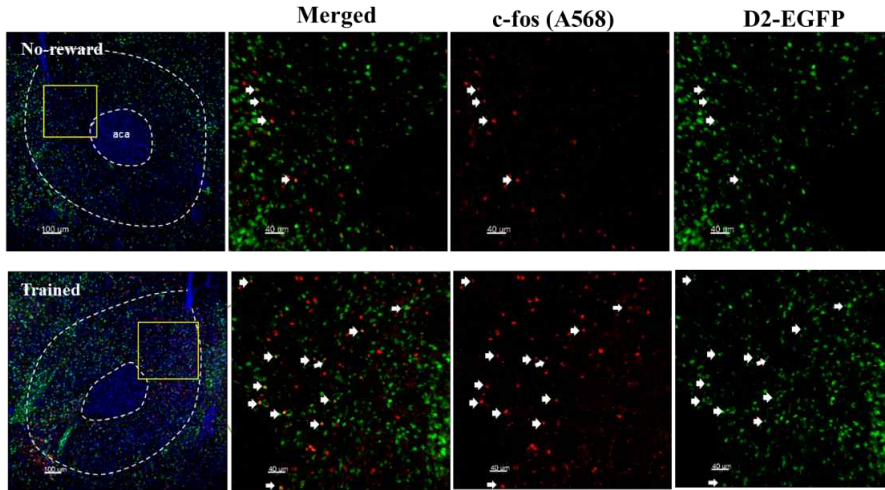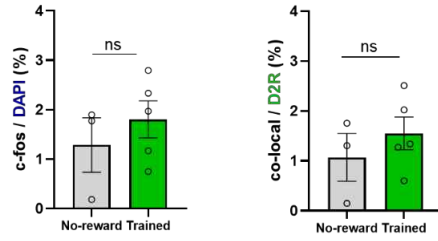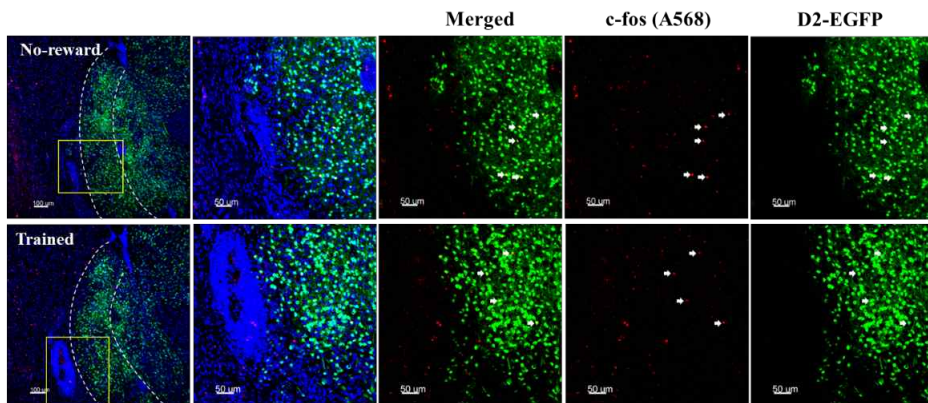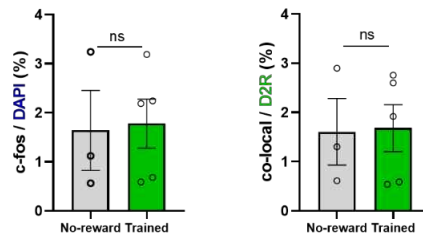| Merged	DAPI	c-fos (A568)
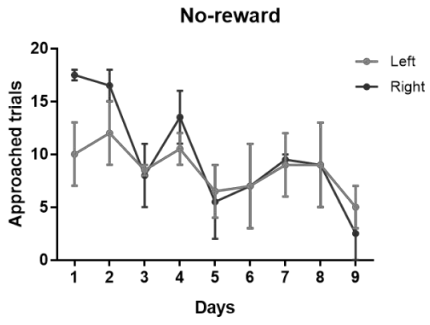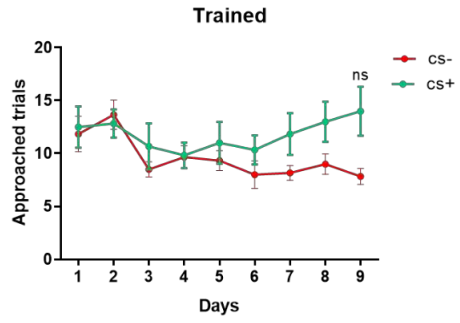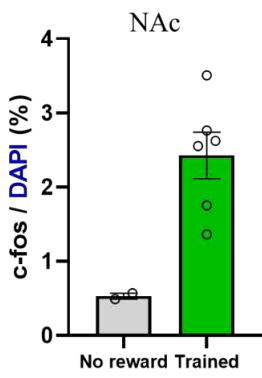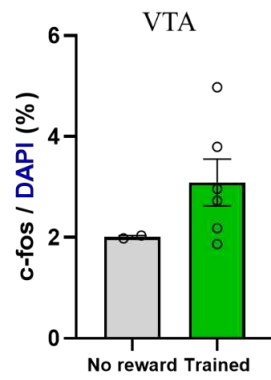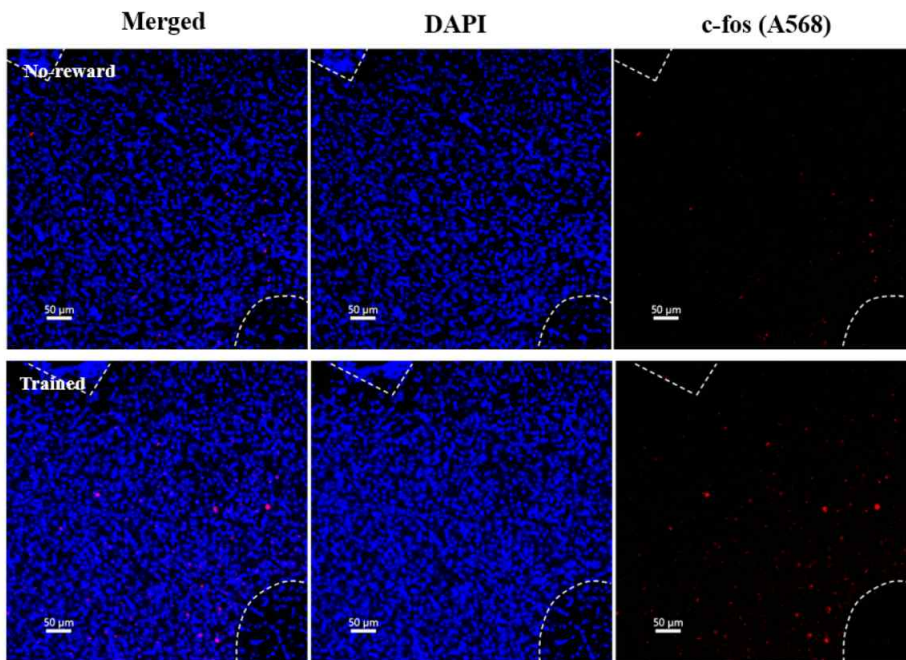
