Lack of functional MHC class Ⅰ leads to altered dopamine system and ADHD-like behavior in mice

Lack of functional MHC class I leads to altered dopamine system and ADHD-like behaviors in mice

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Abstract

Midbrain dopamine systems play a crucial role in the regulation of many behavioral processes and their abnormalities underlie the development of several psychiatric diseases such as attention deficit hyperactivity disorder (ADHD), autism and schizophrenia. These systems express the major histocompatibility complex class I (MHCI), a neuro-immune molecule recently identified to be present in the neurons and glial cells of the brain. The MHCI molecules regulate neural connectivity and plasticity in the developing as well as adult brain. Here we report that mice in which functional MHCI has been lacked exhibit hyperactivity, motor impulsiveness and attention deficits that are three major symptoms of ADHD. They also show alterations in the gene expression of dopamine synthetic enzyme, transporter and receptor subtypes in the cell body and terminal areas of the midbrain dopamine systems. Moreover, their behavioral phenotypes are improved, although not completely reversed, by methylphenidate, an ADHD medicine. These results indicate important behavioral functions for the MHCI molecules within the brain and suggest novel therapeutic targets for ADHD and other psychiatric disorders related to the malfunctions of midbrain dopamine systems.

Introduction

Synapses and receptors are overproduced and subsequently eliminated by approximately half during two stages of life, immediately before birth and during periadolescence (Andersen SL, 2003; Askenasy EP et al, 2007). This developmental process is common to most parts of the mammalian brain such as cortex and cerebellum (Andersen SL, 2005; Giedd JH et al, 1999) Comparable changes also occur within midbrain dopamine circuits (Taraz FI et al, 1998, 1999; Teicher MH et al, 1995) that connect striatum (caudate putamen complex (CPU) and nucleus accumbens (NAc)) and prefrontal cortex, whereby modulating the neuronal activity in these brain regions. Abnormality in synaptic connections of the dopamine circuits during development has been considered as a pathogenesis of idiopathic psychiatric disorders such as schizophrenia (Weinberger DR, 1987), autism (Penzes P et al, 2013), and attention deficit hyperactivity disorder (ADHD) (Andersen SL and Teicher MH, 2000). However, a precise picture of their abnormalities still remains elusive.

Recent evidence has highlighted the non-immune role of major histocompatibility complex (MHC) genes in the central nervous system in addition to their classical immune function (Needleman LA and McAllister AK, 2012). MHC class I (MHCI) mRNAs and proteins are expressed throughout the developing and mature brain (Boulanger LM, 2004, 2009; Fourgeaud L and Boulanger LM, 2010; Liu J et al, 2012; Shatz CJ, 2009) and are directly involved in the formation and plasticity of synaptic connections (Corriveau RA et al, 1998; Goddard CA et al, 2007; Huh GS et al, 2000; McConnell MJ et al, 2009; Shatz CJ, 2009). MHCI, called H2K, H2L and H2D in rodents, consists of two polypeptide chains, heavy chains (α1~α3) and a light chain, β2-microglovulin (β2m) that links to the α3 domain. Antigen peptide transporters 1 (TAP1) and 2 (TAP2) are required to load peptides onto MHCI molecules for delivery to the cell surface (Van Kaer L et al, 1992; Zijlstra M et al, 1990). Thus, in the absence of one of these products, cell-surface expression of MHCI is reduced (Zijlstra M et al, 1990). Suppression of surface MHCI expression in mice through genetic knockouts such as $\beta2m^{-/-}$ (Huh GS et al, 2000), $\beta2m^{-/-}TAP1^{-/-}$ (Huh GS et al, 2000) or complete knockouts of H2K/H2D (Datwani A et al, 2009) severely impairs synapse elimination required during development of ocular dominance in the visual system (Boulanger LM and Shatz CJ, 2004). Moreover, deficiency of neuronal MHCI expression ($\beta2m^{-/-}$) increases synaptic density in the cortex throughout development, whereas its excessive expression (H2K) decreases the density of excitatory as well as inhibitory synapses in cultured neurons (Glynn MW et al, 2011). Similarly, neuronal overexpression of the MHCI gene (H2D) inhibits neurite outgrowth in vitro (Glynn MW et al, 2011). Deficiency of surface MHCI expression also exhibits altered

synaptic plasticity in the adult brain. The β2m$^{-/-}$TAP1$^{-/-}$ double mutant mice show enhanced long-term potentiation with absence of long-term depression in the hippocampus (Huh GS et al, 2000), whereas the H2K$^{-/-}$H2D$^{-/-}$ animals had a lowered threshold for the induction of long-term depression in the cerebellum (McConnell MJ et al, 2009). Thus, MHCI restricts the formation and plasticity of synaptic connections (Glynn MW et al, 2011; Needleman LA and McAllister AK, 2012).

Because MHCI molecules are present in the midbrain dopamine regions, the substantia nigra (SN) and ventral tegmental area (VTA) (Corriveau RA et al, 1998; Linda H et al, 1999), any alteration of their expression could contribute to the aberrant connectivity and plasticity in these regions, causing behaviors characteristic of various psychiatric disorders. Here we tested this hypothesis by determining if functional MHCI deficiency through β2m$^{-/-}$TAP1$^{-/-}$ affects synaptic transmission for midbrain dopamine circuits, thereby causing any abnormality in dopamine-related behaviors of mice.

Methods

Animals

Mice with a constitutive homozygous deletion of the β2m and TAP1 gene (β2m$^{-/-}$TAP1$^{-/-}$ double knockouts) and their wild-type littermates were used throughout the study. Wild-type (WT) and double knockout (DKO) mice were generated by breeding heterozyrous mutants, maintained in a C57BL6/J background, and we used WT littermates as a control group of DKO mice. Mice were housed in groups of four to five animals in standard laboratory Plexiglass cages under a regular 12-hr light/12-hr dark cycle (lights on at 7:00 am) and a temperature (25℃) and humidity controlled (60%) clear facility condition with free access to food and water. All procedures were approved by the Hamamatsu University School of Medicine Animal Care and Use Committee, and carried out in accordance with National Institute of Health general guidelines for the Care and Use of Laboratory animals (NIH Publications No. 86-23). All efforts were made to minimize both suffering and the number of animals used.

Drugs

The D1 receptor agonist SKF81297 (Tocris Bioscience, USA) was dissolved in DMSO (V/V below 5%) and diluted by sterile physiological saline (0.9% NaCl). D2 receptor agonist quinpirole hydrochloride (Sigma Chemical, St. Louis, MO, USA), cocaine hydrochloride (Dai-Nihon, Osaka, Japan), and methylphenidate (Nihon Ciba-Geigy K.K, Hyogo, Japan) were dissolved in 0.9% saline and administered

intraperitoneallly (i.p.) in an injection volume of 10 ml/kg. The dosage was determined based on previous experiments from our and other laboratories.

Open field test

Locomotor activity was assessed in the open filed test box (30cm×30cm×35cm), equipped with infrared sensors attached on the lid and controlled by Windows compatible PC-software (Biotec Co., Ltd., Osaka, Japan). The floor of the test box was covered with approximately 1 cm deep of clean paper bedding. Mice were introduced into the box and locomotor activity was recorded during light phase (10:00-12:00am) or dark phase (19:00-21:00pm) and the data were collected in 15-min blocks over a period of 120 min. Mice treated with SKF81297 (at the light), quinpirole (at the dark), cocaine (at the light) or methylphenidate (at the dark) were immediately subjected to locomotor activity monitoring in the box for 60 or 90 min.

Home cage activity

Mice were placed alone in a home cage (30cm×30cm×35cm) under a 12/12-h light/dark cycle (light on at 07:00 am), whose floor was filled with rodent clean paper bedding. Animals had free access to food and water. After 1 day of acclimation, spontaneous locomotor activity was measured for 7 days with an infrared sensor (Biotec Co., Ltd., Osaka, Japan).

