이학박사학위논문

터치 스크린 기반의 조작 조건화를 이용한 정상 생쥐와 질환 모델 생쥐의 인지적 행동 연구

Studies on cognitive behavior in normal and disease model mice using touch screen-based operant conditioning

2016년 2월

서울대학교 대학원

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Abstract

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Touch screen test is a recently developed cognition testing tool. Based on operant conditioning, touch screen test enables to test cognition of animals by scoring their cognitive response to visual stimulus presented on LCD monitor. Compared to traditional cognition testing tools, touch screen test has its own advantage in testing cognitions of animals, such as automaticity, the variety of testing cognition types, and translation to human cognition.

In spite of these advantages, the real value of touch screen as a cognition testing tool is not fully proven yet. Until a recent date, touch screen has been mostly used to examine the functional roles of a brain region in the specific cognition or to reveal cognitive impairment in transgenic animals, all of which are also possible to investigate by traditional cognition testing tools.

In this study, I evaluated the use of touch screen as a tool of testing mice's cognition with more detail and multiple purposes. To do this, I first showed that mice can perform delayed match to location based working memory task in touch screen. I also showed that touch screen can not only assess working memory itself, but also measure the reaction time of animals during cognitive decision with high accuracy. I found that slight but very significant different reaction time between when mice made correct and incorrect choice, which may have important implication.

I also combined touch screen with optogenetics tool to trigger laser output in automatic and programmed manner. With this automatic optogenetic tool, I tested whether optogenetic activation of dopaminergic neurons can reinforce touching behavior without any natural reward. I first injected adeno-associated virus (AAV) encoding channelrhodopsin 2 (ChR2) into ventral tegmental area (VTA) of tyrosine hydroxylase-cre (TH-cre) mice, to express ChR2 specifically in dopaminergic neurons in VTA. I also designed to trigger optogenetic laser output in contingent with touching visual stimulus. I found that automatic and touch screen mediated optogenetic activation can reinforce touching behavior without any natural reward.

Finally, I investigated cognition disability of Parkinson disease (PD) model animal. Conventional cognition testing tools have limitations in testing cognitions of animal models which have motor impairments. By virtue of low motor demand of touch screen test, I could assess cognitive disability of PD model animals in spite of their motor output deficits.

Key words

Touch screen; Working memory; Delayed match to location; Optogenetics; Reinforcement; Ventral tegmental area; Channelrhodopsin; Reversal learning; attention; 5 choice serial reaction time (5-CSRT); location discrimination; Parkinson

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Introduction

Assessment of Cognition by Touch screen test

Touch screen test is recently developed by Tim Bussey and his colleagues (Bussey et al., 2008). This novel cognition testing tool is based on operant conditioning. Basically, animals, mostly mice and rats, are trained to response with cognition to visual stimulus presented on LCD monitor. When animals make correct response to visual stimulus, they earn reward delivered to reward magazine (figure 1).

Therefore, at first, the most required sensory processing is visual. Whether simple or complex visual stimulus are presented, animals should perceive, discriminate and then react to visual stimuli to get reward (figure 2). Furthermore, animals are required to use their cognitive function, not just simply perceive visual stimulus to response visual stimuli. Repeating trials and errors, animals learn correct responses to given visual stimulus which delivered them reward.

Advantages of touch screen test

Touch screen test provides several advantages compared to traditional cognition testing tools, such as fear memory, and Morris water maze (Bussey et al., 2008, 2012). First, touch screen is almost fully automatic. The only thing required for experimenter is to locate animals into test chamber, and remove them after each session is completed. There are several virtues of this automatic feature beyond reducing labor of experimenters. First, because all experimental procedures are processed by

prepared programed manner, there are little interference possibly given by experimenters during cognition test. It is also possible to provide animals with exact experimental procedures such as delay time, duration of visual stimulus, and the quantity of reward. Finally, the automatic feature of touch screen enables to test many trials of same cognition tests in a session. When cognition of animals is tested by a single trial, a single error, sometimes made both by animals and experimenters can make misinterpretation of cognition of animals. Therefore, the cognition of animals can be measured more exactly by touch screen due to its automatic feature.

Next, touch screen test provides various types of cognition test in a same test chamber. In conventional tests, it is required to prepare each cognition testing devices to test different types of cognition. For example, if both working memory and spatial memory are needed to test in a same time, equipment both for each cognition types should be prepared. However, touch screen can test multiple types of cognition in a same test chamber. The only thing required to test various types of cognition is to change algorithm of visual stimulus and correct response that delivers reward.

Next, touch screen is also highly translational to human cognition. Traditional cognition tests are very useful because they are easy for animals to learn. For example, normal mice do not have any trouble to elicit freezing behavior in the context where they experienced aversive events. However, it is hard to translate that kind of behavior to human cognition. Conditioning to fearful experience is only a small part of human cognition and it is processed unconsciously. Touch screen test challenges these lack

of translation to human cognition belong to traditional cognition testing tools. Similar cognition test can be assessed both in mice and human (Nithianantharajah et al., 2013). Finally, touch screen can test cognition of animals which have impairment in motor output. Most cognition testing tools premise perfect motor outputs. Therefore, it was almost impossible to assess cognitive impairment of animals which have motor output deficits. However, low demand of touch screen test enables to assess cognitive impairment of animal, even they also have cognitive impairment. Touch screen do not require perfect motor outputs. It only requires motor outputs that are needed to touch visual stimulus presented in LCD monitor and ambulate in a small test chamber.

Optogenetics

Optogenetics refers to biological technique to observe or modulate neural activity in the specific types of neurons through optical way. In many cases, optogenetics has been used to modulate neural activity by delivering light to the neurons (Tye and Deisseroth, 2012; Yizhar et al., 2011) (figure 3). Aided by mouse genetics or neuronal type specific expressing virus, opsin proteins can be expressed in the specific type of neurons, therefore, it is possible to regulate neural activity of the specific types of neurons (Gradinaru et al., 2010; Tye and Deisseroth, 2012; Yizhar et al., 2011).

Channelrhodopsin 2 (ChR2), which functions as sensory photoreceptor in unicellular green algae (Nagel et al., 2003), is one of the most commonly used opsin to activate the neurons. Blue light delivered to ChR2 transiently causes opening of cation

channel, therefore, leading to excitation of cell membrane (Boyden et al., 2005). The excitation of cell membrane is highly time locked to blue light delivery, therefore, neuronal activation by ChR2 is useful to activate neurons with high fidelity.

Optogenetics has been widely used to examine causal relationship between a neural circuit and a specific behavior (Tye and Deisseroth, 2012). The necessity of a neural circuit for the specific behavior has managed to be examined by pharmacological lesion or inhibition of the specific neural circuit. However, the sufficiency of a neural circuit for the specific behavior is poorly examined before optogenetics was developed. This is because there has been no way to modulate neural activity, especially activate, in millisecond scale. Optogenetics enables activate neural circuit with high fidelity. Therefore, due to these virtues of optogenetics, sufficiency of a neural circuit for the specific behavior could be investigated.

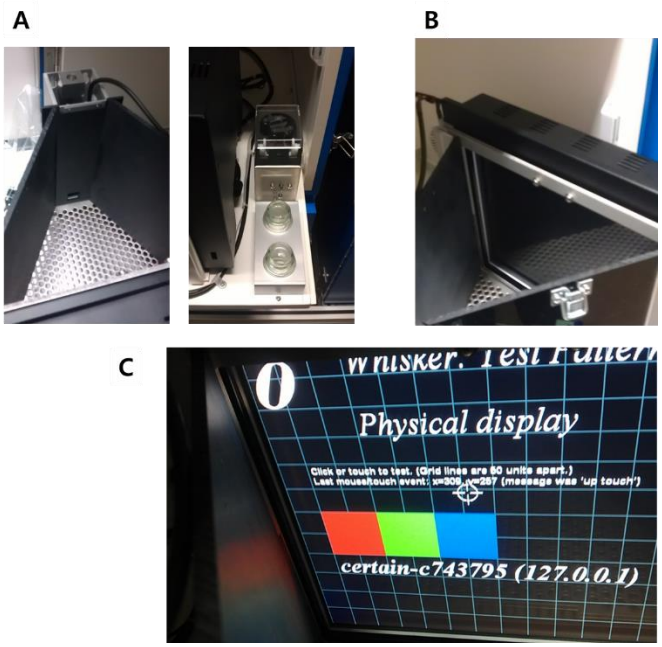

Figure 1

Pictures of touch screen chamber

(A) Reward delivery system

(B) LCD monitor in front of the chamber

(C) Visual stimulus presented in LCD monitor

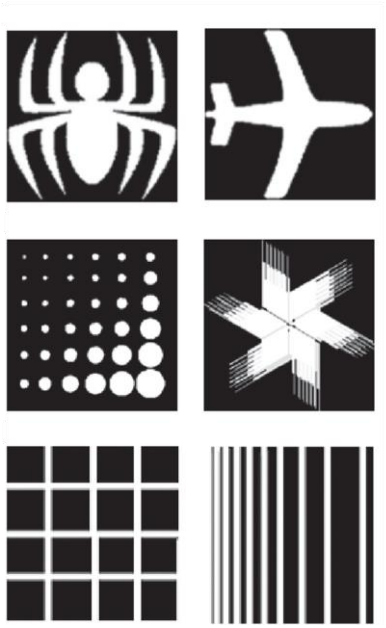

Figure 2

Examples of visual stimulus used in touch screen test (Horner et al., 2013)

Purpose

First, I showed that working memory of mice also can be tested in touch screen. There is already available working memory testing paradigm in touch screen, which is called Trial-unique, delayed nonmatching-to-location (TUNL). However, this paradigm only can assess working memory of rats, but not mice. I purpose to show that mice can perform delayed matched to location paradigm based working memory task in touch screen test and to measure cognitive response time during working memory task.

Next, I purposed to reinforce touching behavior without any natual reward. To do this, I developed and designed to trigger blue laser light in contingent with touching behavior of mice. To reinforce touching behavior, I expressed ChR2 in dopaminergic neurons of ventral tegmental area.

Finally, I clarified that touch screen can be used to assess cognitive function of disease model mice that have also impairment in motor output. To show this, I used Parkinson disease (PD) model mice, of which dopaminergic innervation in dorsal striatum was destroyed pharmacologically. I tested that PD model mice can generate motor outputs which are required for performing touch screen testing. I also found that they showed impairment in specific cognitive function, indicating that their impairment is not off target of all cognition.

Chapter 1

Development of a touch-screen-based paradigm for assessing working memory in the mouse

Abstract

Assessing the working memory of the rodent by using a touch-screen system has several advantages. However, there is currently no available touch screen test based working memory testing paradigm available for mice. In this chapter, I developed a touch-screen testing paradigm in which mice were trained to choose a location that is matched to a sample location after a time delay. Consistent with previous studies, I showed that mice could not only learn the rule in the delayed matched to position (DMTP), but also could retain a transitory memory of the sample position during delay. This indicates that a touch-screen system can provide a DMTP testing platform to assess working memory in the mouse.

Introduction

Working memory is one of memory types, and it allows transitory information to be actively held for several seconds on line (Baddeley, 2003). Testing working memory in the mouse has typically used the delayed non-matched to position (DNMTP) paradigm (Dudchenko, 2004). This paradigm, however, has limitation in accuracy and flexibility of behavioral schedules. For example, during a working memory test in a T-maze, intervention by the experimenter is inevitable because the maze needs to be cleaned and the animal must be relocated to the start box after sampling. Therefore, there is a growing need to test working memory automatically.

Touch screen testing is recently developed for assessing rodent's cognition (Bussey et al., 2008, 2012). This touch screen paradigm has several advantages over traditional behavioral testing paradigms in testing working memory (McAllister et al., 2013; Oomen et al., 2013). First, testing can be scheduled almost automatically, permitting more experimental sessions to be conducted with high accuracy and flexibility. Second, it can provide the animal with multiple choice locations on the screen, which enables the capability of assessing the animal's ability to discriminate between choice locations, such as temporal separation (Kesner, 2013). These advantages would advance research on working memory, if touch-screen-based technology were applied to mice. Finally, touch screen can measure cognitive response of animals with high accuracy, which may critical implication in their

cognitive processing (Yamamoto et al., 2014). Unfortunately, a touch-screen system for the working memory test paradigm for mice is not available yet (Oomen et al., 2013). Therefore, in the present study, I developed a delayed matched to position behavioral assessment paradigm using a touch-screen testing system for evaluating working memory in mice. I also showed that touch screen can measure cognitive response with high accuracy.