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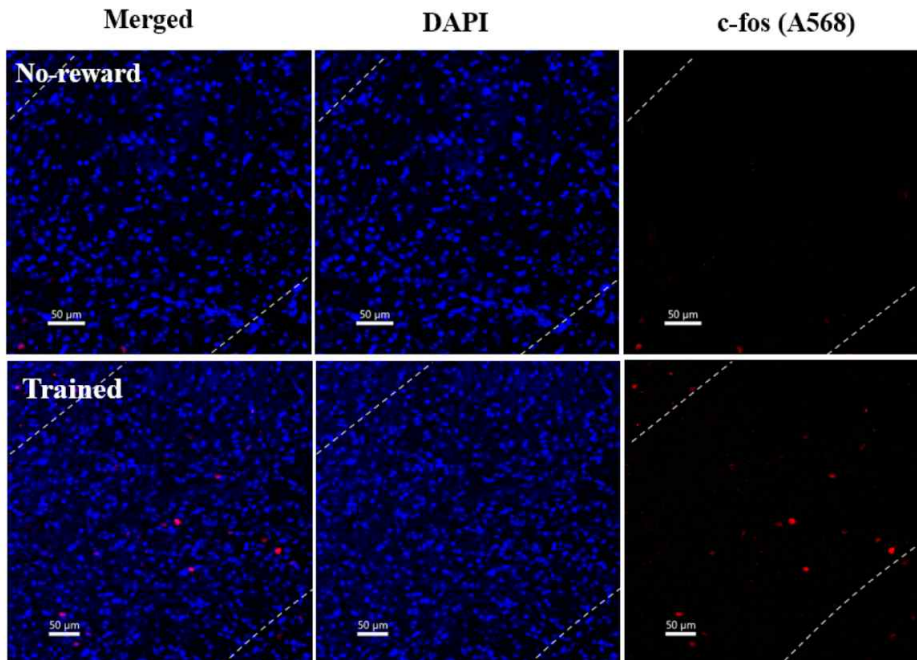
Figure 2. The trained mice increased c-fos expression in the VTA and NAc regions compared to the no-reward control mice.
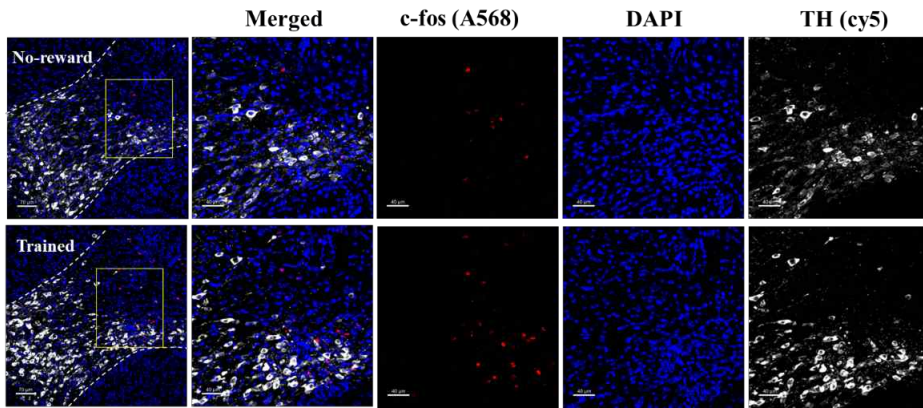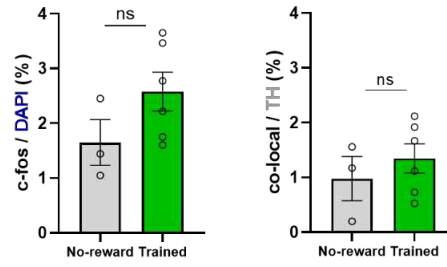
(in collaboration with Min Jung Kim)