Molecular analysis

Basal expressions of mRNAs were analyzed by Quantitative real-time PCR (Q-PCR), and compared between in WT and DKO mice. Briefly, animals were deeply anesthetized with ethyl ether and decapitated. Their brains were quickly removed and stored at -80^{o}C. Coronal brain slices of 1 mm in thickness were made for the entire brain using a mouse brain matrix, and SN, ventral VTA, CPU and NAc were dissected using razor blades. Total RNA was isolated from each brain region using Trizol reagent according to the manufacturer's specifications (Invitrogen, San Diego, CA, USA). The amount and quality of total RNA were determined by a Nanodrop spectrophotometer (Thermo Scientific, Rockford, IL, USA). Total RNA (0.5 µg) from each sample was reverse transcribed using a High-Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's specifications. The reverse transcription reaction is consisted of 10 min at 25^{o}C followed by 2 hrs at 37^{o}C. Samples were diluted with nuclease-free H_2O and stored at -80^{o}C until being assayed. Q-PCR was carried out for the following gene products: TH and DAT in SN and vVTA, DR1 and DR2 in CPU and NAc. As an internal standard, Gusb mRNA levels were also assessed in all regions. Gene expression assays were purchased from Applied Biosystems for each gene product:

TH, Assay ID Mm00447567_m1; DAT, Assay ID Mm00438388_m1; DR1, Assay ID Mm02620146_s1; DR2, Assay ID Mm00438545_m1 and Gusb, Assay ID Mm01197698_m1. Q-PCR reactions were carried out using the Taqman detection system (Step One; Applied Biosystems) in the following conditions: 40 cycles of 2 min at 50°C, 10 min at 95°C, 15 sec at 95°C, and 1 min at 60°C. All Q-PCR reactions were run in triplicate, and relative gene expression levels were calculated by delta Ct (dCt), subtracting the average cycle threshold (Ct) value for each gene product by the average Ct for Gusb mRNA. The amount of each gene product in each brain region was set at 1 in WT using the following formula:

$$F(x)=2^{-(X-Y)}$$

X is the dCT of interest and Y is the averaged dCT of WT.

Cognitive tasks

Apparatus: All the cognitive tasks were carried out with IntelliCage (New Behavior AG, Zurich, Switzerland), a kind of operant box that automates behavioral screening of mice in social group. A schematic aerial view of a cage is shown in Fig. 1A. Briefly, a cage contains 4 corner chambers and each chamber provides with an antenna that recognizes transponders injected in the back of a mouse to identify and monitor each mouse performance. A chamber provides 2 water bottles allocated each right and left side. Mouse can access the bottle through the door front of the nipple of the bottle. Nose-poke sensor is placed just front of the door, and the door is triggered by the nose-poke of mouse inside the chamber. Three LED lights are placed above the door to provide visual stimuli. Various sensors monitor mouse behavior, such as chamber entering, nose-poking, and licking the tips of water bottle. One cage can assess maximum of 16 animals' performance, and in the present study two cages are connected to IntelliCage PC systems. Male animals at the age of 8-12 weeks were used. Before the experiment, 3-4 mice were housed per one breeding cage on a 12:12 hr light-dark cycle (dark phase starts at 19:00) with free access to food and water. Twenty-four hr before introduction into the IntelliCage, each mouse was injected with the transponder in its back. In the IntelliCage, food was freely available but water was restricted in accordance with experiment procedures. Four cognitive tasks were conducted: place learning task, reversal learning task, simple reaction time task (SRTT), and delay discounting task (DDT). Three groups of mice were used for behavioral experiments.

First group (Wild type: n=17, DKO: n=17) experienced spatial learning and reversal learning, and SRTT, second one (Wild type: n=11, DKO: n=11) experienced DDT, and third one (WT: n=8, DKO=7) experienced SRTT following daily methylphenidate treatment. Drug was i.p. injected once daily at 18:00 over 14 days

just before testing but not during the testing period.

Habituation and nose-poke training: Before the cognitive tasks, mice were habituated to the IntelliCage for 5 days. All the doors front of the water bottles were always opened, thus the mice could access water freely. Following 3 days, they were trained for nose-poking to get the water reward. All the doors were closed, and they were opened when the mouse made a nose-poking. Duration of the door opening was 5 sec, and door opening was accompanied with LEDs-on. In one corner entry, the mouse could get reward one time, namely, 5 sec. The mouse needed to exit the chamber and re-enter any chamber to get the reward again.

Place learning and reversal learning task (Fig. 1B)***:*** In the place learning task, mouse can get the reward (i.e. water) in one of the four corners. The mouse learned the location of the rewarding corner chamber to get water, therefore spatial reference memory function was assessed in this task. Following reversal learning task, the rewarding corner was changed to the diagonally placed corner. Mouse needed to notice that the rule is changed and to flexibly learn new rewarding corner.

Cognitive tasks described hereafter were carried out during 19:00-22:00, in other time zone all the doors were closed and never opened by any action of mouse. In the place learning task, rewarding corner for each mouse was set as that was least chosen by the mouse during nose-poke training. Nose-poking in the rewarding corner caused door opening for 5 sec, and the LEDs above the door were switched on for the same duration as door opening. In one visit, the mouse could get reward once, then it needed to re-enter the rewarding corner to get reward again. Nose-poking in other corners caused nothing. Place learning task was carried out for 5 days, and after that, 5-day reversal learning task was initiated. In the reversal learning task, the rewarding corner was changed to diagonally opposite one of the rewarding corner in prior place learning task. Nose-poking to only the new-rewarding corner caused door opening and LEDs-on for 5 sec. The mice were returned to breeding cages after the reversal learning task was over. Percentage of error (the number of trials nose-poked in incorrect corners/the number of trials nose-poked in incorrect and correct corners * 100) in the place and reversal learning tasks were calculated for each training day. Percentage of incorrect response in ex-rewarded corner (the number of trials nose-poked in ex-rewarded corner/the number of trials nose-poked in incorrect and correct corners * 100), which reflects perseverative tendency to the corner, was also calculated in the reversal learning task.

Simple reaction time task (SRTT) (Fig. 1C)***:*** In this task, mouse needs to wait the rewarding signal (LEDs-on) to get the reward. Thus the animals are required not only to inhibit their response, but also to sustain attention to the signal.

Two or three days after reversal learning task, the mice were re-introduced into IntelliCage again and SRTT was started. The mice could get reward in all the corners. A trial started when the mouse made a nose-poke in the corner, then 1, 2, or 3 sec delay was inserted at random. After the delay period, LEDs above the nose-poked side were switched on for 5 sec. If the mouse nose-poked during LEDs-on, the door was opened for 5 sec. The trial ended if the mouse nose-poked before LEDs-on (defined as premature response), or the mouse did not nose-poke during LEDs-on (defined as error of omission). The premature response reflects impulsive action, i.e., motor impulsivity. The latency was recorded for correct responses. The mouse needed to re-enter any corner to start a new trial. SRTT training was carried out for 7 days.

In each delay condition, percentage of premature response (the number of trials with premature response/(the number of trials with premature response + the number of correct trials + the number of trials with omission error) * 100) was calculated in each training day, and average of 7 days training. Response latency (i.e. a millisecond time between onset of the LEDs-on and nose-poke) was rounded off to one decimal place. Since over 90% of responses were made until 2.5 sec from LEDs-on, the data of responses within 2.5 sec were used for analysis. Percentage of response latency in each 0.1 sec was calculated.

Delay discounting task (DDT) (Fig. 1D)***:*** Delay discounting task is one of the most popular task that measures the animal's impulsive choice, i.e., cognitive impulsivity. Mouse chooses immediately small reward (i.e. small immediate reward: S) or large, but delayed, reward (i.e. large delayed reward: L). Impulsive choice is defined as the selection of S reward. Whereas SRTT mentioned above measures the inability to withhold from making a motor response (motor impulsivity), DDT measures impulsive decision making of animals (cognitive impulsivity).

The mice could get reward in all the corners. In the corner, there are two holes (i.e. nose-poke sensors front of door) front of the mouse, the one was assigned to S side and the other to L side. Mice were randomly divided into right-S or left-S groups, but the number of mice was counterbalanced, and the right-left condition was changed in accordance with procedure described below. The mouse could get reward once in one visit, and needed to exit and then re-enter any corner to get reward again. If the mouse nose-poked the S side, the door immediately opened after the nose-poke (thus the delay was 0 sec), and the duration of door opening was always 1.5 sec. In the L side, the delay was 0, 2, 4, 8, or 16 sec and the duration of door opening was always 15 sec. Five delay conditions at the L side were carried out in ascending manner. Each L delay condition was tested for 6 days, and the S and L side was changed in the first and later halves. Percentages of L preferences were

calculated in each L delay conditions.

Behavioral sensitization

The mice used here had already been habituated to the home cage described above for 7 days and divided into four groups: The WT mice treated with saline or cocaine, the DKO mice treated with saline or cocaine. After 1 day of saline injection for acclimation to the injection procedure, animals received 7 daily injections of either saline or cocaine (20 mg/kg) and their locomotor activity was measured for 60 min. Following 10 days without injection, all groups received a challenge injection of cocaine (20 mg/kg) and locomotor activity was again assessed.

Data analysis

Statistical significance for two group comparisons was primarily assessed using ANOVA, followed by post-hoc test when applicable. Student's t-test was applied to locomotor and mRNA results. Data were analyzed with SPSS 17.0 software (SPSS Institute Inc.).