Materials and Methods

Animal

C57BL/6N mice were obtained from Orient Co. (Gyeonggi, Korea). Animals were housed in groups (four mice), maintained on a 12-h light/dark cycle, and food and water were provided ad libitum. All animal procedures were conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee of Seoul National University.

Touch-screen testing

I used Campden Instruments Bussey-Saksida touchscreen chamber (Campden Instruments Ltd, UK) for touch-screen testing. Before mice learned the rules of the cue-response paradigm and DMTP, they were trained to respond to visual stimuli on an LCD monitor to earn a reward. All experiments were conducted as described in the manufacturer's guide and previous studies (Horner et al., 2013; Mar et al., 2013, 2013). Briefly, on day 1, mice were habituated to a touch-screen chamber for 10 min. On day 2, mice were given liquid sweetened milk as a reward. In this phase, the animal's nose poking to the reward magazine located behind the chamber results in delivery of reward. On day 3, in initial touch phase, both nose poking to reward magazine and response to visual stimulus on the LCD monitor delivered the reward.

Next, on days 4 through 6, in the must touch phase, only when mice responded to the visual stimulus was reward delivered. After the must touch phase, mice were trained to avoid responding to a blank window; incorrect punishment. To train this avoidance, when the mice responded to a blank window, the room light was illuminated as a punishment signal. Sixty trials were conducted within 60 min until mice reached the criterion (70 % correct response in consecutive 2 days).

After incorrect punishment, mice were trained to associate central cue and correct location choice for a reward. Two central visual cues have a different shape and color. Guided by the central cues, mice could choose either the left or right location to receive a reward. When the animal made an incorrect choice, the room light was illuminated, and additional correction trials were given until the animal made a correct choice. In cue-response training, 60 trials within 90 min were given for 6 sessions.

Rule learning of DMTP was given after cue-response training. Three touches to the left- or right-positioned sample/cue stimulus on the screen by the mouse resulted in presentation of a visual stimulus in a central window. When the animal responded to the central visual stimulus, visual test stimuli were presented either on the left or right side of the screen. The mouse's correct matched-to-sample response delivered a reward. As in cue-response training, an incorrect choice resulted in room light illumination and additional correction trials. After successful DMTP rule learning,

delay time between final response to the sample location cue stimulus and presentation of the central visual test stimulus was increased to either 3 s or 9 s.

Results

To test working memory, two types of transitory information can be used. First, in the "cue-delay-response" approach, the experimental animal makes a correct response guided by various cues presented at the start of the test trial. After the cue is diminished, the animal should hold a memory of the cue during the delay and up until the animal's choice moment is coming. To use this system, it is necessary to create a reference memory by which the cues can guide the animal to make a correct choice. Second, in the "delayed matched to position (DMTP)" or "non-matched to position (DNMTP)" paradigms, the animal should first learn the basic rule of the delayed matched or non-matched position to make a correct choice response. After the sampling location, a temporal delay is given before the animal is allowed to choose the matching (or non-matching) location.

To test working memory in a touch-screen system, I first tested whether mice can learn a reference memory in which they choose between locations on the left or right, following presentation of a visual cue presented in the center (figure 3A). This task also has been used to test rodent's visuo-motor response (Horner et al., 2013). On a given trial, to receive a reward, the mouse must touch a visual cue presented in the center location on the screen, and then choose either the left or right location of subsequent visual choice stimuli, in accordance with the visual cues (figure 3A) that have different colors and shapes. When choice moments were given 1 second after

touching the center cue, however, mice performed poorly this task (Fig. 3B). Their failure to learn the reference rule indicates that using the cue-delay-response method in the touch-screen paradigm is not an appropriate way to test working memory in mice.

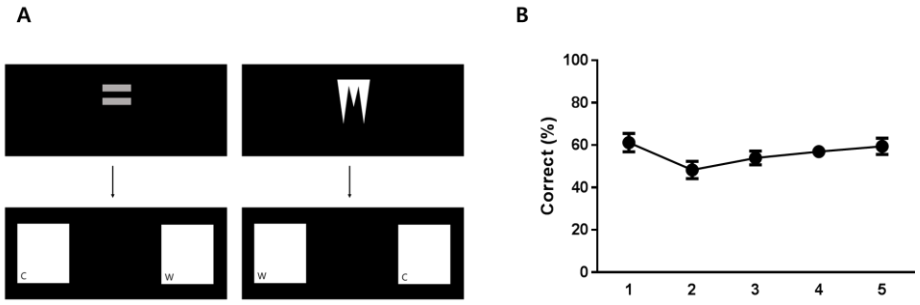

Figure 3

Cue-response structure

(A) Schematic drawings of experimental design. Central cues used in this experiment were provided by manufacturer (Campden Instruments Ltd, UK). C and W indicate 'correct' and 'wrong', respectively.

(B) When mice were guided by a cue to make the correct choice, they performed poorly in associating visual cue and correct choice location. Percentage of correct choice in the first session and last session were 61.3±4.3% and 59.5±3.4%, respectively. Error bars indicate SEM. Eight mice used in this experiment.

I next employed a DMTP paradigm in which working memory of mice can be assessed in a lever-pressing operant chamber (Goto et al., 2010), to determine whether mice can learn a DMTP rule (figure 4). To do sampling, the mouse was required to touch the visual stimulus presented in either the left or right position of the screen three times. One second later, the mouse was required to make a final sample touch of the visual cue presented in the center of the screen. After the mice made this final touch of the center position, they were then presented with two alternative visual stimuli that were positioned in both the left and right position. When mice chose a position that matches the cue's position, a sweet milk reward was delivered (figure 4A). When mice made an incorrect choice, correction trials were given until they made a correct choice. As was true in the lever-pressing operant chamber (Goto et al., 2010), mice successfully learned the matched to position rule (figure 4B and C). After four training sessions, mice scored high in accuracy compared to their first session (figure 4B), and the number of correction trials also diminished (figure 4C), indicating that mice successfully learned the rule of DMTP.

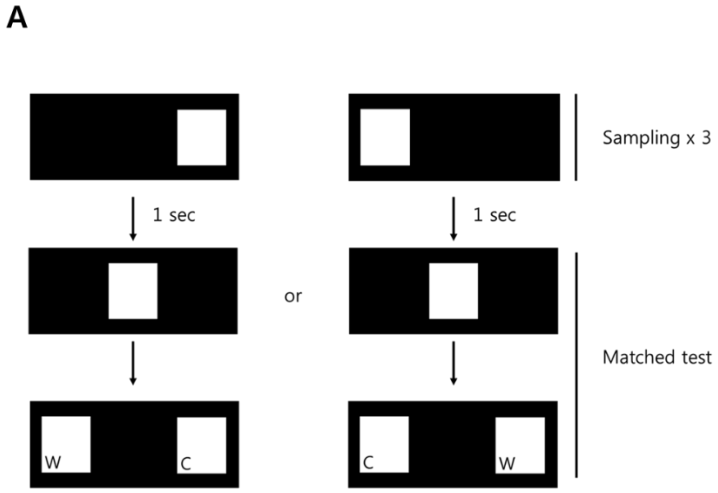

Figure 4A

Rule learning of DMTP

(A) Schematic drawings of experimental design. C and W indicate ‘correct’ and ‘wrong’, respectively.

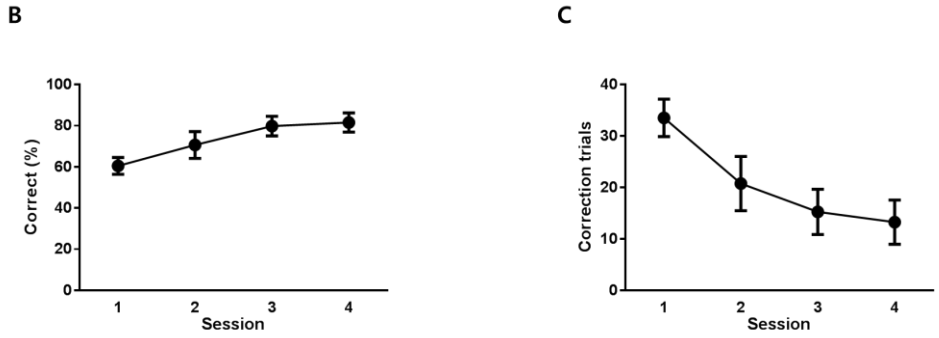

Figure 4B and C

Rule learning of DMTP

(B) Mice successfully made correct choices that match to sample location with session progression. Correct choice rate of the last session was significantly increased compared to that of the first session (paired t-test, $t(7)=6.524$, $p < 0.001$).

(C) The number of correction trials required during DMTP training was significantly reduced (paired t-test, $t(7)=5.435$, $p < 0.05$). Error bars indicate SEM. Eight mice used in this experiment.

After mice learned the matched-to-position rule, to test working memory, I increased delay time between final sample touch and central visual stimulus presentation (figure 5). When delay time was increased to 3 s, there was a slight, but significant, decrease in the rate of correct choice to matched position (figure 5A), compared to the last session of the rule learning. When I further trained mice in a 3-s delay paradigm by twice sessions, animals made correct choices comparable to those of rule learning (figure 5A) with a 1-s delay. Finally, a 9-s delay resulted in a correct matched-to-position learning rate was substantially reduced compared to that in the last session of 3-s delay paradigm (figure 5B).

Prolonged delay time between final sample touch and central visual stimulus presentation reduced the rate of animal's correct matched-to-sample responses. For example, increasing delay time from 1 s to 3 s slightly reduced correct choice rate (figure 5A). As more training sessions were given, however, mice made comparable correct choices to those in the 1-s delay condition (figure 5A). This may indicate, in our experimental condition, that a 3-s delay is not long enough to test the working memory of mice. When delay time was increased to 9 s, mice showed substantial reduced correct choice rate, which indicates that their rate of correct choice of matched position is affected by delay time between sampling and choice moment.

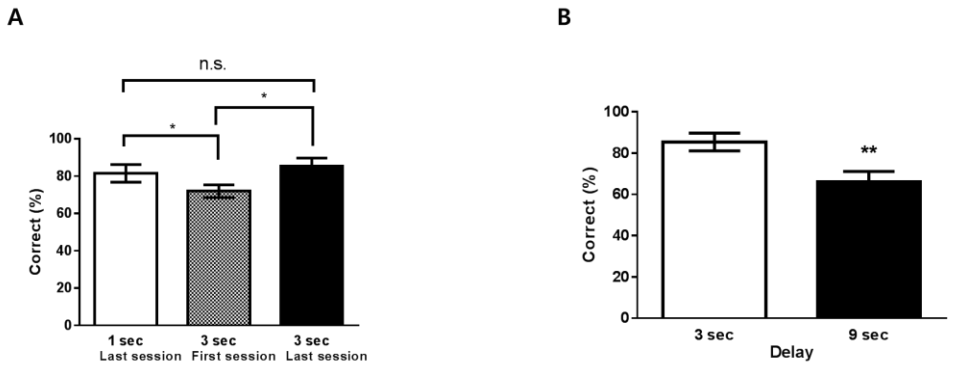

Figure 5

Effect of prolonged delay in DMTP

(A) When delay time was increased from 1 s to 3 s, correct choice rate was slightly, but significantly, reduced (paired t-test, $t(7)= 3.093$, $^{*}p < 0.05$). Percentage of correct choice in the last session of 1 s delay and the first session of 3 s delay were 81.5±4.7% and 71.9±3.4%, respectively. Repetition of DMTP task with 3 s delay increased correct choices comparable to a 1-s delay.