(A)Behavioral scheme. (B,C) Approach trials in the no-reward and the trained group (no-reward, n=2 ; trained, n=6). Two way ANOVA was applied to learning curve in the trained group; n.s (p=0.3319). (D,F) c-fos expression in the NAc region. (E,G) c-fos expression in the VTA region (no-reward, n=2 ; trained, n=6).

A

Food restriction	Habituation 60 trials	Autoshaping 50 trials x 2 /day	Only CS+ exposure	→ Perfusion
3 day	1 day	9 day	1 day	

B

No-reward

C

Trained

D

Merged   c-fos (A568)   D2-EGFP

No-reward

Trained

E

Merged   c-fos (A568)   D2-EGFP

No-reward

Trained

F

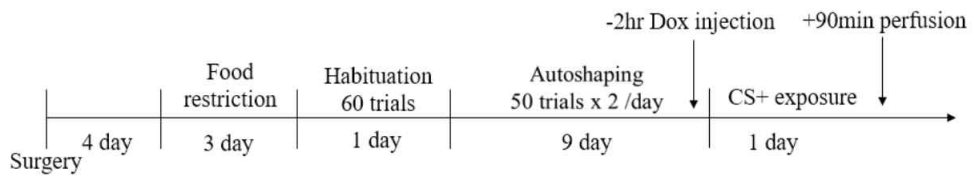| Merged | c-fos (A568) | DAPI | TH (cy5) |
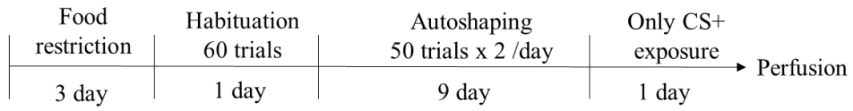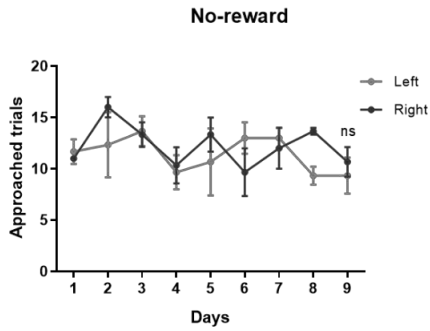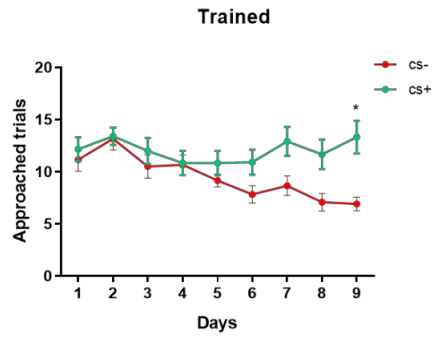
Figure 3. c-fos activity of VTA dopaminergic neurons and NAc D2R neurons in response to the conditioned stimulus after reward associated memory formation.
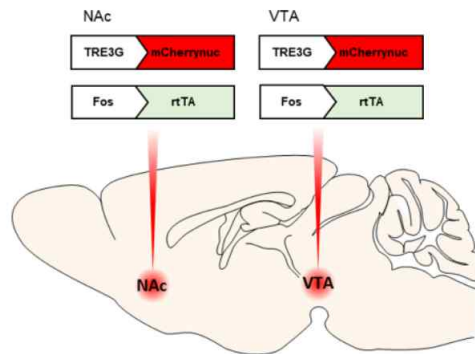
(in collaboration with Min Jung Kim)