Results

DKO mice exhibit increased locomotion

We asked whether the functional MHCI deficiency affects dopamine-related behaviors in mice. We first analyzed the locomotor activity of DKO mice, which are physically healthy and normal in appearance. Locomotor activity of DKO mice was assessed in the open-field test. Eight to 10 week-old DKO mice showed substantially increased locomotor activity compared with WT control littermates during the night (Fig. 2C, D), suggesting that MHCI deficiency leads to hyperactivity in mice exposed to new environments. However, when we examined locomotion during the day, there was no difference between the genotypes (Fig. 2A, B). Likewise, DKO mice showed enhanced locomotor activity in their familiar home cages at the night, but not at the day (Fig. 2E, F). Thus, DKO mice showed the hyperactivity with their nocturnal nature. We next tested whether the enhanced hyperactivity in our DKO mice endures over a lifetime. Consequently, the phenotype difference appeared when mice were 22 days old and persisted at least until they were 70 days old (Fig. 2G), thereby suggesting that the hyperactivity in the DKO mice shows no age-dependent improvement.

DKO mice have altered locomotor responses to dopaminergic drugs

Because midbrain dopaminergic pathways are implicated in regulation of locomotor function, we characterized the impact of D1R and D2R agonists and a

dopamine transporter (DAT) blocker on the locomotor activity of DKO mice. The D1R agonist, SKF 81297 dose-dependently increased locomotion in mice of both genotypes, but it was more effective at the three doses tested (3, 10, 30 mg/kg) in enhancing activities in DKO mice (Fig. 3A). On the other hand, the D2R agonist, quinpirole decreased locomotor activity in a dose-related manner for both the WT and DKO mice. However, the inhibitory effect at any doses tested (0.3, 1, 3 mg/kg) did not differ between genotypes (Fig. 3B). The DAT blocker, cocaine, dose-dependently increased locomotion for both WT and DKO mice (Fig. 3C). Notably, the effect on the DKO mice was greater at the higher doses tested (20, 40 mg/kg). It is thus suggested that altered D1R and DAT expression may contribute to locomotor hyperactivity in the DKO mice.

DKO mice have alterations in D1R, D2R, TH and DAT gene expression

Because dopamine synthesizing enzyme, transporter and receptors could be strongly associated with locomotor activity, we tested if DKO brains show alterations in these dopamine measures. Eight to 10 week-old mice were used for mRNA expression experiments to evaluate the expression levels of dopamine synthesis enzyme tyrosine hydroxylase (TH), DAT and dopamine receptor subtypes. The DKO mice showed a significant increase in D1R expression and a marginal increase in D2R expression of the CPU (Fig. 4A) whereas no apparent change in those of the NAc (Fig. 4B) when compared with WT controls. On the other hand, DKO mice exhibited a significant decrease in DAT gene expression in both the SN and the VTA. They also showed a significant decrease in TH gene expression in the VTA, while a trend towards a decrease in the SN. These modifications of mRNAs are in partial support of the conclusion that DKO mice displayed increased hyperlocomotion to dopaminergic drugs.

DKO mice exhibit normal place learning and reversal learning

Midbrain dopamine systems regulate not only motor function but also cognitive function and their malfunctions are associated with learning disorders, increased impulsivity and attention deficits (Cardinal RN et al., 2004). Therefore, we wanted to know if the DKO mice have any abnormalities on learning, impulsivity, and attention. We utilized the Intellicage test, which measures place learning and reversal learning. The DKO mice learned as quickly as WT littermate controls in a place learning task with similar learning curves (Fig. 5B). In the reversal learning, in which the reward corner is changed to the one placed diagonally, both genotypes again responded with similar learning curves, which show normal extinction for the previous location in which water had been available (Fig. 5C). Normal learning in

spatial and reversal tasks in the DKO mice suggests no apparent impairment in fundamental cognitive ability and flexibility of these mice.

DKO mice exhibit increased impulsivity and impaired attention

Next, to determine if functional MHCI deficiency would alter impulsivity and attention, we employed a SRTT paradigm with the Intellcage in which water-deprived mice were trained to make a nose-poke into the hole to initiate a trial and then lick the feed-water nozzle inside the hole while the LEDs are switched on after a random delay duration of 1, 2 or 3 sec to get water rewards. In this task, mice were needed to inhibit nose-poking during the delay period before LEDs-on. Responses recorded during this period were considered premature and provided a measure of impulsive action i.e., motor impulsivity. Both WT and DKO mice exhibited a significant increase in premature response (response before LEDs-on), in a delay-dependent way (Fig. 6B). The percentage of premature response in the DKO mice was significantly higher at the longer delays of 2 and 3 sec, thus indicating increased impulsive action (diminished inhibitory control) for DKO mice (Fig. 6C). Mice were also needed to nose-poke for water reward as soon as possible after LEDs-on. The latency to nose-poke into the hole after the light onset provided a measure of attention. The distribution of response latency in WT mice exhibited single peaking at around 0.3 sec post-LED onset, whereas in DKO mice double peaking at approximately 0.3 and 0.8 sec (Fig. 7). Thus WT mice quickly initiated licking after the onset of LEDs, whereas DKO mice often started licking long after the light onset, suggesting deficits in sustaining attention over a delay in the DKO mice.

DKO mice exhibit normal performance on delay discounting

Because impulsivity contains motor and cognitive aspects (Brevers D et al, 2012; Dalley JW et al, 2011), we asked if the DKO mice have any alteration in cognitive impulsivity. We employed the delayed discounting task to measure impulsive choice, a small immediate reinforcer in preference to a large delayed reinforcer. Both genotypes discount future reinforcers (reduced preference for delayed reinforcement) as a function of the delay from the time of choice, choosing small immediate reinforcers (Fig. 8B). The DKO mice exhibited a pattern of choice, which is indistitinguishable from that of the WT controls, suggesting no impairement of impulsive choice, i.e., cognitive impulsivity, in the DKO mice.

DKO mice display augmented cocaine sensitization

The DKO mice displayed motor hyperactivity, decreased sustained attention and motor impulsiveness, which are core symptoms of ADHD-like behavior. As ADHD in humans is highly comorbid with addiction of drugs such as nicotine, methamphetamine and cocaine (Andersen SL and Navalta CP, 2004), we utilized the behavioral sensitization, a well-known animal model of drug addiction, to determine if the DKO mice have enhanced behavioral sensitization to cocaine. After one day of saline injection to acclimatize animals to the activity test box, locomotor response to the drug increased across days of testing in both WT and DKO mice (Fig. 9). Furthermore, DKO mice showed a much larger increase in locomotor response to cocaine than WT mice on days 4, 5, 6 and 7. To test whether this method produced long-lasting sensitization, we administered a challenge dose of cocaine (20 mg/kg) to both saline- and cocaine-treated animals of WT and DKO groups after 10 days of withdrawal from the last drug injection. Mice pretreated with chronic cocaine exhibited a much greater locomotor response to the drug than did saline-treated animals in both groups of WT and DKO mice (Fig. 9). Again, this response in DKO mice was remarkably large compared with WT animals. These findings indicate that behavioral sensitization to repeated exposure of cocaine is more enhanced in DKO mice, and that the sensitization lasts long.

ADHD-like symptoms of DKO mice are reversed by methylphenidate

We hypothesized that methlphenidate, a psychostimulant used to treat ADHD, would normalize ADHD-like phenotypes in the DKO mice. Indeed, MPD at low dose tended to suppress hyperactivity in DKO-mice as compared with saline treatment (Fig. 10A, B). In contrast to DKO mice, methlphenidate -treated WT mice showed enhanced locomotor activity (Fig. 10A, B). Despite this evidence, methlphenidate at higher doses markedly increased locomotor activity in both genotypes (Fig. 10A, B). However, it should be noted that this enhancement relative to saline-treatment was significantly smaller in the DKO mice (Fig. 10C). These findings are analogous to the reported effects by methlphenidate or amphetamine on WT and ADHD model mice (Gong R et al, 2011; Trinh JV et al, 2004; Zhou M et al, 2010; Zhuang X et al, 2001). We next wanted to know if methlphenidate also improves impulsivity and attention deficits in DKO mice. In the SRTT test, DKO mice treated with repeated methlphenidate showed a similar score in premature response rate compared with methlphenidate-treated WT mice (Fig. 11A). As in WT mice (Fig. 11B), the distribution response latency in methlphenidate-treated DKO mice clearly exhibited single peaking at 0.3 sec, and their latency was not different from that of methylphenidate-treated WT mice (Fig. 11C). This is reminiscent of the methylphenidate-induced normalization of impulsivity and inattention in human

subjects with ADHD. It is thus suggested that the psychostimulant treatment normalizes hyperactivity, impulsivity and inattention in DKO mice.

Disucussion

The present study demonstrated, for the first time, that double mutant mice lacking β2m and TAP1 (β2m$^{-/-}$TAP1$^{-/-}$), which consequently show diminished surface expression of MHCI, display hyperlocomotion, impulsive action, i.e., motor impulsiveness, and deficits in sustained attention. They exhibit altered behavioral responses to dopamine agonists. Although the mutant mice show impulsive action, they do not exhibit impulsive choice, i.e., cognitive impulsiveness. Furthermore, the β2m$^{-/-}$TAP1$^{-/-}$ animals have a normal phenotype in learning ability and behavioral flexibility. Thus, the β2m$^{-/-}$TAP1$^{-/-}$ mice appear to be an animal model that exhibits some but not all of the behavioral and pharmacological characteristics of ADHD.