(B) When delay was prolonged to 9 s, correct choice rate was significantly reduced (paired t-test, $t(7) = 3.842$, $^{**}p < 0.01$). Percentage of correct choice in the last session of 3 s delay and 9 s delay were 85.4±4.3% and 66.2±4.9%, respectively Error bars indicate SEM. Eight mice were used in this experiment.

Due to automatic measure of cognitive behavior in touch screen, I also could measure response latency to correct or incorrect choice. Interestingly, mice spent slight but significantly longer time to response to correct location compared to incorrect choice (figure 6). The amount of time spent more during choosing correct location seems to be the time which required to retrieve the its previous sampling memories (Yamamoto et al., 2014).

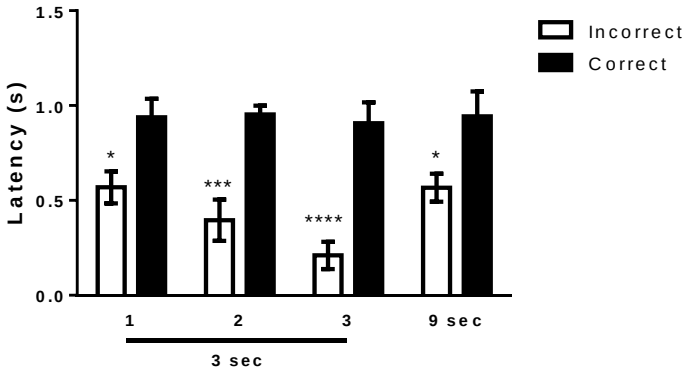

Figure 6

Response latency to correct or incorrect location

Mice showed prolonged latency to response when they make correct choice compared to incorrect choice (Two-WAY ANOVA test, interaction, $F(3, 42) = 2.921$, $P = 0.045$, followed by a Bonferroni post-hoc test. Error bars indicate SEM. Eight mice were used in this experiment.

Discussion

My present approach to assess working memory in mice using touch-screen testing could be used to characterize several features of working memory. First, how long mice can retain the memory of a sample position could be tested. Although not well investigated, 10 s is commonly used as the time limit in current T-maze-based experiments assessing working memory in mice (Suh et al., 2011). Using a similar testing protocol in my present study, mice could make correct matched-to-sample choices with as long as a 9-s delay (figure 5). Therefore, with my highly flexible and accurate system, the duration of transitory memory during DMTP tasks could be determined for mice. Second, the effect of pattern separation on working memory could be studied in mice (Gilbert et al., 2001; McAllister et al., 2013; Talpos et al., 2010). The hippocampal CA1 region is required to make non-matched choices between highly separated choices but not between close choices in the radial-arm maze task (Kesner, 2013). There is no study that assesses whether mice also have hippocampal CA1-dependent temporal separation memory. Using my present paradigm, it could be examined whether mice have temporal separation memory, and, if they do, which brain regions are involved in temporal separation memory.

Finally, it is also would be interesting the neural circuits which are involved during they choose correct location. In my study, I could find that mice spent more time to choose correct location (figure 6). This critical period seems to have important

implication in processing working memory. The combination of observational tool, such as in vivo recording or micro-endoscope and optogenetic tool, which can control neural activity during this critical period may be used to examine the necessity and sufficiency of the neural circuit during this critical period.

Chapter 2

Reinforcement of touching behavior

by touch screen based automatic optogenetics tool

Abstract

Dopaminergic circuit in midbrain brain is thought to be required for motivation of a behavior. To examine roles of dopaminergic circuits in motivated, most of studies have employed conditioned place preference (CPP) paradigm. Conditioning preference to a specific context is formed in a passive way, therefore, it is not clear whether contingent activation of dopaminergic can reinforce a specific behavior actively. In this chapter, I developed automatic and touch screen based optogenetic tool. With this tool, I designed to activate dopaminergic neurons in VTA in contingent with touching behavior of mice. I found that this contingent activation of dopaminergic neurons increased touching behavior without any natural reward. This indicate that activation of dopaminergic neurons is sufficient to reinforce a specific behavior.

Introduction

Dopaminergic neurons in ventral tegmental area send their projections to nucleus accumbens consisting of mesolimbic pathway. This pathway is suggested to be underlying neural circuits of motivated behaviors. To answer whether this circuit is necessary for motivated behaviors, lesion, electrical stimulation, and pharmacology experiments have been conducted. However, these methods have limitations in fidelity and cell type specificity. It is important to activate dopaminergic neurons with high fidelity given that in vivo recording showed that dopaminergic neurons are have different activation patterns according to absence, presence or prediction of rewards. These different activation patterns are thought to play different roles in motivated behaviors. Therefore, it is required to control activation patterns of dopaminergic neurons with high fidelity to examine causal relationship between dopaminergic neurons and motivated behaviors. In this regard, the use of optogenetics is essential to examine the causal relationship between activation of dopaminergic neurons and motivated behaviors.

In previous, it was shown that phasic activation of dopaminergic circuit was sufficient to condition preference to a context (Tsai et al., 2009). This was the first study that used optogenetic tool to activate dopaminergic neurons to condition preference to a specific context. This showed that phasic, but not tonic, activation of dopaminergic neuron can increase release of dopamine in nucleus accumbens, forming preference

memory to the context in which animals received phasic activation of dopaminergic neurons. Similar results was also shown by wireless optogenetic tools (Kim et al., 2013). All of these studies, however, did not show clearly that dopaminergic neurons could actively reinforce a specific behavior, because all of behaviors were based on passive conditioned to context.

Touch screen is well established automatic tools. All of its outputs are controlled in programmed automatic manner. Therefore, it is possible to trigger optic laser by output signal coming from touch screen. To do this, I designed that optic laser could be triggered contingent with animals' touching behavior. Using this automatic optogenetic tool, I showed that touching behavior of mice was reinforced by this contingent activation of dopaminergic neuron. I also found that this touching behavior was specific to visual stimulus, which triggered optic laser. On the other hand, the number of touching blank window, which did not trigger optic laser, did not change. Therefore, it indicates that my optogenetic touch screen tool can reinforce touching visual stimulus specifically, but not overall touching both visual and blank windows.

Materials and Methods

Animals

Tyrosine hydroxylase-cre (TH-cre) mice were obtained from Jackson Laboratory (B6.Cg-Tg(Th-cre)1Tmd/J). They were back-crossed to at least 5 generations. During the back-crossing male TH-cre male mice were mated with C57BL/6N female mice. Mice were fed ad libitum and maintained under 12 hours light/dark cycle. Four to five mice were housed in a same cage.

Stereotaxic surgery

To express ChR2 in dopaminergic neurons within VTA, I injected DIO-ChR2 virus into VTA unilaterally (AP, -3.15mm; ML, right to 0.5mm; DV, 4.4mm). Injected volume was 0.5µl and the viral titer was 8 x 10^11/ml. Optic cannula were made as previously described (Ung and Arenkiel, 2012). Optic cannula with diameter 200µm were placed above VTA (AP, -3.15mm; ML, right to 0.5mm; DV, 3.9mm) and chronically implanted by dental cement.

Immunohistochemistry

After reinforcement experiments, I removed brains and fixed them in 4% paraformaldehyde at 4℃, and overnight. After the fixation, I maintained fixed brain in 30% sucrose solution one or two days. Next, I sectioned brain with 40μm thickness. To immune-stain tyrosine hydroxylase, I first treated brain section with tyrosine hydroxylase antibody (ratio, 1:1000; millipore) at 4℃ and overnight. After that, I treated them with secondary antibody (anti-rabbit, alexa-594).

Electrophysiology recording

300μm thick horizontal slices of VTA were prepared with Leica VT-1000S and incubated in 25~26°C for 1hr. The artificial cerebrospinal fluid(aCSF) containing 124mM NaCl, 2.5mM KCl, 1mM NaH2PO4, 25mM NaHCO3, 10mM Glucose, 2mM CaCl2, 2mM MgSO4 was used for incubation and bath solution. Oxygenated bath solution with 95% O2, 5% CO2 mixed gas was perfused 2 ml/min at 25~26°C (TC-324B, Warner). The slices were transferred to the recording chamber of BX51WI microscope(Olympus) and TH-cre and DIO-ChR2-eYFP double positive cells were visualized with ProgRes MFcool CCD microscope camera.

For current clamp recording, the brorosilicate glass recording pipettes were pulled on a horizontal pipette puller (P-1000, Sutter instrument) to a resistance of 3~4 MΩ when filled with internal solution containing 145 K-Gluconate, 5mM NaCl, 0.2mM

EGTA, 10mM HEPES, 2mM MgATP, 0.1mM Na3GTP, 1mM MgCl2 (pH 7.2 with KOH, 280~290 mOsm). For tonic activation, TH(+) neurons visualized with eYFP signal were current clamped and 473 nm laser was delivered to the VTA area with 5Hz. For Phasic activation, TH(+) neurons were current clamped and 473 nm laser was delivered to VTA area with 20Hz(10ms pulse width) for 280ms.

Reinforcement behavior by optogenetic touch screen

About 4 weeks after stereotaxic surgery, I performed reinforcement behavior in the optogenetic touch screen chamber. Before conditioning, mice were handled in consecutive 4 days. After the handling, mice were habituated to touch screen chamber for 10 minute. One day after the habituation, mice were conditioned for consecutive 2 days. When mice touched visual stimulus presented LCD monitor, they received optic laser stimulus for 10 second (20 ms, 20 Hz). Next visual stimulus was followed 20 second after previous visual stimulus touching. Visual stimulus was presented either left or right side of LCD monitor and the location of visual stimulus was random.

Results

ChR2 was specifically expressed in tyrosine hydroxylase positive neurons

First, I confirmed the selectivity and efficacy of viral expression in dopaminergic neuron. To express ChR2 specifically in dopaminergic neurons, I injected AAV encoding Cre inducible ChR2 in its open reading frame into VTA of TH-cre mice. Consistent with previous reports (Tsai et al., 2009), I found that high proportion of TH-positive neurons was positive for ChR2-EYFP, indicating that ChR2 was specifically expressed in TH-positive, dopaminergic neurons (figure 7).

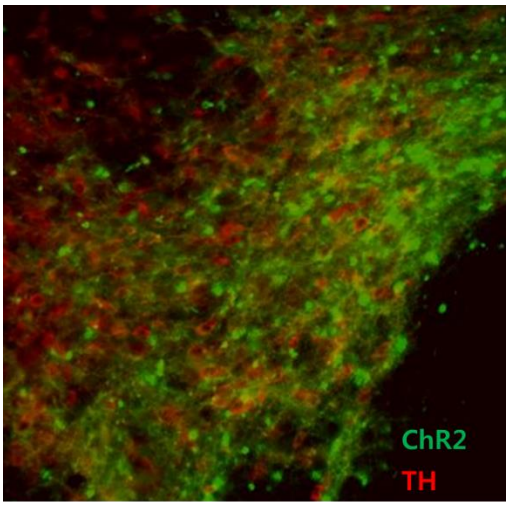

Figure 7

ChR2 expressed in tyrosine positive neurons in VTA

Blue light activated dopaminergic neurons in VTA

Next, to test whether blue light can activate neural activity, I performed whole cell patch recording (in collaboration with *Jaehoon Shim*) (figure 8A). I found that blue laser not only activated dopaminergic neurons in VTA, but also this activation was highly time locked to phasic and tonic blue light (figure 8B and C). This indicates that my blue light delivery can activate dopaminergic neurons in VTA with high fidelity.