(A)Behavioral scheme. (B,C) Approach trials in the no-reward and the trained group (no-reward, n=3 ; trained, n=5). Two way ANOVA was applied to learning curve of each group; n.s (p=0.9997) for no-reward group; * (p=0.0178) for trained group. (D) c-fos expression in the NAc region. Unpaired t test was applied to fos expression of each group; NAc neurons, n.s (p=0.4492); NAc D2R neurons, n.s (p=0.4250). (E) c-fos expression in the NAc shell region. Unpaired t test was applied; NAc shell neurons, n.s (p=0.8829); NAc shell D2R neurons, n.s (p=0.9273). (F) c-fos expression in the VTA region. Unpaired t test was applied; VTA neurons, n.s (p=0.1559); VTA TH neurons, n.s (p=0.4589).
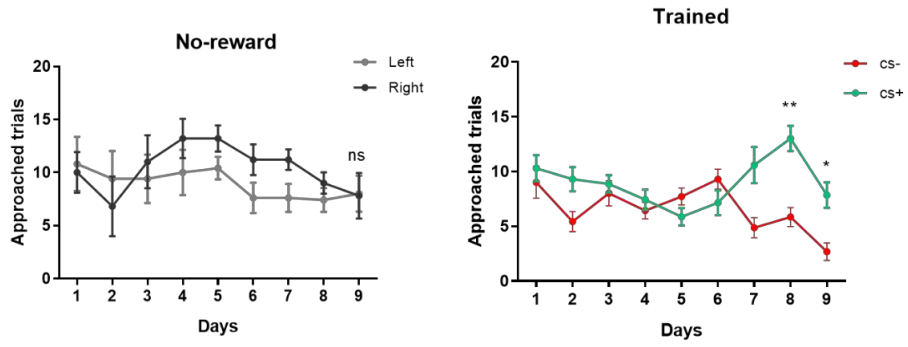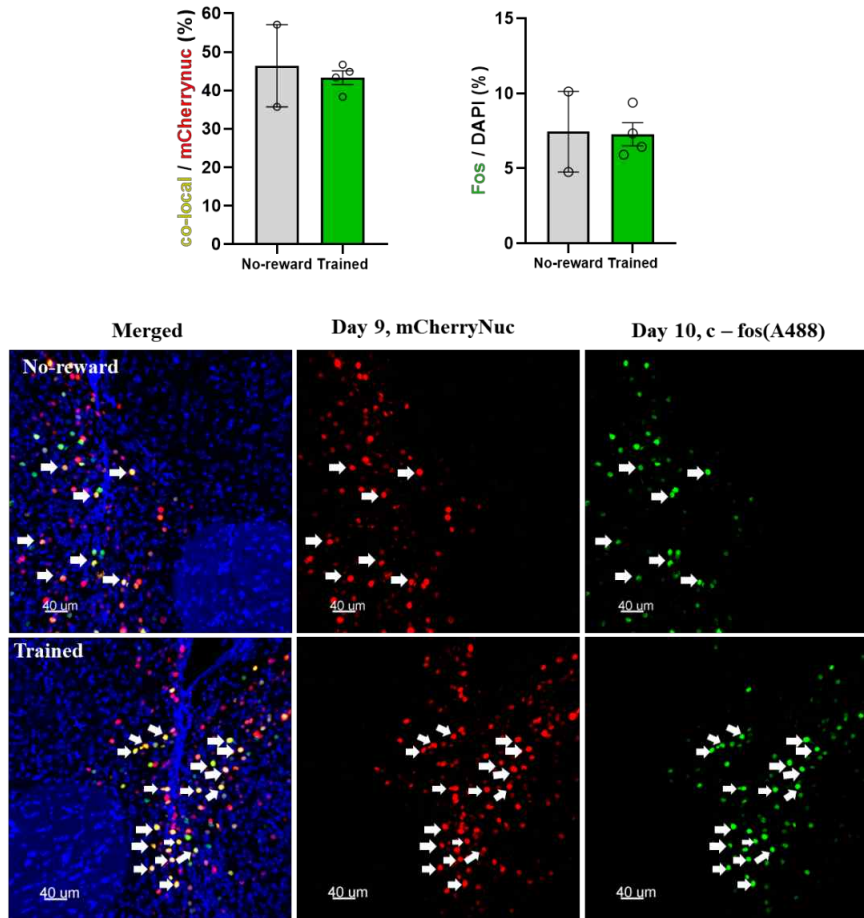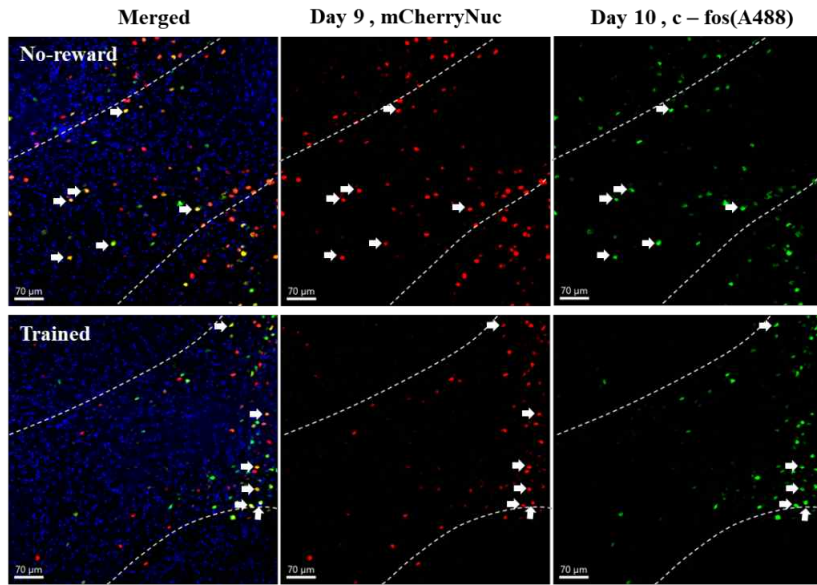
A

B

29

C

No-reward

Left
Right

Approached trials

ns

Days

Trained

cs-
cs+

Approached trials

**

*

Days

D

co-local / mCherrynuc (%)

No-reward Trained

Fos / DAPI (%)