ADHD is associated with physiological dysfunction of the midbrain dopamine circuits, but whether ADHD is caused by a hyperdopaminergic (Drerup JM et al, 2010; Gainetdinov and Caron, 2000; Krapacher FA et al, 2010; Trinh et al, 2003; Zhuang X et al, 2001) or hypodopaminergic (Barr CL et al, 2000; Gong R et al, 2011; Mill J et al, 2002; Raber J et al, 1997) transmission is still highly disputable (Swanson J, 1998; Zhuang X et al, 2001). We measured TH mRNA levels in the β2m$^{-/-}$TAP1$^{-/-}$ mice, and a marked decrease was found in the midbrain compared to WT mice. Thus our model appears to be in line with the hypodopaminergic hypothesis. The weakened dopamine activity has previously been observed in a genetic mouse model of ADHD, the Coloboma mutant mice (Barr CL et al, 2000; Mill J et al, 2002; Raber J et al, 1997).

The β2m$^{-/-}$TAP1$^{-/-}$ mice display an enhancement in locomotor activating response to a D1 receptor agonist SKF8197 and also to a DAT inhibitor cocaine. However, they are apparently normal in locomotor suppressing response to a D2 receptor agonist quinpirole. This suggests that D1 receptor signaling is heightened in the β2m$^{-/-}$TAP1$^{-/-}$ mice while D2 receptor signaling is unchanged, and also that DAT function might be lowered. In fact, we detected a significant increase in D1 receptor mRNA in the CPU and a decrease in DAT mRNA in the SN and the VTA. Thus the augmentation of locomotor response to the D1R agonist and DAT blocker in the β2m$^{-/-}$TAP1$^{-/-}$ mice might be due to an alteration of transcription to these mRNAs, primarily in the nigrostriatal dopamine circuit.

While we can attribute these dopamine-related transcriptional modifications and behavioral abnormalities to a disruption in MHCI function, the underlying mechanisms are presently undetermined. MHCI acting as an eliminator of excessive synapses (Glynn MW et al, 2011; Needleman LA and McAllister AK, 2012) may

directly or indirectly regulate expression of dopamine receptor subtypes. The unexpected decrease in the gene expression of TH and DAT observed here, therefore, may represent an attempt of the circuits to compensate for an overall increased activity of dopamine receptors in excessive synapses of the β2m$^{-/-}$TAP1$^{-/-}$ brain. Future studies will be needed to elucidate this possibility.

ADHD symptoms can be treated by low but not high doses of the psychostimulant methylphenidate, which increases dopamine concentrations at synaptic clefts through inhibition of dopamine uptake by blocking the activity of the dopamine transporter. The dose-dependency of methylphenidate on treating hyperactivity is commonly used to evaluate the validity of ADHD animal models (Beaulieu JM et al, 2006; Drerup JM et al, 2010; Furuse T et al, 2010; Krapacher FA et al, 2010; Siesser WB et al, 2005; Zhou M et al, 2010). Treatment with methylphenidate at the dose range used for curing human ADHD (2.5 mg/kg) caused a tendency to decrease the locomotor activity of β2m$^{-/-}$TAP1$^{-/-}$ mice but not WT mice in a familiar environment. In contrast, high doses of methylphenidate (>2.5 mg/kg) potentiated locomotion for both WT and β2m$^{-/-}$TAP1$^{-/-}$ mice, but the mutant mice showed a smaller percent increase in locomotor activity compared to the WT controls. Since there was a change in mRNA levels of D1 and D2 receptors in the β2m$^{-/-}$TAP1$^{-/-}$ mice, this will likely lead to an unbalance between presynaptic and postsynaptic dopamine receptor functions which act in the opposite way on locomotor activity (Jones SR et al, 1999), contributing to the reduced locomotor response to a low dose of methylphenidate (2.5 mg/kg) in the β2m$^{-/-}$TAP1$^{-/-}$ mice. Furthermore, we examined if repeated exposure to a lower dose of methylphenidate (1 mg/kg) can reverse deficits of motor impulsivity and poor attention in β2m$^{-/-}$TAP1$^{-/-}$ mice. Consequently, we found that chronic methylphenidate reduced motor impulsivity and remarkably improved poor attention of these mice, which, to the best of our knowledge, is the first observation in the mouse ADHD model. Such a finding is in good agreement with the well-known therapeutic action of this drug in ADHD patients.

Notably, high rates of comorbidity between ADHD and substance use have been documented in humans. About 20% of people with substance use disorder has ADHD and these patients often have a more severe and complicated course of substance use disorder than those without ADHD (Wilens and Upadhyyaya, 2007). Conversely, adolescents with ADHD have a two-fold prevalence of cigarette smokers compared to those without ADHD (Wilens TE, 2007), thus suggesting directionality of ADHD recognized as a risk factor for substance use disorder. When β2m$^{-/-}$TAP1$^{-/-}$ mice were repeatedly exposed to cocaine, they showed a long-lasting hypersensitized locomotor response to the drug, indicating that β2m$^{-/-}$TAP1$^{-/-}$ mice are highly vulnerable to cocaine. Taken together, the ADHD-like phenotypes of

β2m$^{-/-}$TAP1$^{-/-}$ mice suggest that these mice may be useful for a mouse model of ADHD, particularly with substance use susceptibility.

ADHD has a substantial genetic component, with heritability of around 0.79 (Lichtenstein P, 2010). However, ADHD is a complex psychiatric disorder that is most likely heterogenous and polygenic (Tripp G and Wickens JR, 2009). Therefore, discrete genetic manipulations may only contribute a small percent to the symptoms (Comings DE, 2001; Faraones SV and Mick E, 2010; Franke B et al, 2009). This could explain the moderate behavioral effects in the β2m$^{-/-}$TAP1$^{-/-}$ mice. Neverthless, the human MHCI region at 6p21.3 that is related to immune system has recently been implicated as a susceptibility locus for ADHD in a study of ADHD within sibling pairs identified for reading disability (Willcutt EG et al, 2002). In addition, ADHD and autoimmune diseases such as asthma and atopic dermatitis have often been found to co-occur in the same individuals (Chen MH et al, 2012). Thus, the study of the β2m$^{-/-}$TAP1$^{-/-}$ mice may provide insights into the mechanisms by which a neuro-immune system may impair a fundamental behavioral process such as locomotor activity, self-control, and attention, and may direct us to a new target for treatment of ADHD.

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Figure legends

Figure 1 A: Aerial view of Intellicage apparatus. **B, C, D:** A schematic drawing illustrates the procedure of place learning and reversal learning task (B), simple reaction time task (C) and delay discounting task (D).

Figure 2 β2m$^{-/-}$TAP1$^{-/-}$ mice are hyperactive. A, C: Time course of spontaneous locomotor activity during the first 120 min following placement in the novel environment at light (A) or at dark (C) for β2m$^{-/-}$TAP1$^{-/-}$ (DKO, red line) and its littermate control group (WT, blue line). **B, D:** Mean locomotor activity accumulated over 2 hr during light (B) and dark (D) phase. The DKO mice displayed a higher level of locomotor activity only in the dark phase (Student's t-test, $t(22)=2.647$ $p<0.05$). **E:** Daily pattern of locomotor activity in WT and DKO mice during a 12/12-hr light/dark cycle in the home cage. **F:** Mean locomotor activity accumulated over 12 hr during the light vs. dark phase of the cycle. Both genotypes displayed a rhythmic circadian pattern of locomotion. However, DKO mice showed a higher activity during the dark phase compared with WT mice. Two way ANOVA revealed a main effect of phase ($F(1,22)=380.309$, $p<0.001$), genotype ($F(1,22)=14.041$, $p<0.001$) and a significant interaction of phase × genotype ($F(1, 22)=14{,}219$, $p<0.001$). Significant genotype-differences were observed only during the dark phase (Bonferroni post-hoc test, $p<0.01$). **G:** Time course of daily locomotor activity at ages between postnatal 22 and 70 days in WT and DKO mice. The DKO mice displayed hyperlocomotion over a lifetime from weaning age to adult age. Two way ANOVA revealed a main effect of genotype ($F(1,14)=15.984$, $p=0.001$) and day ($F(48,671)=37.995$, $p<0.001$) and a significant interaction of genotype X day ($F(48,672)=1.405$, $p=0.04$). Values are presented as mean ± SEM (n=6-12 for each genotype). ZT: zeitgeber time. *$p<0.05$, **$p<0.01$.