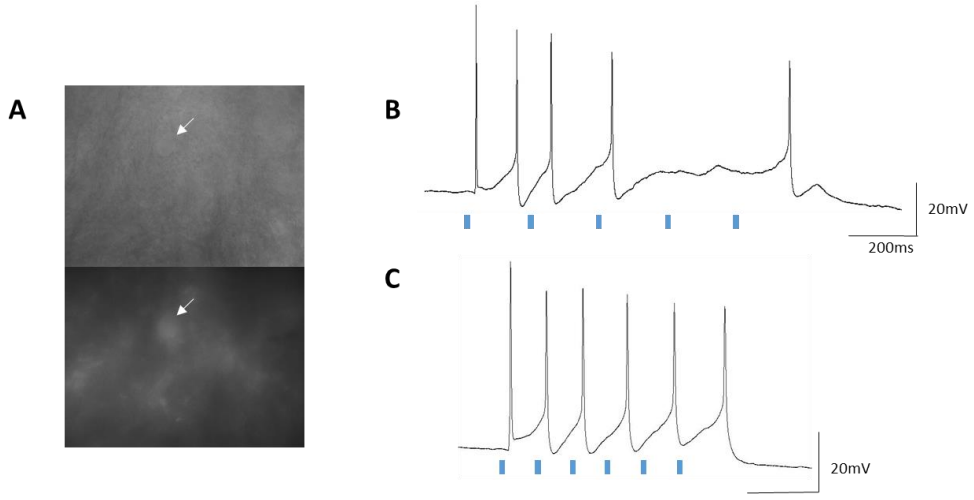

Figure 8

Blue light activated dopaminergic neurons in VTA in vitro.

(A) IR image of VTA (top) and eYFP signal in TH(+) neuron in VTA (bottom).

(B) Sample trace for tonic activation.

(C) Cample trace for phasic activation.

Optogenetic activation can reinforce touching behavior

Finally, I conditioned touching behavior of mice with blue light delivery to VTA. To do this, I designed that TTL output signal from touch screen could trigger optic laser output. The printed circuit board (PCB) circuit was located between pulse generator and optic laser. When the output from touch screen is not released, TTL signal from pulse generator is not delivered to optic laser in my PCB circuit design. Only when TTL signal was released from touch screen, TTL signal from pulse generator was allowed to be delivered to optic laser.

One day after habituation, I conditioned touching behavior of mice with blue laser for consecutive 2 days (figure 9A). To trigger blue laser output in contingent with touching behavior of mice, I designed output signal generated by touching visual stimulus screen to be delivered to the PCB circuit (figure 9B). Therefore, by my design, touching visual stimulus triggered blue laser output.

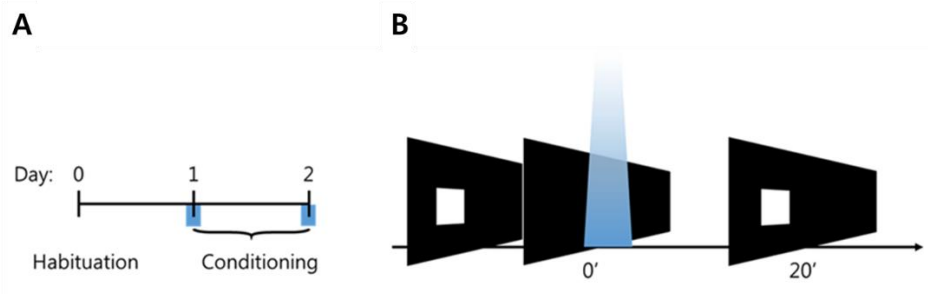

Figure 9

Behavior schedule and laser delivery scheme

(A) Behavior schedule

(B) Laser delivery scheme

When dopaminergic neurons were activated in contingent with touching visual stimulus, mice showed the increase in the number of visual stimulus (figure 10). Mice increased touching visual stimulus at the first day of conditioning compared to their control group (figure 10B). This indicates that my optogenetic reinforcement is very effective compared to previous study (Kim et al., 2013). It is also notable that mice did not show any difference in the number of touching blank window (figure 10A). This means that mice learned that touching visual stimulus, but not blank window, resulted in 'optogenetic reward'. The optogenetic reward motivated mice to reinforce touching visual stimulus.

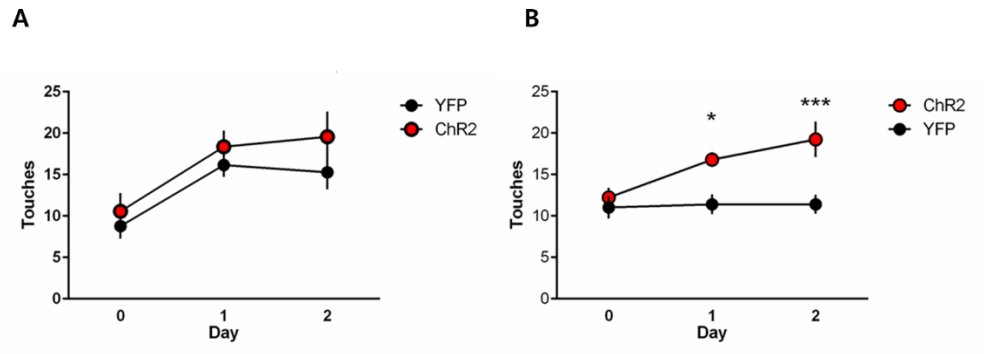

Figure 10

Contingent optogenetic activation reinforced visual stimulus touching.

(A) The number of touching blank window [two-way ANOVA: effect of injected virus, F (2, 30) = 0.3023, p = 0.7413, YFP, n = 8 and ChR2, n = 9]. The data are presented as the mean ± SEM.

(B) The number of touching visual stimulus [two-way ANOVA: effect of injected virus, F (2, 30) = 3.518, p = 0.0424, YFP, n = 8 and ChR2, n = 9]. The data are presented as the mean ± SEM.

Discussion

The dopaminergic circuit in midbrain have long been suggested to be responsible for motivation of behaviors. To prove this hypothesis, lesion, electrical stimulation, or pharmacological activation have been used. However, these ways cannot control neurons both with high fidelity and cell type specificity. Optogenetics enables to control activity of specific types of neurons with high fidelity. Activating dopaminergic neurons with optogenetic showed that this circuit is sufficient to form preference to a specific place (Kim et al., 2013; Tsai et al., 2009). However, it was not clear whether dopaminergic circuits are sufficient to reinforce a specific behavior in real time. In this chapter, I developed touch screen based optogenetic tool by which optic laser is triggered automatically and in programmed manner. With this automatic optogenetics tool, I showed that activation of dopaminergic circuit in VTA can reinforce a specific behavior without any natural reward.

In previous, it was shown that optogenetic activation of dopaminergic neuron seems to reinforce nose poking behavior (Kim et al., 2013). However, by the nature of experimental design, the causal relationship between optogenetic activation of dopaminergic neurons and the increase of nose poking was not clear. First, mice learned to nose poke too slowly. Therefore, it is hard to say that mice associate their nose poking with the optogenetic reward in real time. Next, nose poke devices were located end of maze arm and their locations were not changed through conditioning.

In this experimental design, mice showed not only the increase in the number of nose poking but also preference to the specific arm. Therefore, it was also possible that the increase of nose poking was accidental as mice spent more time in the specific arm.

In this study, however, I showed that optogenetic activation of dopaminergic neurons was sufficient to reinforce touching visual stimulus by employing automatic optogenetic tool combined with touch screen. Contrast to previous study, the present result showed that the number of touching behavior increased at the first day of conditioning, proving that activation of dopaminergic neuron is sufficient to reinforce in real time. Interestingly, there was no change in the number of touching blank window, which was not associated with optogenetic activation. The locations of visual stimulus presented in LCD monitor were randomly changed through trials. Therefore, these results indicate that mice specifically associated touching visual stimulus with the optogenetic reward.

The use of my automatic optogenetic tool is not limited to examine reinforcement behavior. This tool has more potential to dissect neural circuits involved in complex cognitive function. A single cognition is contributed by multiple cognitive components. For example, to perform working memory, subject should encode sample, hold the memory of sample for a delay, and retrieve the working memory after delay (Baddeley, 2003). It was also shown that distinctive neural circuits are responsible for each cognitive component during working memory performance (Fujisawa et al., 2008; Spellman et al., 2015; Yamamoto et al., 2014). All of these

cognitive components are recruited successively or intermingled within millisecond scale. To control neural circuits involved in each cognitive component by optogenetics, therefore, the programmed and automatic optogenetic tool is necessary. In this regard, my automatic optogenetic can be used to automatically control neural activity through optogenetic in various cognition tasks.

Chapter 3

Assessments of cognitive abilities in a mouse model of Parkinson's disease with touch screen test

Abstract

Parkinson's disease (PD) patients suffer both from motor output deficits and cognition disabilities. Various types of PD model rodents have been developed to contribute to research and clinical fields, figuring out genetic and circuitry causes of PD devising therapeutic strategies. Most studies using PD model rodents have focused on motor output deficits. Relative less attention have been paid to cognition disability of PD model rodents. A reason of this uneven attention is lack of appropriate cognition testing tools which require low motor demand. In this study, I embarked on assessment of cognition disability in PD model mice. To do this, I employed touch screen test, which needs low motor ability to perform cognition task. First, I clarified that PD model mice with unilateral striatal dopaminergic degeneration successfully performed operant conditioning. This indicates that that motor ability in PD model mouse used in this study was not disordered to the extent that mice cannot perform touch screen test. Next, I tested cognition disability in PD model mouse. I found that PD model mice had impairment in location discrimination, whereas attention, and reversal learning is intact. My present study indicates that touch screen test is useful tool to assess cognitive disability hidden in disease model animals due to their motor impairment.

Introduction

The main neurological feature of Parkinson's disease (PD) is dopaminergic degeneration, which has long been considered the main cause of the symptoms that distress PD patients. PD rodent models have contributed to our understanding of the pathophysiology and therapeutics of PD (Duty and Jenner, 2011). One of the most common methods for generating PD model mice is to destroy dopaminergic circuits genetically or pharmacologically. For example, neurotoxins, such as 6-hydroxydopamine (6-OHDA) or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), are widely used to ablate dopaminergic neurons selectively, thereby reproducing the motor impairments that are observed in PD patients (Schober, 2004). These neurotoxin-induced motor impairments can be ameliorated by pharmacological correction of the dopamine deficiency or optogenetic activation of striatal medium spiny neurons (Kravitz et al., 2010; Park et al., 2015).

Many studies on the importance of the dopaminergic circuit in cognition (Nieoullon, 2002) and the cognitive disabilities of PD patients (Muslimović et al., 2005) suggest the need for studies of such disabilities in PD rodent models. However, the investigation of cognitive disabilities in PD rodent models is limited, because most cognitive tests require normal motor function for performing the given task (Lindgren and Dunnett, 2012). Therefore, more consideration should be taken before drawing conclusions on cognitive abilities in PD rodent models, as poor performance on cognitive tasks might be due to motor impairments rather than cognitive disabilities.

However, to date, no tools for testing cognition in disease model animals with motor impairments have been available.

The touch screen test was recently developed to test complex cognitive abilities in mice and rats (Bussey et al., 2012). In the touch screen test, the animals are required to perform a cognitive task in order to obtain a reward by touching a visual stimulus that is shown on a LCD monitor located in the front of the chamber. The touch screen test has several advantages over conventional cognitive test tools for the testing the cognitive disabilities of PD rodent models. First, it requires relatively lower motor output (Bussey et al., 2008), only ambulation in the small chamber and touching the LCD monitor in the front of the chamber. This low motor demand can reduce the possibility that poor performance in a cognitive task is due to motor impairments. Next, various types of cognition tasks can be tested with the touch screen test, and these include conventional cognition tasks (Bussey et al., 2008), such as operant conditioning, delayed matching-to-position (DMTP), and 5-choice serial reaction time (5-CSRT) tasks, or novel complex tasks, such as paired-associate learning (PAL), visual discrimination and its reversal learning, and trial-unique delayed non-matching-to-location (TUNL) tests. Because these complex tasks can also be used to test human cognition (Nithianantharajah et al., 2013), the touch screen test is also useful for testing cognitive deficits in many human disease models.

In this study, we employed a touch screen test paradigm to assess cognitive disabilities in PD model mice, which were generated by unilateral injections of 6-

OHDA into the dorsal striatum. We found that these mice did not show abnormal performance in a simple operant conditioning task in the touch screen test. However, they showed impairments in location discrimination, which may not be due to motor impairments, but rather to cognitive impairments. Therefore, the touch screen test can be used to test and investigate the mechanisms underlying cognitive disabilities that are concealed by motor impairments in many disease models.