No-reward Trained

| Merged | Day 9, mCherryNuc | Day 10, c − fos(A488) |

No-reward

40 µm

Trained

40 µm

E

| | Merged | Day 9 , mCherryNuc | Day 10 , c − fos(A488) |

No-reward

Trained

70 µm

31
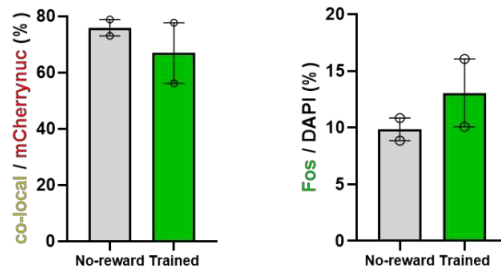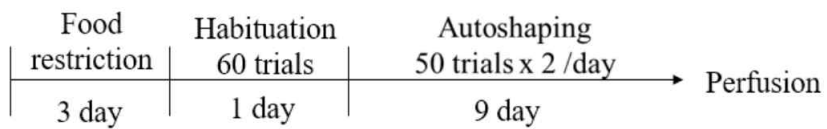
Figure 4. overlapping population between the activated population during training and the activated population in response to the conditioned stimulus after training.
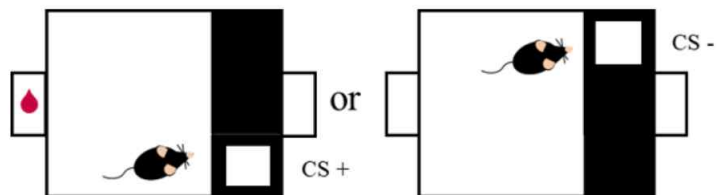
(in collaboration with Min Jung Kim)

(A)Behavioral scheme. (B) Schematic illustrations of injected AAVs and brain sites. (C) Approach trials in the no-reward and the trained group (no-reward, n=3; trained, n=5). Two way ANOVA was applied to learning curve of each group; n.s (p=0.9862) for no-reward group; * (p=0.00481), ** (p=0.0064) for trained group. (D) overlapping population between the activated population during training and the activated population in response to the conditioned stimulus after training in the NAc region (no-reward, n=2; trained, n=4). (E) overlapping population in the VTA (no-reward, n=2; trained, n=2).

A

Food restriction	Habituation 60 trials	Autoshaping 50 trials x 2 /day	Perfusion
3 day	1 day	9 day	

B

CS +

CS -

C

Left visual stimulus

or

Right visual stimulus

D

E

F

G

H

| | Merged | c-fos (A568) | TH (cy5) |
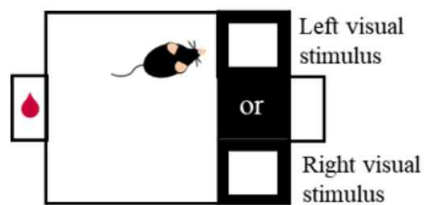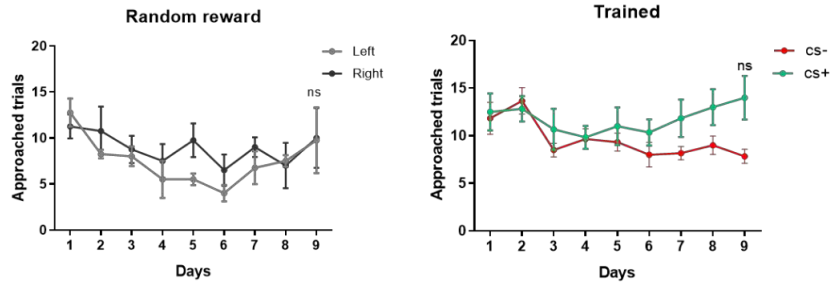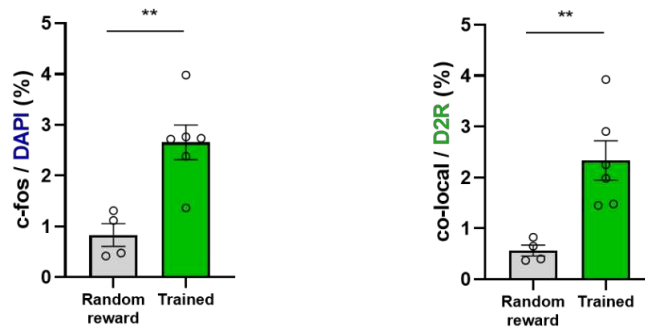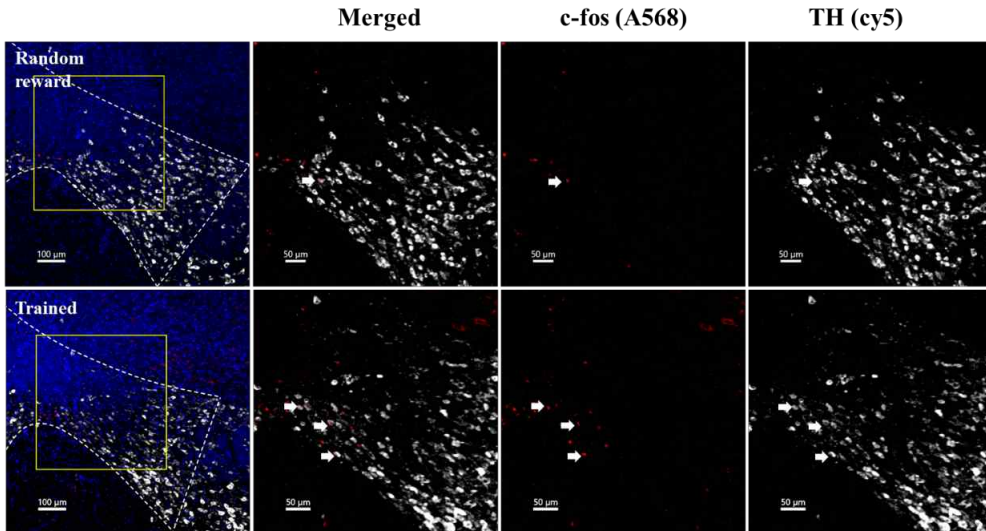
Figure 5. NAc D2R neurons are highly activated compared to the random reward group when the reward-associated memory is formed.