Figure 3 Locomotor responses to dopaminergic drugs are enhanced in β2m$^{-/-}$TAP1$^{-/-}$ mice. A: Effects of DR1 receptor agonist SKF81297 on locomotion in wild-type (WT) and β2m$^{-/-}$TAP1$^{-/-}$ (DKO) mice. After 1-hr habituation in the light phase, WT and DKO mice were injected intraperitoneally with either vehicle or 0.3, 3.0, 10, 30mg/kg SKF81297, and locomotor activity was measured for 60 min. SKF81297-induced hyperlocomotion was greater in DKO mice than in WT mice. Two-way ANOVA with repeated measures revealed a significant main effect of dose

(F(3, 42)= 18.049, p<0.001), genotype (F(1, 14)= 9.916, p<0.01) and a significant interaction of genotype × dose (F(1, 14)=7.622, p<0.001). Significant genotype-differences were observed at doses of 10mg/kg (Bonferroni post-hoc test, p<0.05) and 30 mg/kg (p<0.05). **B:** Effects of DR2 receptor agonist quinpirole on locomotion in WT and β2m$^{-/-}$TAP1$^{-/-}$ mice. After 1 hr habituation in the dark phase, WT and DKO mice were injected intraperitoneally with either saline or 0.3, 1.0, 3.0 mg/kg quinpirole, and locomotor activity was measured for 60min. Quinpirole-induced hypolocomotion was similar in both genotypes of mice. Two-way ANOVA with repeated measures revealed a significant main effect of dose (F(3, 42)= 88.658, p<0.001) but genotype (F(1, 14)=0.897, p=0.360) and no significant interaction of genotype × dose (F(1, 42)=1.987, p=0.131). **C:** Effects of dopamine transporter blocker cocaine on locomotion in WT and β2m$^{-/-}$TAP1$^{-/-}$ mice. After 4 hr habituation, mice received an injection of either saline or 10 mg/kg, 20 mg/kg, 40 mg/kg cocaine, and locomotor activity was monitored for 90 min. Cocaine-induced hyperlocomotion was larger in β2m$^{-/-}$TAP1$^{-/-}$ mice than in WT mice. Two-way ANOVA with repeated measures revealed a significant main effect of genotype (F(1, 40)= 5.954, p<0.05), dose (F(3, 40)= 104.982, p<0.001) and a significant interaction of genotype × dose (F(3, 40)= 3.021, p<0.05). Significant genotype differences were observed at doses of 20mg/kg (Bonferroni post-hoc test, p<0.01) and 40mg/kg (p<0.05), but not 10mg/kg (P = 0.800). Values are expressed as mean ± SEM (n=6-9 for each genotype). *p<0.05, **p<0.01, ***p<0.001.

Figure 4 D1 and D2 receptor mRNA expression is upregulated, whereas TH and DAT mRNA expression is downregulated in midbrain dopamine circuits of β2m$^{-/-}$TAP1$^{-/-}$ mice. **A, B:** DR1 and DR2 mRNA expression in CPU (A) and NAc (B). In CPU, β2m$^{-/-}$TAP1$^{-/-}$ (DKO) mice showed higher expression levels of both D1R (Student's t-test, t(14)=2.517, p=0.0246) and D2R (t(14)=2.016, p=0.0634) mRNA than wild-type (WT) mice. However, in the NAc, there was no genotype-difference in both dopaminergic measures (D1R: t(14)=0.8873, p=0.3899; D2R: t(14)=0.4197, p=0.6811). **C, D:** TH and DAT expression in SN (C) and VTA (D). There were genotype-differences in expression level of DAT mRNA in both SN (t(13)=2.297, p=0.0389) and VTA (t(13)=2.399, p=0.0321), and of TH mRNA in VTA (t(13)=2.991, p=0.0104) but not SN (t(13)=1.390, p=0.1878). Values are expressed as mean ± SEM (n=6-9 for each group). *p<0.05, #p<0.10.

Figure 5 Performance on place and reversal learning is normal in β2m$^{-/-}$TAP1$^{-/-}$ mice. **A:** Experimental timeline illustrating when place learning and reversal learning was analysed. **B:** Percentage of incorrect responses on place and reversal

learning tasks. Cognitive ability and flexibility of β2m$^{-/-}$TAP1$^{-/-}$ (DKO) mice is not different from wild-type (WT) mice. Before analysis of variances, the data were transformed using arc-sine transformation. Place learning: Two-way ANOVA with repeated measures revealed a significant main effect of day ($F(4,128)=64.736$, $p<0.01$) but not genotype ($F(1,32)=2.593$, $p=0.117$) and no significant interaction of day x genotype ($F(4,128)=0.224$, $p=0.924$); Reversal learning: Two-way ANOVA with repeated measures revealed a significant main effect of day ($F(4,128)=18.058$, $p<0.01$) but genotype ($F(1,32)=3.949$, $p=0.056$) and no significant interaction of day x genotype ($F(4,128)=0.182$, $p=0.948$); **C:** Percentage of responses to originally rewarded corner during reversal learning. Perseveration of DKO mice is not significantly different from WT mice. Two-way ANOVA revealed a significant main effect of day ($F(4,128)=67.26$, $p<0.001$) but genotype ($F(1,32)=2.20$, $p=0.148$) and no significant interaction of day x genotype ($F(4,128)=0.20$, $p=0.937$). Dotted lines indicate chance level of corresponding responses. Values are expressed as mean ± SEM (n=17 for each genotype).

Figure 6 Increased premature responses in β2m$^{-/-}$TAP1$^{-/-}$ mice. A: Timeline illustrating when performance on simple reaction time task was analysed. **B, C, D:** Time course of premature responses (%) in simple reaction time task at delays of 1 sec (B), 2 sec (C) and 3 sec (D). Both genotypes displayed a similar learning pattern of premature responses at either delay durations. However, β2m$^{-/-}$TAP1$^{-/-}$ (DKO) mice showed enhanced impulsive action during the delay compared with wild-type (WT) mice. Delay 1 sec: Two way ANOVA revealed a significant main effect of day ($F(6,192)=75.749$, $p<0.01$) but not genotype ($F(1,32)=1.080$, $p=0.306$) and a significant interaction of day × genotype ($F(6,192)=3.093$, $p<0.01$). Significant genotype-differences were observed on days 5 and 7 (Bonferroni post-hoc test, $p<0.05$); Delay 2 sec: Two way ANOVA revealed a main effect of day ($F(6,192)=86.140$, $p<0.01$) and genotype ($F(1,32)=13.137$, $p<0.01$) and no significant interaction of day × genotype ($F(6,192)=1.829$, $p=0.095$); Delay 3sec: Two way ANOVA revealed a main effect of day ($F(6,192)=93.339$, $p<0.01$) and genotype ($F(1,32)=12.688$, $p<0.01$), and no significant interaction of day × genotype ($F(6,192)=2.014$, $p=0.066$). **E:** Mean premature responses accumulated over 7 days at each delay. Two way ANOVA revealed a main effect of delay ($F(2,64)=934.355$, $p<0.001$), genotype ($F(1,32)=9.438$, $p<0.01$) and a significant interaction of delay × genotype ($F(2,64)=6.023$, $p<0.01$). Significant genotype-differences were observed at delays of 2 sec (Bonferroni post-hoc test, $p<0.05$) and 3 sec ($p<0.05$). Values are expressed as mean ± SEM (n=17 for each genotype). *$p<0.05$, ***$p<0.001$.

Figure 7 Increased response latency in β2m$^{-/-}$TAP1$^{-/-}$ mice. A, B, C: Distribution of response latency (%) in simple reaction time task at delays of 1 sec (A), 2 sec (B) and 3 sec (C). Response latency exhibited monomodal distribution in WT mice, while bimodal distribution in DKO mice. Thus DKO mice had difficulty sustaining attention. Delay 1sec: Two way ANOVA revealed a significant main effect of latency ($F(24,768)=18.349$, $p<0.001$) but not genotype ($F(1,32)=0.000$, $p=0.994$) and no significant interaction of latency × genotype ($F(24,768)=1.357$, $p=0.119$); Delay 2 sec: Two way ANOVA revealed a main effect of latency ($F(24,768)=19.998$, $p<0.001$) but not genotype ($F(1,32)=0.475$, $p=0.496$), and a significant interaction of latency × genotype ($F(24,768)=2.071$, $p=0.01$). Significant genotype-difference was observed at latency of 0.4 and 1.0 sec (Bonferroni post-hoc test, $p<0.05$); Delay 3sec: Two way ANOVA revealed a main effect of latency ($F(24,768)=20.574$, $p<0.001$) but not genotype ($F(1,32)=0.001$, $p=0.970$), and no significant interaction of latency × genotype ($F(24,768)=1.059$, $p=0.386$). **D:** Distribution of cumulative response latency. Two way ANOVA revealed a main effect of latency ($F(24,768)=35.300$, $p<0.001$), but not genotype ($F(1,32)=0.010$, $p=0.921$), and a significant interaction of latency × genotype ($F(24,768)=1.600$, $p<0.05$). Significant genotype-difference was observed at latency of 0.4 and 0.8 sec (Bonferroni post-hoc test, $p<0.05$). Values are expressed as mean ± SEM (n=17 for each genotype). *$p<0.05$.