Materials and Methods

Animals

C57BL/6N mice were obtained from Orient Bio Co. (Gyeonggi, Korea). The animals were kept in groups (3 to 4 mice) and maintained on a 12-h light/dark cycle as previously described. Food and water were provided ad libitum except during the touch screen test. All of the animal procedures were conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee of Seoul National University.

Stereotaxic 6-OHDA injections

6-OHDA (Sigma-Aldrich Co. LLC, St. Louis, MO, USA) was dissolved in phosphate-buffered saline (PBS) in which ascorbic acid was added to stabilize the dissolved solution. Either one microliter of the 6-OHDA solution (4 μg/μL) or same volume of PBS, as a control were injected into the right dorsal striatum. For the stereotaxic injections of 6-OHDA, the mice were first anesthetized with an intraperitoneal injection of a ketamine and xylazine cocktail. The stereotaxic coordinates were established according to previous studies (Kravitz et al., 2010) (from bregma, anterior to posterior, +0.4 mm; midline to right, 1.5 mm; dorsal to ventral, -3.0 mm).

Rotarod test

The rotarod test was performed as previously described (Park et al., 2015). Briefly, mice were training for consecutive 5 days. On the first day, animals were placed rotating rod with low speed (4 rpm). For every 30 second, rotating speed was increased by 1 rpm, and final rotating speed was end up to 15 rpm. In the second day, mice were placed rotating rod with 4 rpm speed for 90 second, and rod speed was increased every 30 second by 1 rpm, finally reaching to 20 rpm. Through 3-5 days of training, falling latency was measured as rotating speed of rod increased linearly from 4 rpm to 40 rpm. After the training, 6-OHDA were injected. After a week, falling latency was measure in the same condition of last 3 days of training.

Open-field test

The mice were placed in the open field for 15 min under dim light. The open field consisted of an opaque white floor and walls (40 cm × 40 cm × 40 cm). Center area was defined as central rectangular region consisting of 20 cm × cm. EthoVision XT video tracking software (Noldus Information Technology, Wageningen, The Netherlands) was used to track and analyze the thigmotaxis and movement distance.

Round Chamber test

The activities of the mice in the round chamber were recorded as described in a previous study with slight modification (Kravitz et al., 2010). Briefly, the mice were

placed in a small 15-cm-diameter cylinder for 10 min. The mobility, movement distance, and rotational behaviors of the mice were tracked and analyzed by the EthoVision XT video tracking software.

Touch screen test

The touch screen test was performed as previously described (Kwak et al., 2015). Briefly, I used the Bussey-Saksida touch screen chamber (Campden Instruments, Ltd., Loughborough, UK). Before the testing, the animals' access to food was limited in order to increase their motivation for reward. The weights of the mice were maintained at about 80% of their initial weight by limiting the animals' access to the food to 1 to 2 h/day. Sweetened condensed milk was given to the mice as a reward (SeoulMilk, Seoul, Korea).

Operant training test

For the operant conditioning test, the mice were trained to touch a visual stimulus that was presented in 1 of 12 blanks. For the first operant conditioning test (figure 15), 6-OHDA was injected into the right dorsal striatum. After a week of recovery, the mice were habituated to the touch screen chamber for 10 min. The next day, the mice were moved to the reward collection-learning phase, during which a nose poking reward magazine resulted in reward delivery for 40 min. On day 3, in the initial-touch phase, either nose poking or touching the visual stimulus resulted in reward delivery. On

days 4 to 6, in the must-touch phase, the mice were required to touch the visual stimulus to earn reward. On days 7 to 11, in the incorrect-punishment phase, the mice were required to not only touch the visual stimulus but also avoid touching the blanks to earn reward.

For the second operant conditioning test, the mice were first trained to touch the visual stimulus as in the first operant conditioning test except that they were not injected with 6-OHDA. After completion of the operant conditioning, the mice were injected with 6-OHDA. After a week of recovery, the mice were tested again in the incorrect-punishment phase.

Visual discrimination test

After completion of the operant conditioning, 6-OHDA was injected into the right striatum. After a week of recovery, the mice were tested as to whether their response to a visual stimulus that was presented in 1 of the 2 blanks remained intact and was the same as the responses in the operant conditioning test. During the visual discrimination learning, two complex visual stimuli were presented on the LCD monitor. Touching the one visual stimulus delivered reward, whereas touching the other resulted in looming room light and white noise, which were wrong signals. The locations of the visual stimuli (either left or right) were changed throughout the trials. When the wrong visual stimulus was touched, the mice were moved to the correction trials instead of being moved to the next trial. In a day, the mice underwent 30 trials

within 60 min. After 12 days of visual discrimination learning, the correct and wrong visual stimuli were reversed in order to test reversal learning. The mice were trained to touch the correct visual stimulus, which used to be the wrong stimulus, and a correction trial was given when the mice chose the wrong visual stimulus, which used to be the correct stimulus, as in the initial visual discrimination task for 18 days.

5-CSRT task

The 5-CSRT task was performed as in previous studies (Nithianantharajah et al., 2013). Briefly, after completion of the operant conditioning during which the animals were required to touch a white square that was presented in 1 of the 5 blanks, the mice were trained on the 5-CSRT task. First, the mice were trained to delay their immediate response to the LCD monitor and to respond to the white square that was illuminated for a given time (4 s). Each trial was initiated by nose poking. Once initiated, the mice were required to hold their response to the LCD monitor for 5 s (holding time) during which no visual stimulus was presented on the LCD monitor. If the mice touched the LCD monitor during the holding time, the response was counted as a premature response. After the holding time, the visual stimulus was presented on 1 of 5 blanks for 4 s. The percentage of correct responses among all of the responses that was calculated after the holding time was considered the accuracy, and failures to respond within the time were considered omissions. After the mice were trained with this 4-s protocol for 10 days, they were moved to a 2-s protocol in which the visual stimulus

was presented for 2 s. After 8 days of training with the 2-s protocol, 6-OHDA was injected into the right striatum. After a week of recovery, the mice were first tested with the 2-s protocol (data not shown) for 3 consecutive days. Next, the mice were tested with a visual stimulus protocol with the duration reduced to 1 s and 0.5 s. The tests with the 1-s duration were conducted first for 3 consecutive days, and the scores were averaged. After the test, the mice were again trained with the 2-s protocol in order to attain the baseline performance for 3 days. Finally, the mice were tested with the 0.5-s duration for 3 consecutive days. Fifty trials were conducted within 60 min during all of the 5-CSRT training and test sessions.

Location discrimination test

After completion of the operant conditioning, 6-OHDA was injected into the right striatum. After a week of recovery, the mice were tested on whether their response to a visual stimulus that was presented in 1 of 5 blanks remained intact according to the operant conditioning test. Next, the mice were moved to the location discrimination test. To do this, both the left or right location were associated with reward delivery, and the opposite side was associated with the wrong signals, including looming room light and white noise. Thirty trials were conducted within 60 min each day. As in the visual stimulus tests, correction trials were also conducted. For 4 consecutive days, initial location training was performed. Subsequently, the correct location was reversed in order to test behavioral flexibility. On the first day of the reversal, 60 trials

were conducted to facilitate reversal learning, and correction trials were also conducted. Subsequently, two more days of tests with 30 trials within 60 min per day were conducted.

Results

Motor output deficits in PD model mice

To selectively ablate dopaminergic neurons in mesostriatal pathway, I injected 6-OHDA into dorsal striatum, which is well known to be responsible for various types of cognition as well as motor output regulation. To reduce mortality caused by 6-OHDA injection, I chose low dose of 6-OHDA (4μg/μl), and unilateral injection (right hemisphere only). In my preliminary experiment, I found that my 6-OHDA injection ablated dopaminergic innervation in striatum, especially limited to dorsal area (figure 11).

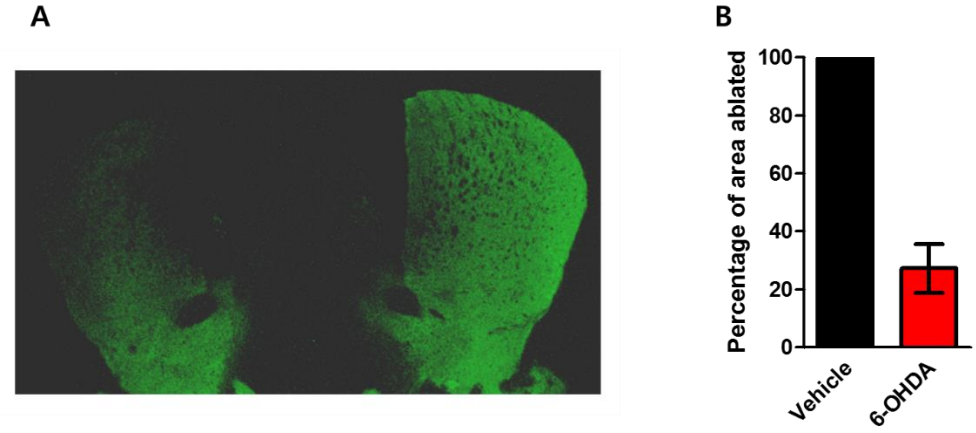

Figure 11

Injection of 6-OHDA into stratum ablated dopaminergic innervation

(A) Injections of 6-OHDA ablated the dopaminergic neurons in the dorsal striatum. Green color indicates tyrosine hydroxylase-immunopositive signal.

(B) The degree of dopaminergic ablation in 6-OHDA injected striatum, compared to vehicle injection. The data are presented as the mean ± SEM.

Next, I validated whether my 6-OHDA injection causes motor output deficit, which is main feature belong to PD model animal. First, to test motor output coordination, I performed rotarod test. After 5 days of rotarod training, I injected 6-OHDA into right dorsal striatum. Following a week of recovery, I performed rotarod probe test. No deficit in motor coordination was found in PD mice, however (figure 12). This is consistent with the previous study, which showed that similar PD model mouse did not show any deficit in gait parameter, which also tests locomotor coordination (Kravitz et al., 2010).

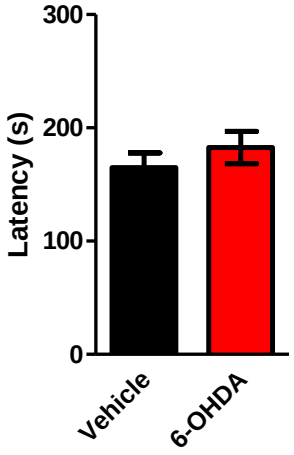

Figure 12

Injections of 6-hydroxydopamine (6-OHDA) resulted in no effect in motor coordination in the Parkinson's disease (PD) model mice.
(In collaboration with Jihye Park)

The latency of the PD model mice to fall off the rotating rod was comparable to that of the control mice [unpaired t-test: $t(22) = 0.2408$, $p = 0.8119$; vehicle, $n = 12$ and 6-OHDA, $n= 12$].

Two days after rotarod probe test, I performed open field test to test basal locomotion and anxiety. I did not find any change in anxiety (figure 13A). However, I found that total moved distance of PD mice reduced (figure 13B), indicating that basal locomotion was reduced by my 6-OHDA injection. Next, I tested locomotion and rotational behavior in PD mice. To do this, I compared animals' locomotive activity before and after 6-OHDA injection (figure 14) in small round chamber. As observed in open field test, PD mice showed reduced locomotion and time spent in mobility (figure 14A and B). PD mice also showed biased rotational behavior, rotating less to counter-clock wise direction (figure 14C and D). This biased rotating to clock-wise direction is thought to be result of unilateral (right) 6-OHDA injection.