(in collaboration with Min Jung Kim)

(A)Behavioral scheme. (B,C) illustrations of trained group and random reward group. (D) Approach trials in the random reward and the trained group (random reward, n=4 ; trained, n=6). Two way ANOVA was applied to learning curve of each group; n.s (p>0.9999) for no-reward group; n.s (p=0.3319) for trained group. (E,F) c-fos expression in the NAc region. Unpaired t test was applied to fos expression of each group; NAc neurons, ** (p=0.0043); NAc D2R neurons, ** (p=0.0070). (G,H) c-fos expression in the VTA region. Unpaired t test was applied; VTA neurons, * (p=0.0261); VTA TH neurons, n.s (p=0.5003).

A

Food restriction | Habituation 60 trials | Autoshaping 50 trials x 2 /day → Perfusion
3 day | 1 day | 7 day

B

CS +

CS -

or

C

Left visual stimulus

or

Right visual stimulus
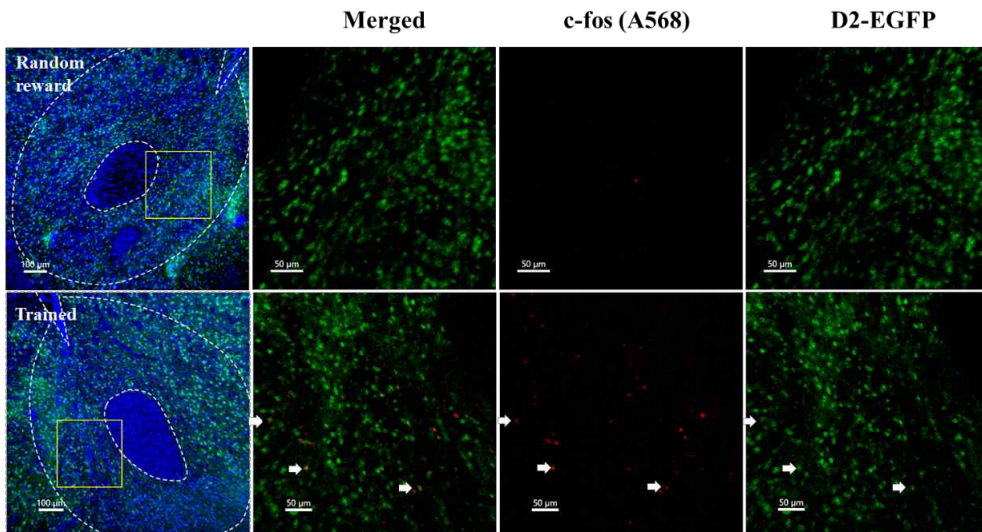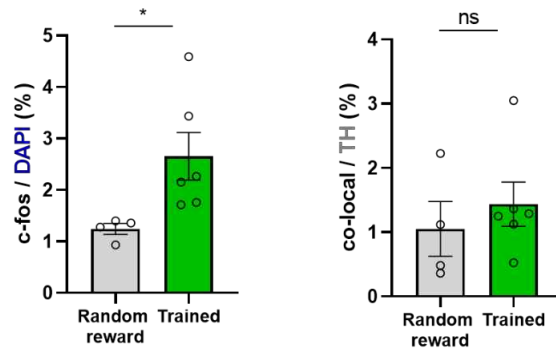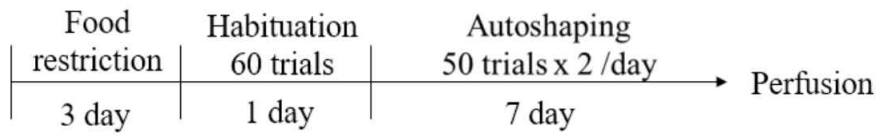
D