Figure 8 Performance on delay discounting task is normal in β2m$^{-/-}$TAP1$^{-/-}$ mice. A: Timeline illustrating when performance on delayed discounting task was analysed. **B:** Percent choice of the large reward when that option was delayed from 0 to 16 sec. All mice discounted the value of the large reward as the delay increased. Both genotypes showed a similar discounting of delayed rewards. Two way ANOVA revealed a main effect of delay ($F(4,72)=24.019$, $p<0.001$) but not genotype ($F(1,18)=0.542$, $p=0.471$), and no significant interaction of delay × genotype ($F(4,72)=0.502$, $p=0.734$). Values are expressed as mean ± SEM (n=11 for each genotype).

Figure 9 Cocaine-induced behavioral sensitization is enhanced in β2m$^{-/-}$TAP1$^{-/-}$ mice. After one day of saline injection to habituate to the procedure, mice were divided into 4 groups that received daily injections of saline or cocaine (20 mg/kg) for 7 days: Wild-type (WT) treated with saline (WT-Saline), WT treated with cocaine (WT-Cocaine), β2m$^{-/-}$TAP1$^{-/-}$ (DKO) treated with saline (DKO-Saline) and DKO cocaine (DKO-Cocaine). After 10 days without injection, all the mice were challenged to 20 mg/kg of cocaine. The locomotor activity was measured over 60 min after each injection. Mice of both genotypes showed increased locomotor

activity following cocaine, but not saline, injections. Moreover, DKO mice exhibited higher levels of hyperlocomotion than WT mice. Two-way ANOVA with repeated measures revealed a main effect of genotype ($F(1, 16)=9.649$, $p<0.01$) and day ($F(7, 112)=49.736$, $p<0.001$), but no significant interaction of day × genotype ($F(7, 112)=1.534$, $p =0.163$). Significant differences were observed between genotypes treated with chronic cocaine on days 4, 5, 6 and 7 (Bonferroni post-hoc test, $P<0.05$ or $p<0.001$). Following another 10 days without injections, mice that were given 7-day cocaine injections still exhibited larger locomotor activity in response to a single cocaine injection than mice that were given 7-day saline injections. Moreover, the magnitude of hyperlocomotion was larger in DKO mice than in WT mice. Two-way ANOVA revealed a main effect of genotype ($F(1, 32)=6.979$, $p<0.05$) and treatment ($F(1, 32)=58.124$, $p<0.001$), and no significant interaction of day × genotype ($F(1, 32)=0.540$, $p =0.468$). Significant differences were observed between genotypes treated with chronic cocaine (Bonferroni post-hoc test, $P<0.05$) and between treatments irrespective of genotypes ($P<0.001$). Values are expressed as mean ± SEM ($n=9$ for each genotype). *$p<0.05$, **$p<0.01$, ***$p<0.001$.

Figure 10 Acute methylphenidate dose-dependently produces hypoactivity and hyperactivity in β2m$^{-/-}$TAP1$^{-/-}$ mice. A: Time course of locomotor activity after methylphenidate exposure at various doses. Metylphenidate at the doses indicated was injected intraperitoneally to the mice and 10 min later their locomotor activities were measured in the home cage for 60 min. When vehicle was injected, DKO mice displayed a higher level of locomotor activity during the dark phase than WT mice. Two-way ANOVA revealed a significant main effect of genotype ($F(1,10)=4.904$, $p=0.05$) and time ($F(5,50)=7.239$, $p<0.001$) and no significant interaction of genotype x time ($F(5,50)=0.982$, $p=0.438$). As compared with basal (vehicle-induced) levels of locomotor activity, methylphenidate injected at a low dose (2.5 mg/kg) slightly increased locomotor activity in WT mice whereas decreased it in DKO mice. On the other hand, methylphenidate at higher doses increased locomotor activity in mice of both genotypes. However, there was no genotype-difference at any doses. **B:** Mean locomotor activity accumulated over the 60 min at the doses indicated. Two-way ANOVA revealed a significant main effect of dose ($F(4,52)=34.236$, $p<0.001$) but not genotype ($F(1,52)=3.168$, $p=0.081$), and no significant interaction of genotype x dose ($F(4,52)=0.675$, $p=0.613$). Significant dose-differences were observed at doses of 5, 10, and 20 mg/kg compared with vehicle injection (Bonferroni post hoc tests, $p<0.001$). **C:** Normalized locomotor activity at the doses indicated. Locomotor activities were normalized to the mean activity of each individual mouse with no methylphenidate (vehicle, dashed line). Compared with vehicle, a low dose of methylphenidate (2.5

mg/kg) reduced their locomotor activity after injection, whereas higher doses (5.0 mg/kg to 20.0 mg/kg) enhanced the locomotor activity. Two-way ANOVA revealed a main effect of genotype ($F(1,52)=45.090$, $p<0.001$) and dose ($F(4,52)=26.882$, $p<0.001$), and a significant interaction of genotype × dose ($F(4,52)=4.938$, $p=0.002$). Significant genotype differences were observed at higher doses of 5 mg/kg (Bonferroni post-hoc test, $p<0.01$), 10 mg/kg ($p<0.001$) and 20 mg/kg ($p<0.001$). A trend towards a significance was also seen at 2.5mg/kg ($p=0.097$). Values are expressed as mean ± SEM (n=6-7 for each genotype). *$p<0.05$, **$p<0.01$, ***$p<0.001$, #$p<0.10$.

Figure 11 Chronic methylphenidate reduces premature responses and response latency in β2m$^{-/-}$TAP1$^{-/-}$ mice. A: Mean percentage of premature responses accumulated over 7 days at each delay. Two way ANOVA revealed a main effect of delay ($F(2,26)=90.351$, $p<0.001$) but not genotype ($F(1,13)=0.001$, $p=0.971$), and no significant interaction of delay × genotype ($F(2,26)=0.884$, $p=0.425$). **B:** Distribution of cumulative response latency in wild-type (WT) mice. As in WT controls (WT-CON: n=17), response latency exhibited monomodal distribution in WT mice treated with chronic methylphenidate (WT-MPH: n=8). There was no difference in latency between both groups of mice. Two way ANOVA revealed a main effect of latency ($F(24,552)=27.648$, $p<0.001$) but no treatment ($F(1,23)=2.500$, $p=0.127$), and no significant interaction of latency × treatment ($F(24,552)=0.588$, $p=0.942$). **C:** Distribution of cumulative response latency in β2m$^{-/-}$TAP1$^{-/-}$ (DKO) mice. Response latency exhibited bimodal distribution in DKO controls (DKO-CON), while monomodal distribution in DKO mice treated with methylphenidate (DKO-MPH). Thus methylphenidate remarkably improved the capacity of DKO mice to sustain attention. Two way ANOVA revealed a main effect of latency ($F(24,528)=22.255$, $p<0.001$) but not treatment ($F(1,22)=0.271$, $p=0.608$), and a significant interaction of latency × treatment ($F(24,528)=3.067$, $p<0.001$). Significant treatment-differences were observed at latencies of 0.3, 0.4, 0.9, 1.1, and 1.3 sec (Bonferroni post-hoc test, $p<0.05$). Control data for each genotype were obtained from the first group of mice experienced cognitive tasks. Values are expressed as mean ± SEM. *$p<0.05$.