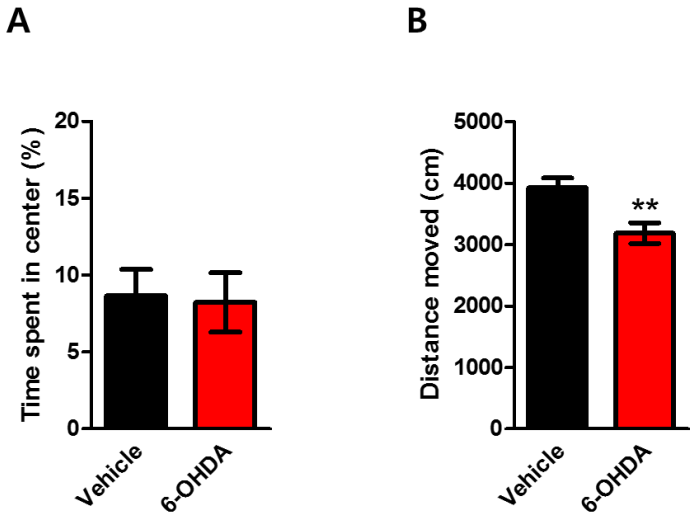

Figure 13

Injections of 6-hydroxydopamine (6-OHDA) resulted in impaired motor output in locomotion in open field test.

(A) The PD model mice spent similar time in center area compared to the control mice in the open-field [unpaired t-test: $t(22) = 0.1638$, $p = 0.8714$, vehicle, $n = 12$ and 6-OHDA, $n= 12$].

(B) The PD model mice showed reduced locomotion in the open-field compared to the control mice [unpaired t-test: $t(22) = 3.216$, $p < 0.005$, vehicle, $n = 12$ and 6-OHDA, $n= 12$]. The data are presented as the mean ± SEM.

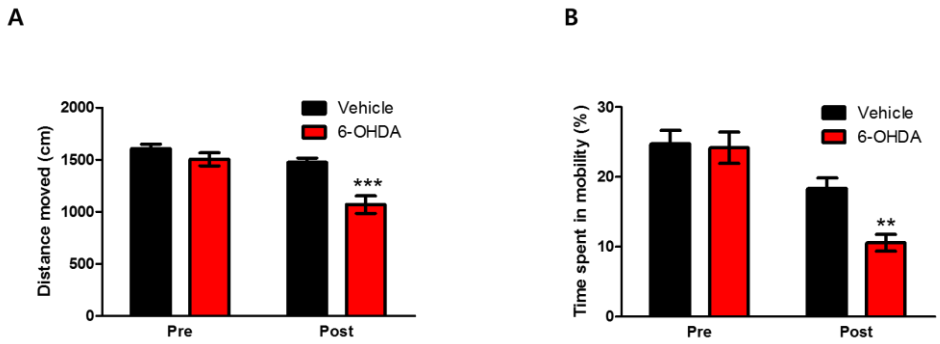

Figure 14A and B

Injections of 6-hydroxydopamine (6-OHDA) resulted in impaired motor output in locomotion and rotation in round chamber.

The PD model mice showed reduced locomotion in the round chamber compared to the control mice. The PD model mice exhibited less ambulation (A) and spent less time in mobility (A) [A, two-way ANOVA: interaction, $F(1, 13) = 10.860$, $p < 0.01$; B, two-way ANOVA: interaction, $F(1, 13) = 9.049$, $p < 0.01$, vehicle, $n = 8$ and 6-OHDA, $n = 7$]. The data are presented as the mean ± SEM.

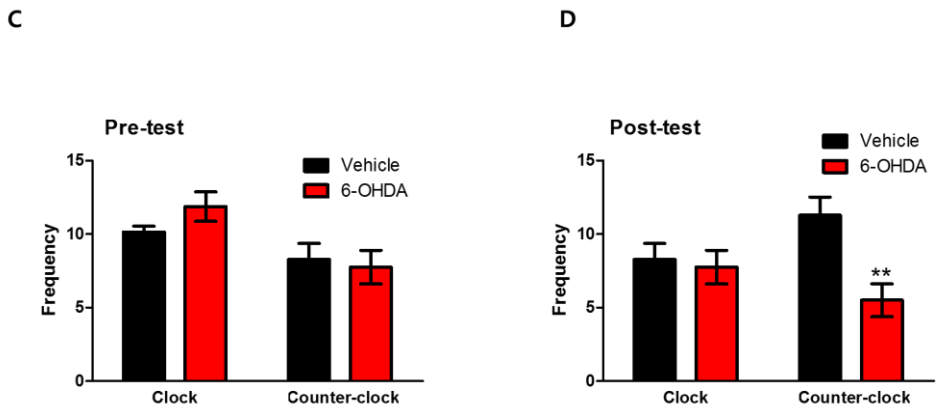

Figure 14C and D
Injections of 6-hydroxydopamine (6-OHDA) resulted in impaired motor output in locomotion and rotation in round chamber.

In the post-test (D), the PD model mice showed rotational bias in the round chamber. The PD model mice rotated less in the counterclockwise direction compared to the control mice [C, pretest, two-way ANOVA: interaction, $F(1, 13) = 1.563$, $p = 0.233$; D, post-test, two-way ANOVA: interaction, $F(1, 13) = 6.573$, $p < 0.05$, vehicle, $n = 7$ and 6-OHDA, $n = 8$]. The data are presented as the mean ± SEM.

Normal performance of PD mice in operant touch screen test

Although problems in motor coordination were not observed in rotarod test, there was also possibility that motor outputs necessary to perform touch screen task, such as touching LCD monitor, and ambulating back and forth in a chamber, can be impaired by my 6-OHDA injection, leading to poor performance in touch screen test. To address this issue, I tested simple operant conditioning task before or after 6-OHDA injection (figure 15 and 16). First, I trained animals to associate touching visual stimulus with reward delivery (operant conditioning) a week after 6-OHDA injection (figure 15). In reward collection phase, in which animal's head entry into reward magazine results in reward delivery (therefore, no touching behaviors are required), there was no difference in latency to reward (figure 15B), and the number of head entry (figure 15A). This indicates that animals' motivation was not affected by 6-OHDA injection. Through pre-training, PD mice showed reduction in the number of blank touch in initial touch (figure 15D), and the number of touching visual stimulus in the first day of must touch (figure 15E). After pre-training, animals were moved onto incorrect punishment phase. In this incorrect punishment phase, PD mice showed less correct touching visual stimulus (figure 15G).

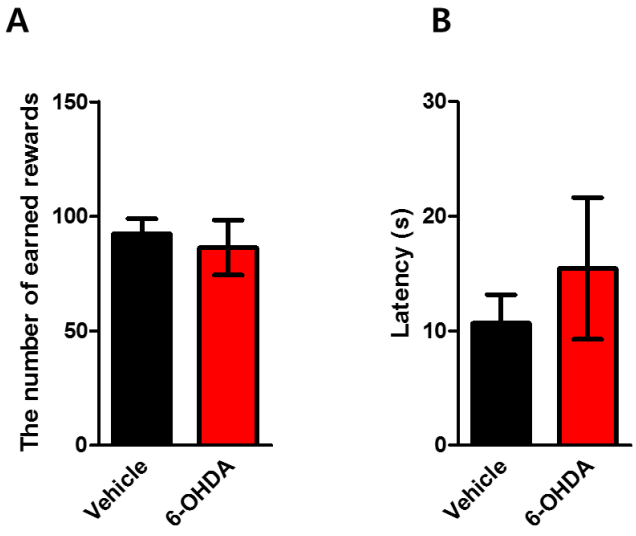

Figure 15A and B

The simple operant conditioning of the PD model mice was tested after 6-OHDA injection.

The number of earned reward by head entry into reward magazine (A) and the latency to head entry into the reward magazine (B) were similar in the control and PD model mice [A, unpaired t-test: $t(12) = 0.459$, $p = 0.654$; B, unpaired t-test: $t(12) = 0.790$, $p = 0.443$, vehicle, $n = 8$ and 6-OHDA, $n = 6$]. The data are presented as the mean ± SEM.

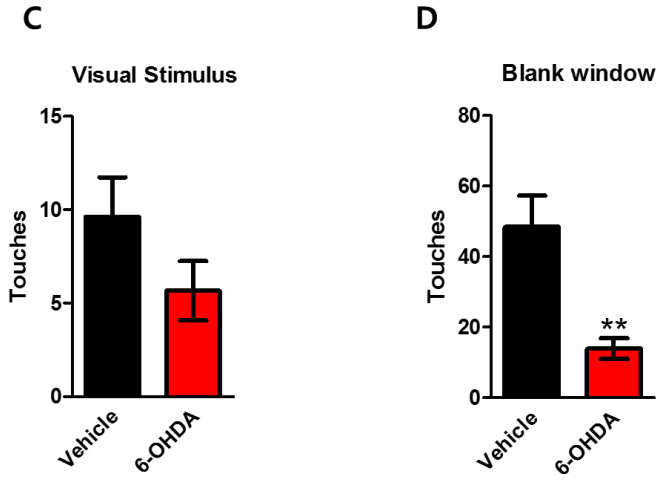

Figure 15C and D
In the touch screen test, the simple operant conditioning of the PD model mice was tested.

The number of touches during the initial touch was tested. The number of touches of the visual stimulus on LCD monitor was not changed (C), whereas the touches of the blank LCD monitor was reduced in the PD model mice (D) [C, unpaired t-test: $t(12) = 1.404$, $p = 0.186$; D, unpaired t-test: $t(12) = 3.243$, $p < 0.01$, vehicle, $n = 8$ and 6-OHDA, $n = 6$]. The data are presented as the mean ± SEM.

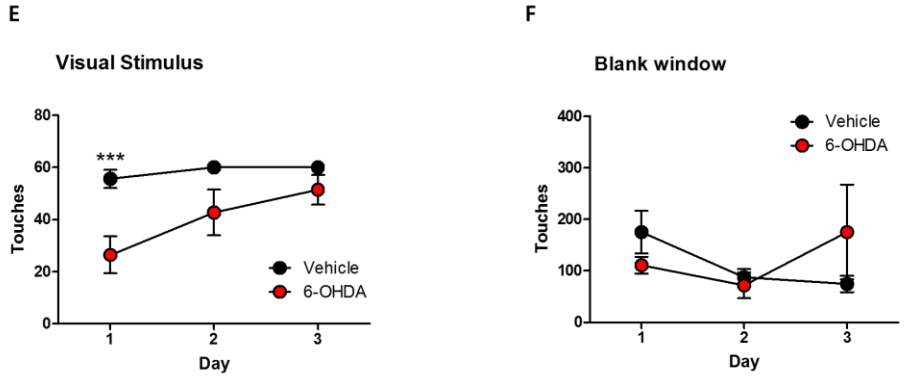

Figure 15E and F
In the touch screen test, the simple operant conditioning of the PD model mice was tested.

The number of touches in response to the must-touch stimulus was analyzed. The PD model mice touched the visual stimulus less on the first day of the must-touch session (E), whereas the PD model mice touched the blank LCD monitor a similar number of times (F) [E, two-way ANOVA: interaction, $F(2, 26) = 4.848$, $p < 0.05$, followed by a Bonferroni post-hoc test; F, two-way ANOVA: interaction: $F(2, 24) = 3.069$, $p = 0.065$, vehicle, $n = 8$ and 6-OHDA, $n = 6$]. The data are presented as the mean ± SEM.

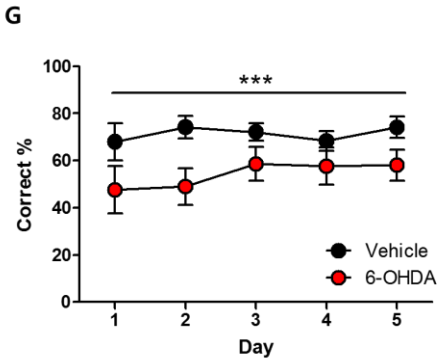

Figure 15G
In the touch screen test, the simple operant conditioning of the PD model mice was tested.

The PD model mice showed less correct touches of the visual stimulus during the incorrect-punishment phase [two-way ANOVA: effect of injected drug, $F(1, 65) = 16.920$, $p < 0.0001$, vehicle, $n = 8$ and 6-OHDA, $n = 6$]. The data are presented as the mean ± SEM.