E
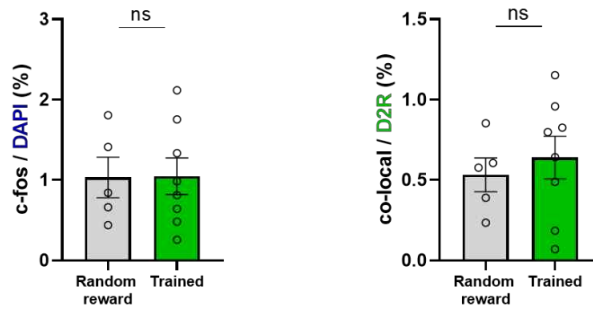
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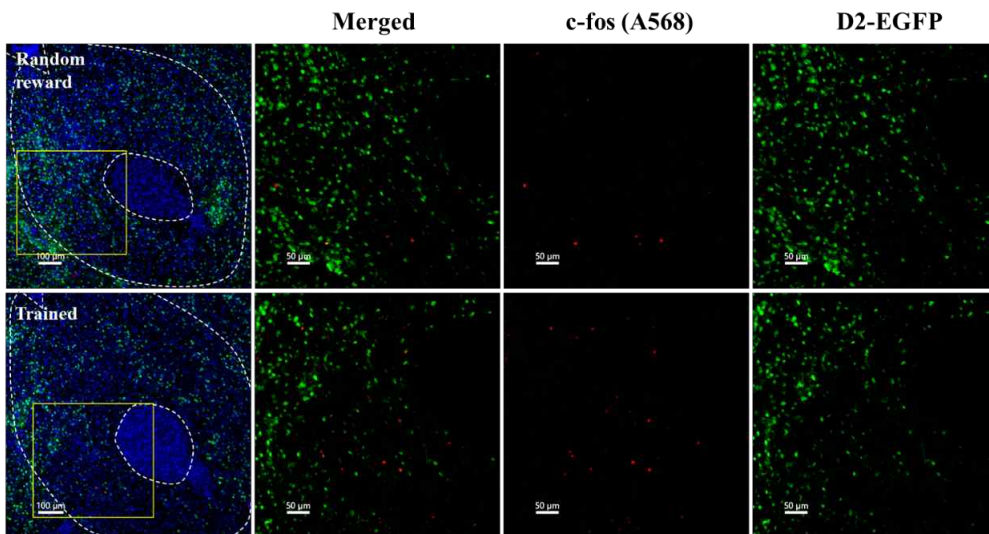
G
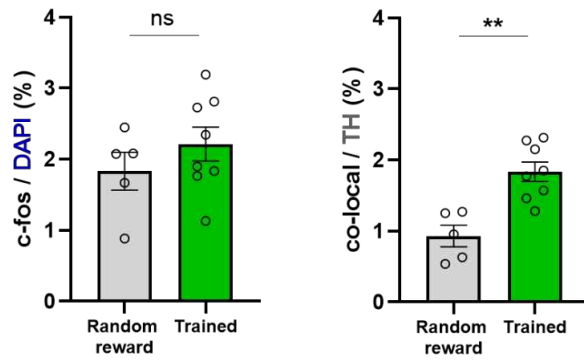
H
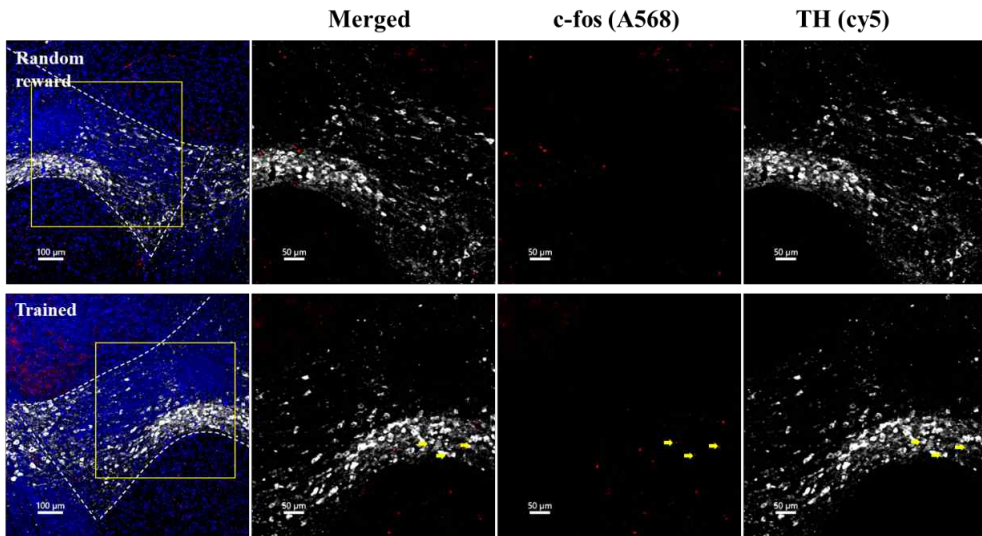
Figure 6. VTA dopaminergic neurons are highly activated compared to the random reward group during early learning stage.
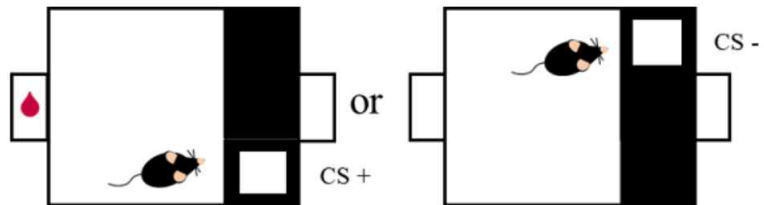
(in collaboration with Min Jung Kim)

(A)Behavioral scheme. (B,C) illustrations of trained group and random reward group. (D) Approach trials in the random reward and the trained group (random reward, n=5 ; trained, n=8). Two way ANOVA was applied to learning curve of each group; n.s ($p > 0.9662$) for random reward group; n.s ($p = 0.1715$) for trained group. (E,F) c-fos expression in the NAc region. Unpaired t test was applied to fos expression of each group; NAc neurons, n.s ($p = 0.9655$); NAc D2R neurons, n.s ($p = 0.5785$). (G,H) c-fos expression in the VTA region. Unpaired t test was applied; VTA neurons, n.s ($p = 0.3250$); VTA TH neurons, ** ($p = 0.0013$).
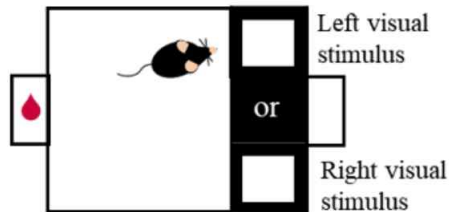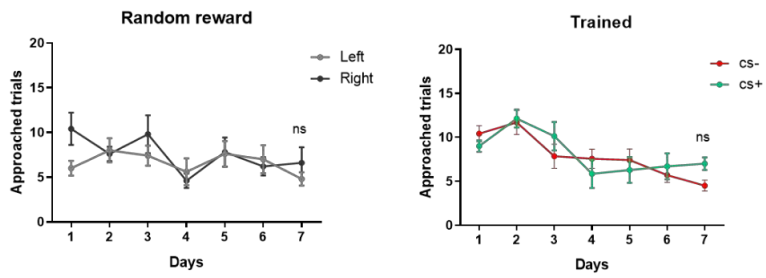
Discussion