Fig.1

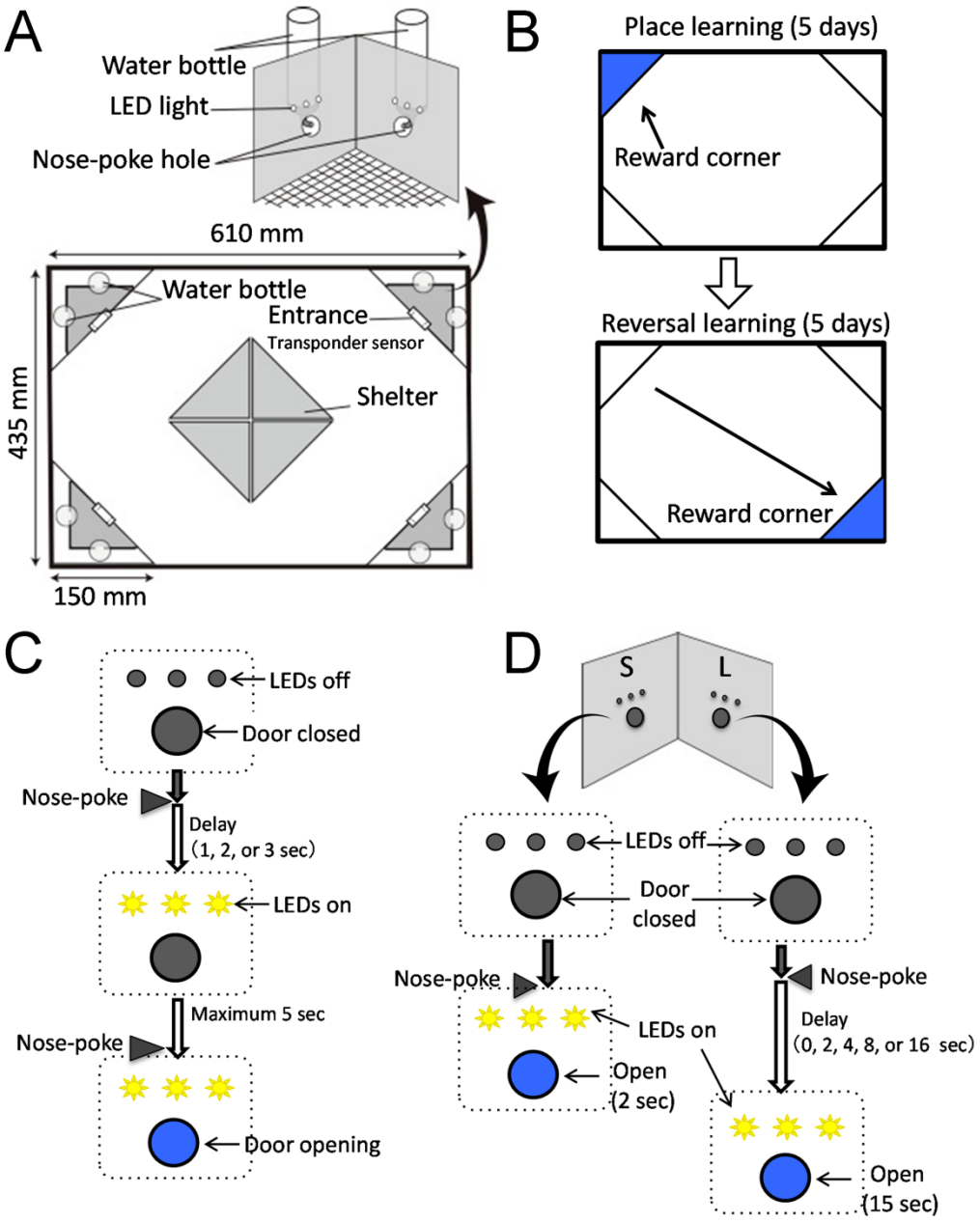

A
Water bottle
LED light
Nose-poke hole
610 mm
Water bottle
Entrance
Transponder sensor
Shelter
435 mm
150 mm
B
Place learning (5 days)
Reward corner
Reversal learning (5 days)
Reward corner
C
LEDs off
Door closed
Nose-poke
Delay
(1, 2, or 3 sec)
LEDs on
Maximum 5 sec
Nose-poke
Door opening
D
S
L
LEDs off
Door
closed
Nose-poke
LEDs on
Open
(2 sec)
Nose-poke
Delay
(0, 2, 4, 8, or 16 sec)
Open
(15 sec)

Fig.2

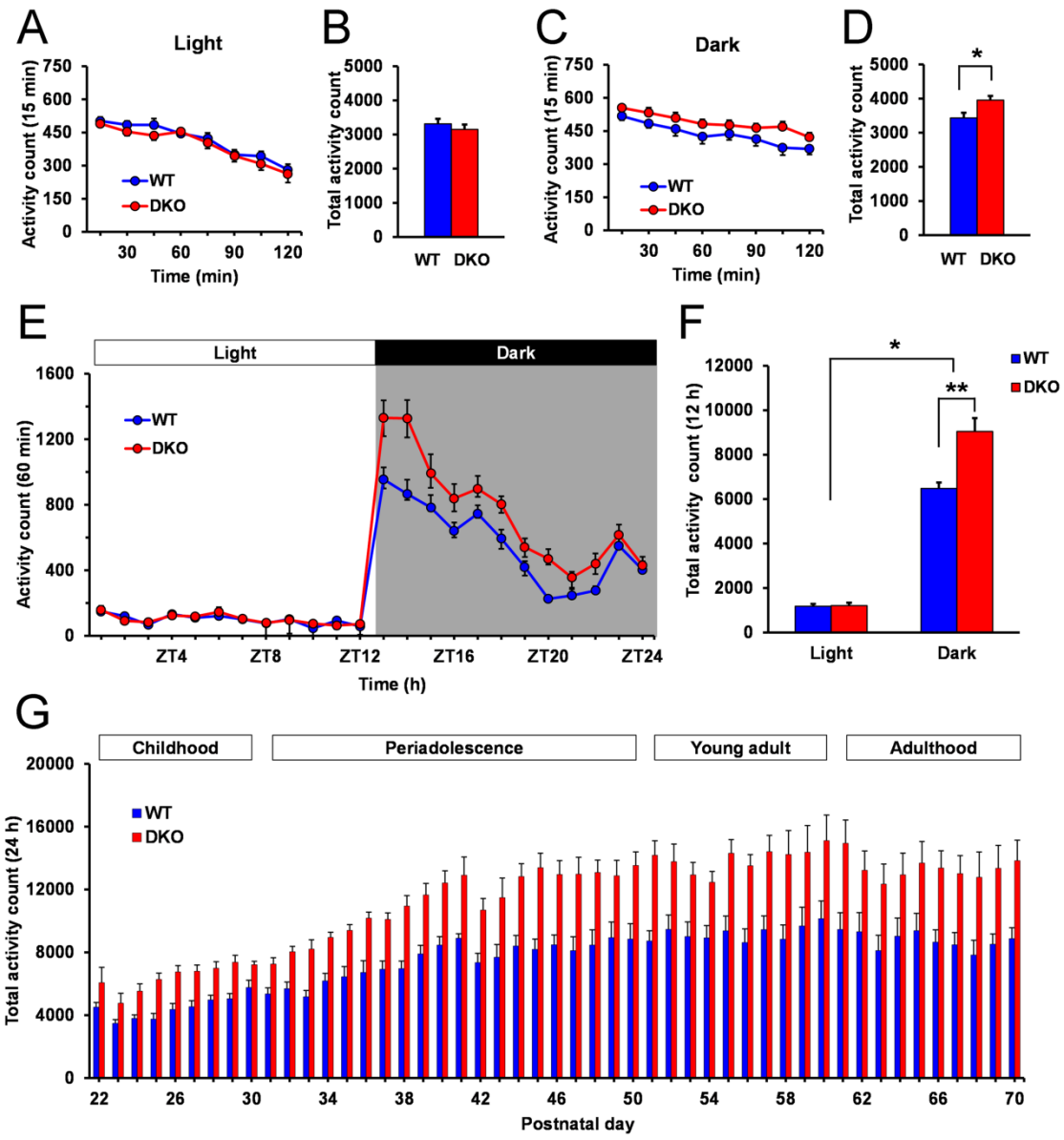

A
Light
Activity count (15 min)
750
600
450
300
150
0
WT
DKO
30
60
90
120
Time (min)
B
Total activity count
5000
4000
3000
2000
1000
0
WT
DKO
C
Dark
Activity count (15 min)
750
600
450
300
150
0
WT
DKO
30
60
90
120
Time (min)
D
*
Total activity count
5000
4000
3000
2000
1000
0
WT
DKO
E
Light
Dark
Activity count (60 min)
1600
1200
800
400
0
WT
DKO
ZT4
ZT8
ZT12
ZT16
ZT20
ZT24
Time (h)
F
*
**
WT
DKO
Total activity count (12 h)
12000
10000
8000
6000
4000
2000
0
Light
Dark
G
Childhood
Periadolescence
Young adult
Adulthood
Total activity count (24 h)
20000
16000
12000
8000
4000
0
WT
DKO
22
26
30
34
38
42
46
50
54
58
62
66
70
Postnatal day