There are two alternatives that can explain poor performance of PD mice in operant conditioning. First, it may be results of impairment in motor output even though I found no change in motor output coordination in rotarod test. If it is true, touch screen test cannot be used to assess cognition disability in PD model mice, because poor motor output can be scored as poor cognition in further cognition test. Second, low locomotion belong to PD mice may reduce chances to associate touching visual stimulus and reward delivery, because the less mice ambulate in touch screen chamber, the less mice would have reward contingency with touching visual stimulus. The latter possibility is supported by the observation that PD mice showed the reduced number of touching during initial touch and must touch, even before they were moved onto incorrect punishment phase (figure 15E).

To distinguish these possibilities, I injected 6-OHDA after animals reached criteria in incorrect punishment. A week after injection, I tested whether animals showed impairment in incorrect punishment (figure 16). If 6-OHDA injection caused problematic motor output required for correct touching, PD mice would show poor performance even 6-OHDA is injected after training of operant training. I found that, at this time, 6-OHDA injection did not affect performance in incorrect punishment (figure 16). This indicates that PD mice have normal motor outputs necessary to perform touch screen task such as touching LCD monitor and ambulation in a chamber, in spite of their motor output deficit in ambulation and rotational bias in

open field and round chamber. It further indicates that cognitive disability belong to PD model mice can be assessed in advantage of touch screen test.

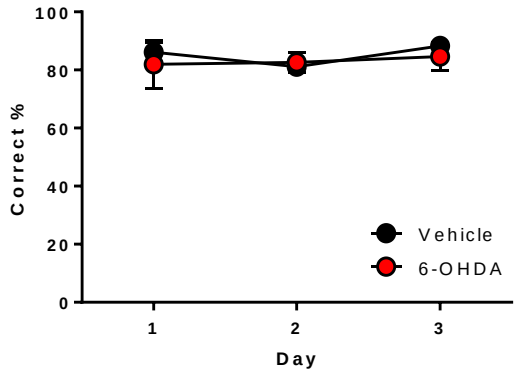

Figure 16

In the touch screen test, the simple operant conditioning of the PD model mice was tested.

The PD model mice showed normal touches of the visual stimulus after 6-OHDA was injected after the completion of operant conditioning training [two-way ANOVA: interaction, $F(2, 18) = 0.296$, $p = 0.747$, vehicle, $n = 5$ and 6-OHDA, $n = 6$]. The data are presented as the mean ± SEM.

Assessment of cognition disability in visual discrimination and its reversal learning

Next, I tested cognition of PD mice in visual discrimination and its reversal (figure 17), 5-choice serial reaction time test (5-SCRT) (figure 18), and location discrimination (figure 19). First, I conducted visual discrimination and its reversal learning test to see complex learning and reversal learning in PD mice. In this task, animals must learn to discriminate two complex visual stimuli (figure 17A). To do this, I injected 6-OHDA after completion of incorrect punishment. After 1 week of injection, I clarified that PD mice did not have any impairment in incorrect punishment phase (data not shown). Next, I trained animals to discriminate two complex visual stimuli. Through 12 days of visual discrimination training, PD mice did not show any impairment in visual discrimination (figure 17B). Because PD patient have problems in behavioral flexibility, I next test whether my PD mice also have impairment in behavioral flexibility. To test behavioral flexibility, I reversed reward contingency of initial learning after animals reached criteria in initial visual discrimination (figure 17A). As did in initial learning, PD mice did not show any impairment in reversal learning (figure 17C). This indicates that at least in my experimental condition, dopaminergic circuit disruption is not involved in visual discrimination and its reversal learning.

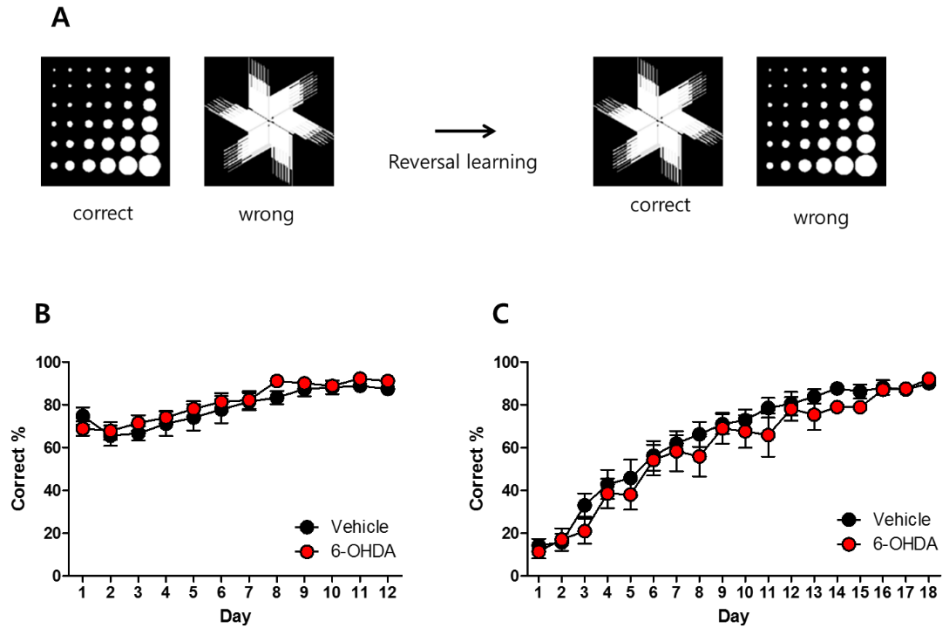

Figure 17

The PD model mice were tested on the visual discrimination and its reversal learning tasks.

(A) Schematic drawing of the experimental design.
(B and C) The PD model mice showed normal performances both in the visual discrimination (B) and its reversal learning (C) tasks [B, two-way ANOVA: interaction, $F(11, 176) = 0.542$, $p = 0.873$; C, $F(17, 272) = 0.611$, $p = 0.8824$, vehicle, $n = 9$ and 6-OHDA, $n = 9$]. The data are presented as the mean ± SEM.

Assessment of cognition disability in 5-choice serial reaction time

Next, I tested attention and compulsivity of PD mice in advantage of 5-CSRT. Requirement to hold reaction during delay time and respond to visual stimulus in limited time allow to test compulsivity and sustained attention during 5-CSRT (figure 18). It is well known that PD patients have attentional deficit. There have been also lots of studies reporting that PD model rodents have impairment in attention and striatal circuit plays critical roles in attention and compulsivity (Robbins, 2002). To see attentional performance of PD mice during 5-CSRT probe, rather during 5-CSRT training, I injected 6-OHDA after mice completed 5-CSRT training. A week after 6-OHDA injection, I performed 5-CSRT test reducing visual stimulus duration from 2 second, through 1 second to 0.5 second. However, I did not found any changes in attention (figure 18B and C) and compulsive behavior (figure 18D). This indicates that in my experimental condition, PD mice did not have impairment in attention and compulsivity.

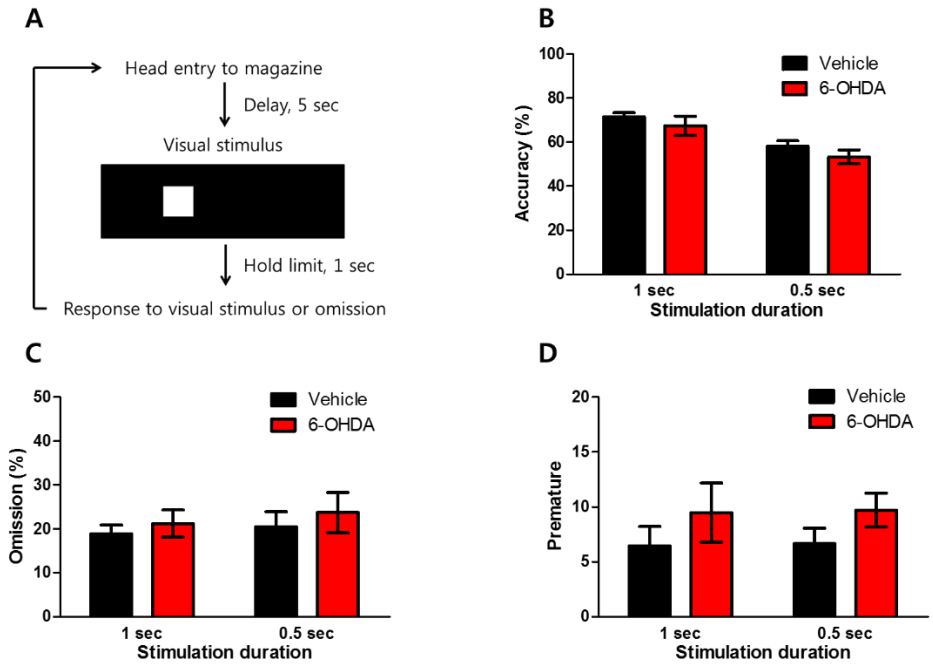

Figure 18

The PD model mice were tested on the 5-choice serial reaction time (5-CSRT) task.

(A) Schematic drawings of the experimental design. (B and C) The PD model mice showed normal performances during the 5-CSRT test in accuracy (B), omissions (C), and premature responses (D) [B, two-way ANOVA: $F(1, 11) = 0.0134$, $P = 0.910$; C, $F(1, 11) = 0.013$, $p = 0.910$; D, $F(1, 11) = 1.920$, $p = 0.997$, $n = 7$ and 6-OHDA, $n = 6$]. The data are presented as the mean ± SEM.

Assessment of cognition disability in location discrimination and its reversal learning

Finally, I examined whether PD mice have impairment in discrimination of spatial location. To this end, I first injected 6-OHDA after mice reached criteria in incorrect punishment. After a week of 6-OHDA injection, I clarified that performance of PD mice in incorrect punishment is comparable to vehicle control (data not shown). Next, mice were moved to location discrimination task. In location discrimination task, two rectangular visual stimuli were presented at the both sides of LCD monitor, one of the visual stimuli is correct, and the other is incorrect location (figure 19A). Because my PD mice showed rotational bias to clock-wise direction, I balanced correct location (correct location is either left or right) to avoid possibility that deficit in location discrimination came from rotational bias, instead of cognitive disability. Interestingly, in the first session of location discrimination, PD mice showed lower correct percentage in discriminating correct location (figure 19B). Location of correct side was irrelevant to lower correct percentage of PD mice (figure 9C), indicating that the impairment in location discrimination is result of cognitive disability, rather than their rotational bias. In following sessions, PD mice showed normal location discrimination. This means that long term memory of location discrimination was not affected.

Next, I examined behavioral flexibility in location discrimination. To do this, I reversed correct location after 4 sessions of initial learning (figure 19D and E). To

facilitate reversal learning, I doubled learning trials (from 30 trials to 60 trials) in reversal learning session. As did in visual discrimination, mice did not show any impairment in reversal learning and following additional sessions (figure 19D). To see results of the reversal learning in more detail, I split 60 trials of reversal learning into 6 blocks (figure 19E). PD mice showed comparable performance through all blocks relative to control animals, indicating that PD mice showed not only normal behavioral flexibility and but also normal new learning during reversal learning.