Based on previous studies that dopamine projection from the VTA TH to the NAc D2R neurons participates in reward-seeking behavior, this study aims to provide information of the dynamics in the IEG activity of TH neurons and D2R neurons during reward-associated learning. Over the reward-associated learning, the trained mice showed the propensity of approach to CS+ and increased neuronal activity in the VTA and the NAc compared to unlearned mice (Fig. 2C-2E). To show the fos activity of VTA TH and NAc D2R neurons response to CS+ itself, the mice were exposed to only CS+ after the autoshaping task was completed. The trained group showed that fos expression of VTA TH neurons and D2-MSNs was similar to no-reward control (Fig. 3D,3F). Furthermore, I compared the activated population at day 9 of training and a day after autoshaping learning was completed to confirm whether the overlapping activated population represents information about the specific cue-associated reward. Unexpectedly, there was no significant difference in fos expression between the control and the trained group (Fig. 4D,4E). Exposure for 3mins of a visual cue may not be enough to induce a significant IEG change. In the next experiment, I investigated the cell activity in the early- and late learning stage depending on the learning progress. When the reward-associated memory was formed for a specific visual stimulus, the fos activity of D2R-MSNs was increased

but the activity of TH neurons was similar to the activity in the random-reward group (Fig. 5E,5G). Interestingly, when learning was not completed but mice discriminate visual cues behaviorally, only the fos level of the dopaminergic neurons of the VTA significantly increased compared to the random-reward control group (Fig. 6G). In conclusion, this study suggests that VTA TH and NAc D2R neurons participate in reward-associated learning and have different activity patterns during reward-associated learning. Dopamine is thought to control reward-related learning through G-protein coupled receptor signaling. However, the study of molecular mechanism for dopamine release is poorly understood. The secretory biology of dopamine release has unique properties (Liu and Kaeser 2019). Dopamine in the midbrain also is released from somata and dendrites (Beckstead, Grandy et al. 2004, Li, Waites et al. 2005, Gantz, Bunzow et al. 2013). It has remained unclear how axonal dopamine releases shape and regulate signaling (Sulzer, Cragg et al. 2016). Studies about molecular mechanisms will be needed. I hope this IEG study will help to understand the relationship between dopamine and reward-associated learning in the cellular level. Future work will need to study the cellular activity of non-dopamine neurons in the VTA.

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국 문 초 록

복측피개영역은 보상 연관 도파민을 방출하는 뇌영역으로 잘 알려져 있다. 복측피개영역 도파민 뉴런은 측좌핵 도파민 수용체 뉴런과 강하게 연결되어 있다. 측좌핵 도파민 수용체 뉴런은 D1 수용체를 가진 뉴런과 D2 수용체를 가진 뉴런으로 구성되어 있다. 고전적 관점에서, 이 두 종류의 뉴런은 반대적인 기능을 매개한다고 받아들여지고 있다. D1R 뉴런은 보상관련 행동을, D2R 뉴런은 혐오적 반응을 매개한다고 알려져 있지만 최근 연구에서 D2R 뉴런이 보상 관련 행동에도 참여함이 밝혀지고 있다. 본 연구는 복측피개야 도파민 뉴런과 측좌핵 D2R 뉴런의 활성을 중점으로, 보상 관련 학습동안에 이러한 뉴런의 활성에 대한 정보를 밝히는데 목적을 두고 있다. 도파민과 IEG 활성 간의 관계는 아직 밝혀지지 않았고 본 연구가 세포적 레벨에서 보상 관련 학습 동안에 도파민의 역할을 이해하는데 도움을 줄 수 있을 것이다. 행동 실험으로써 특정 자극에만 보상이 나오는 autoshaping을 선택하였다. 학습 과정동안 3가지 시점에서 복측피개영역 도파민 뉴런과 측좌핵 도파민 D2 수용체 뉴런의 IEG 활동을 연구했다. 3가지 시점은 1) 마지막 훈련일, 2) 훈련이 끝난 다음날, 3)쥐가 특정 시각적 신호에 접근하는 경향성을 보이는 초기 학습 단계다. 훈련된 마우스는 대조군 마우스에 비해 복측피개영역과 측좌핵 영역에서 fos 활성이 증가되어 있었다. 복측피개영역 도파민 뉴런은 초기 학습 단계에서 증가된 활성, 측좌핵 D2R 뉴런은 보상 관련

기억이 형성되었을 때 증가된 활성을 보였다. 결론적으로, 본 실험은 복측피개영역 도파민 뉴런과 측좌핵 D2R 뉴런이 보상 관련 학습에 참여하며, 보상 연관 기억이 형성되는 동안 다른 활성 패턴을 가지고 있음을 보여주었다.

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