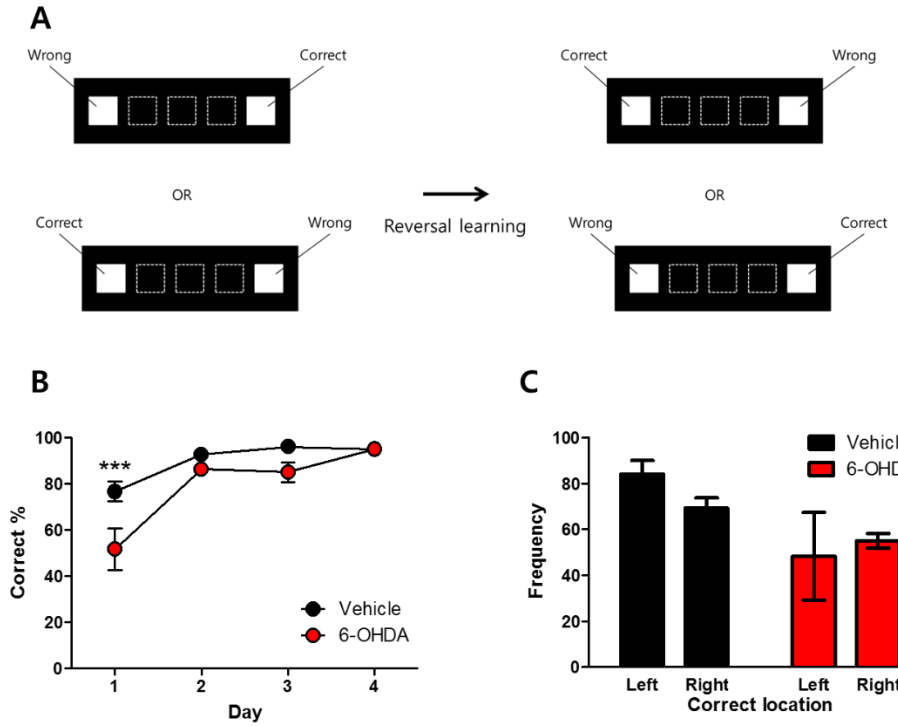

Figure 19A, B and C

The PD model mice were tested on the location discrimination and its reversal learning tasks.
(A) Schematic drawings of the experimental design.
(B) The PD model mice showed impairments on the first day of location discrimination [two-way ANOVA: interaction, $F(3, 48) = 4.832$, $p < 0.01$, followed by a Bonferroni post-hoc test, vehicle, $n = 10$ and 6-OHDA, $n = 8$].
(C) Impairments during location discrimination occurred regardless of the correct location [two-way ANOVA: interaction, $F(1, 7) = 1.825$; $p = 0.219$, vehicle-right, $n = 5$, vehicle-left, $n = 3$, 6-OHDA-right, $n = 4$, and 6-OHDA-left, $n = 4$]. The data are presented as the mean ± SEM.

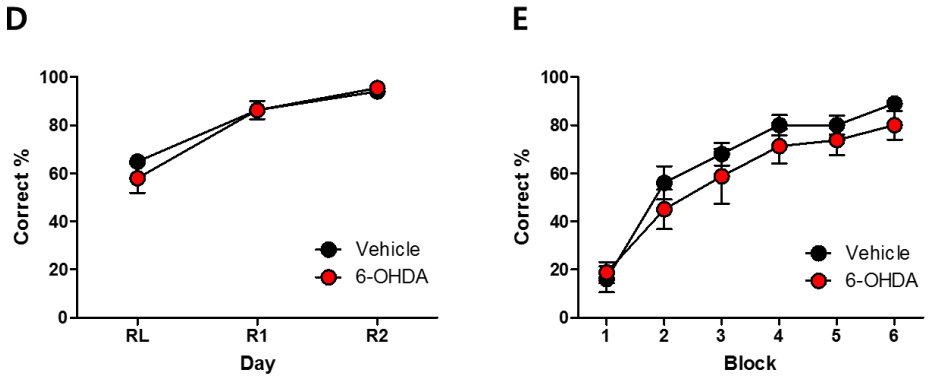

Figure 19D and E
The PD model mice were tested on the location discrimination and its reversal learning tasks.

(D) The PD model mice showed comparable performances on the reversal learning of location discrimination relative to the control mice [two-way ANOVA: interaction, $F(2, 32) = 1.504$, $p = 0.238$, vehicle, $n = 10$ and 6-OHDA, $n = 8$].
(E) During the reversal learning of location discrimination, the PD model mice showed normal performances both in the reversal and new learning [two-way ANOVA: interaction, $F(5, 80) = 0.575$, $p = 0.719$, vehicle, $n = 10$ and 6-OHDA, $n = 8$]. The data are presented as the mean ± SEM.

Discussion

In present study, I showed that touch screen test can be used to assess cognition disability belong to PD model mouse. To generate PD model mice, I injected 6-OHDA into dorsal striatum in low dose and unilaterally. This mild way of generating PD model mice was used to reduce mortality (Thiele et al., 2012). Similar to previous studies, my PD model mice showed impairment in locomotion and rotational bias, whereas motor coordination was not affected (Kravitz et al., 2010). When PD mice were trained to associate touch visual stimulus and reward, they showed normal motivation for reward, whereas they showed less touching number in initial touch/must touch, and less correct touching in incorrect punishment. When 6-OHDA was injected after operant conditioning training, however, my PD mice showed comparable correct touching in incorrect punishment to their vehicle control. Therefore, impairments of PD mice during operant conditioning training seem to be a result of low locomotion (thereby low chance to associate touching visual stimulus and reward), rather than motor output deficits. It also indicates that my PD mice manage to generate normal motor output required for touch screen task, such as touching LCD monitor, ambulating in a chamber, and earning reward, in spite of their motor output impairment found in open field and round chamber activity.

Next, I assessed cognition disability of my PD model mice. To do this, I tested cognitive paradigms, visual discrimination, 5-CSRT, and location discrimination, which are known that striatum plays critical role. In my experimental condition, PD

mice showed comparable performance in visual discrimination and 5-SCRT test relative to vehicle control. These normal cognitive functions seem to be inconsistent with previous studies (Nieoullon, 2002; Robbins, 2002). Especially, the fact that PD patients suffer from impairment in attention, and many types of PD model animals have showed poor performance in attentional test such as 5-CSRT is inconsistent with my present study (Robbins, 2002; Zhou et al., 2012). These inconsistent results may come from my way of 6-OHDA injection. I chose low dose of 6-OHDA and unilateral injection to reduce mortality. Therefore, relative low dose may remained minimal dopaminergic neurons required for 5-CSRT task. It is also possible that intact striatum hemisphere not injected with 6-OHDA, may compensate attentional function of 6-OHDA injected striatum.

It should be noted that my PD mice showed impairment in location discrimination task in spite of intact cognition in visual discrimination and 5-CSRT. These results indicate that my PD mice did not have target-off disability in their cognition. Rather, my way of producing PD mice, injecting 6-OHDA in mild dose and unilaterally, caused impairment in the specific cognition. Unlike visual discrimination and 5-CSRT, in which correct locations changed randomly through test, correct location was fixed in location discrimination task. Rotational bias observed in my PD mice raised possibility that cognitive impairment in location discrimination may be result of their biased motor output, not result of cognition. However, my PD mice showed impairment both in right and left correct location, excluding the possibility that

rotational bias caused poor performance in location discrimination. It is also interesting that PD mice showed impairment only during the first session of location discrimination. This indicates that cognitive disability was only limited to location-reward contingency, and that long term memory on correct location was not affected by 6-OHDA injection.

Studying cognitive deficit in PD model animals has been tried in previous studies (Lindgren and Dunnett, 2012; Tadaiesky et al., 2008). But employing touch screen has a few advantages over conventional learning tasks in testing cognitive disability in PD model animals (Bussey et al., 2008). First, touch screen test requires lower motor output compared to conventional cognition test. All the required motor outputs are touching LCD monitor, and ambulating in a small touch screen chamber. However, in most conventional cognition test, such as Morris water maze and contextual fear memory, normal motor outputs are essential prerequisite for testing cognition. For example, in cued version of Morris water maze, PD model rats showed increased latency to find target platform in a previous study (Tadaiesky et al., 2008). I concern that more considerations were not given before drawing conclusion that these PD model rats had cognitive deficit, because there were a few alternatives that can explain increased latency: first, like human PD patients, some PD model animals show depression like symptoms. Depressive emotion of PD model animals can increase immobility in water maze, thereby increasing latency to escape water maze. More importantly, motor deficit in PD model animals can reduce swimming speed in

water maze task. Therefore, latency to target platform may increase as a result of reduced swimming, rather than cognitive deficit (Lindgren and Dunnett, 2012). On the other hand, in touch screen test, I clarified that PD animal have normal motivation toward reward and they manage to touch LCD monitor with similar correctness compared to control animals. This results exclude possibility that poor performance of PD mice was result of low motivation and motor output deficit.

Second, touch screen test can assess not only diverse, but also translational cognition test. Human PD patients show various types of cognition disability according to their causes and progress of the disease. Therefore, testing a single type of cognition may not be enough to reveal cognition of PD model animals, given that it could miss another cognition disability that cannot be assessed by the single cognition test. On the other hand, touch screen test enables testing various types of cognition with comparable easiness. Working memory (by DMTP and TUNL), pattern separation (by TUNL and location discrimination), perceptual discrimination (by visual discrimination), object location memory (by PAL), and attention (by 5-CSRT) can be tested in touch screen chamber. All these various cognition can be tested in the same behavior apparatus, with same visual stimulus and reward, simply by changing programmed algorithm for animals to get reward. More notable feature of touch screen test in assessing cognition of disease model animal is its translational utility. Face validation of disease model indicates that the animals share pathological biochemical, circuitry, and behavioral features of the disease with human patients

(Nestler and Hyman, 2010). Therefore, a useful disease model should meet this face validation, especially, common cognitive deficit with human disease patients. Recent studies have proven that touch screen test which have been used to measure human cognition can also assess cognition of rodents. Furthermore, touch screen based translational research showed that human and mice both carrying Dlg2 mutant have similar cognition disability, validating the mutant mice as a disease model (Nithianantharajah et al., 2013). Taken together, touch screen is more useful to test cognition of PD disease model rodents given that its low motor output requirement and multi-domain translational tests.

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국문 초록

Touch screen test 는 최근에 개발된 인지능력 시험 도구이다. 보상적인 학습을 기본으로 하여, touch screen 은 실험 동물이 LCD 모니터의 시각 자극에 반응하는 것의 점수를 매겨 인지능력을 확인한다. 기존의 전통적인 인지능력 시험 도구와 비교하였을 때 touch screen test 는 모든 시험이 자동적으로 이루어 진다는 것, 시험할 수 있는 인지능력 종류가 다양하다는 것, 인간과 동물 모두에게 동일한 종류의 인지능력 시험을 할 수 있다는 장점이 있다.

이러한 장점들에도 불구하고 touch screen 은 그 동안 충분히 잘 활용되지 않았다. 지금까지 touch screen 은 특정 뇌 영역이 어떤 인지 기능을 수행하는지 혹은 유전자 변형 생쥐가 어떤 인지 능력 장애를 가지고 있는지를 시험하는데 쓰여 왔는데, 이런 것들은 기존의 전통적인 인지 시험 도구를 통해서도 충분히 가능한 것들이다.

본 연구에서 나는 인지능력을 시험하는 도구로서의 touch screen 을 더욱 잘 활용할 수 있는 방안을 연구하고 개발하였다. 먼저 나는 touch screen 을 이용해 생쥐의 작동 기억 (working memroy)을 시험할 수 있다는 것을 보였다. 더 나아가 생쥐가 인지 결정을 내리는데 걸리는 시간을

측정하여, touch screen 을 통해 인지 행동을 매우 정교하게 측정 할 수 있다는 것을 확인하였다.

나는 또한 광유전학(optogenetics)을 touch screen test 에 도입하여 광학 레이저를 자동으로 조정할 수 있는 도구를 개발하였다. 이를 이용해 ventral tegmental area 의 도파민 뉴런에 채널로돕신을 선택적으로 발현시킨 생쥐의 행동을 긍정적 강화할 수 있다는 것을 확인하였다.

마지막으로 나는 파킨슨 모델 생쥐의 인지기능을 touch screen test 를 이용해 시험 하였다. 그 동안 파킨슨 모델 생쥐의 인지기능을 시험하려는 시도는 있었지만, 파킨슨 생쥐가 가지고 있는 운동 장애 때문에 정확한 인지 기능을 시험할 수는 없었다. Touch screen test 는 실험 동물이 가지는 운동 능력에도 불구하고 인지능력을 훌륭히 시험할 수 있었으며, 나는 파킨슨 모델 생쥐가 가지고 있는 특정 인지 능력 장애를 확인할 수 있었다.

Key words

Reconsolidation; synapses; de-stabilization; re-stabilization; proteasome; Aplysia; sensitization; touch screen; working memory; delayed match to location; reversal learning; attention; 5 choice serial reaction time (5-CSRT); location discrimination; Parkinson