Fig.3

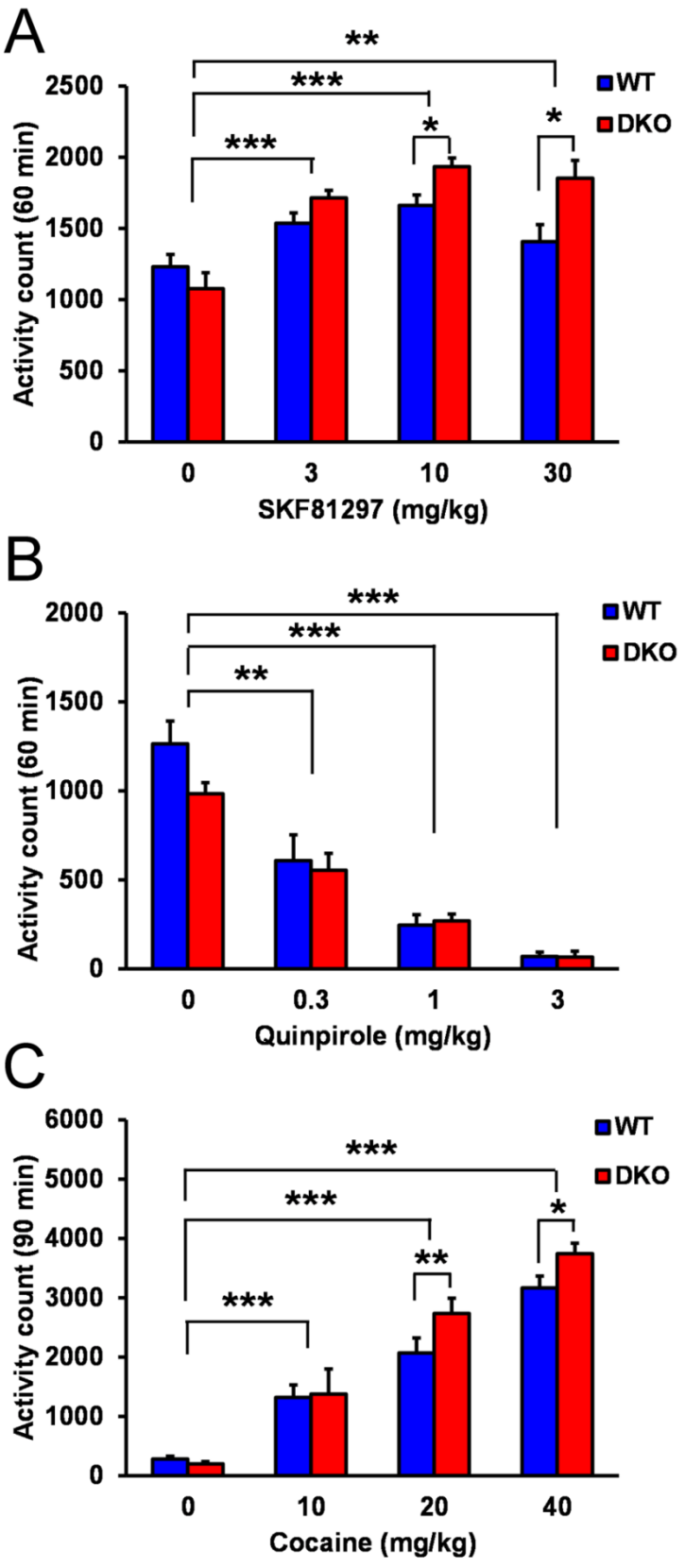

A
**
***
***
*
*
WT
DKO
Activity count (60 min)
2500
2000
1500
1000
500
0
0
3
10
30
SKF81297 (mg/kg)
B
***
***
**
WT
DKO
Activity count (60 min)
2000
1500
1000
500
0
0
0.3
1
3
Quinpirole (mg/kg)
C
***
***
**
*
***
WT
DKO
Activity count (90 min)
6000
5000
4000
3000
2000
1000
0
0
10
20
40
Cocaine (mg/kg)

Fig.4

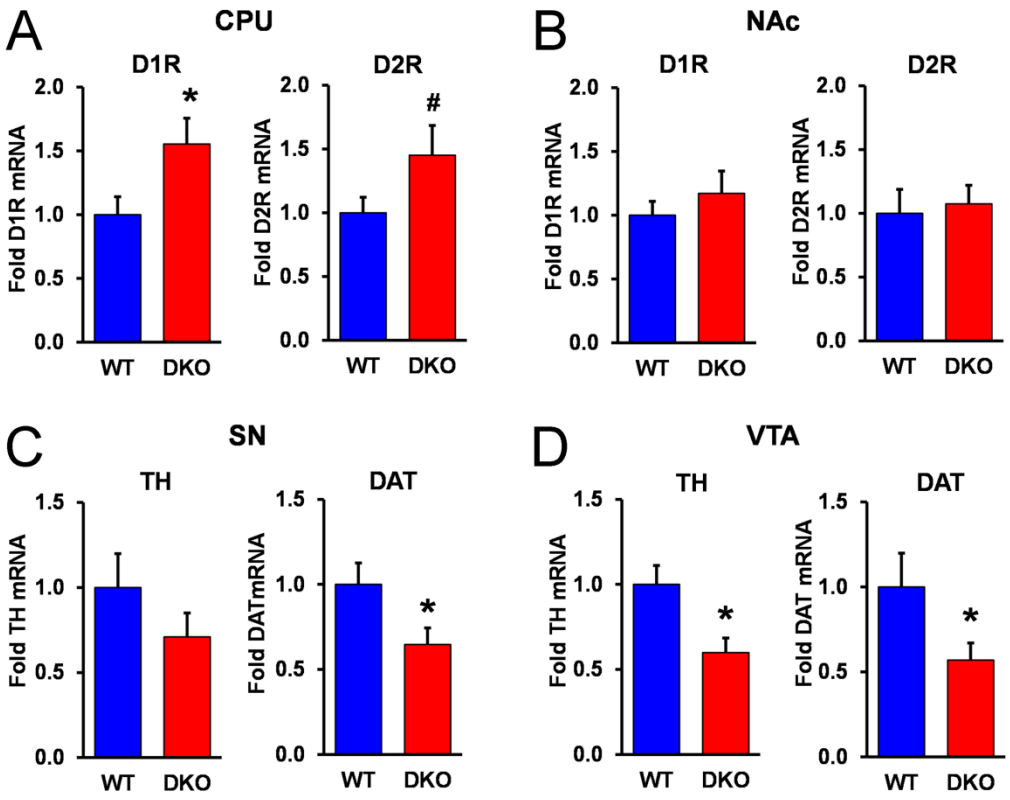

A
CPU
D1R
D2R
Fold D1R mRNA
Fold D2R mRNA
2.0
1.5
1.0
0.5
0.0
*
#
WT
DKO
B
NAc
C
SN
TH
DAT
Fold TH mRNA
Fold DATmRNA
D
VTA

Fig.5

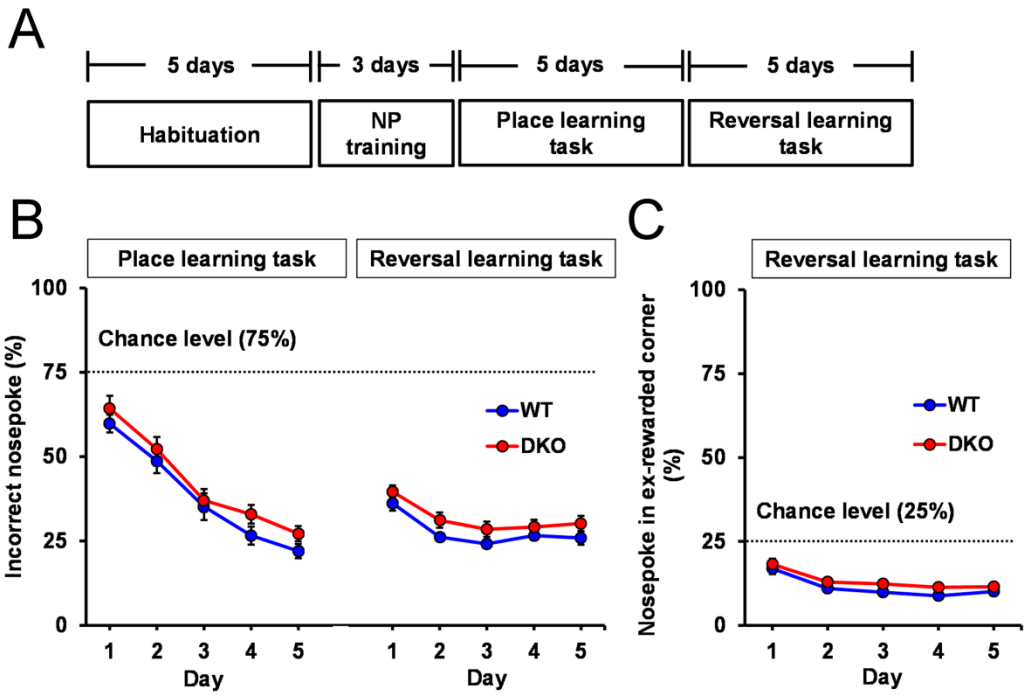

A
5 days
3 days
5 days
5 days
Habituation
NP training
Place learning task
Reversal learning task
B
Place learning task
Reversal learning task
Incorrect nosepoke (%)
100
75
50
25
0
Chance level (75%)
WT
DKO
1 2 3 4 5
Day
1 2 3 4 5
Day
C
Reversal learning task
Nosepoke in ex-rewarded corner (%)
100
75
50
25
0
WT
DKO
Chance level (25%)
1 2 3 4 5
Day

Fig.6

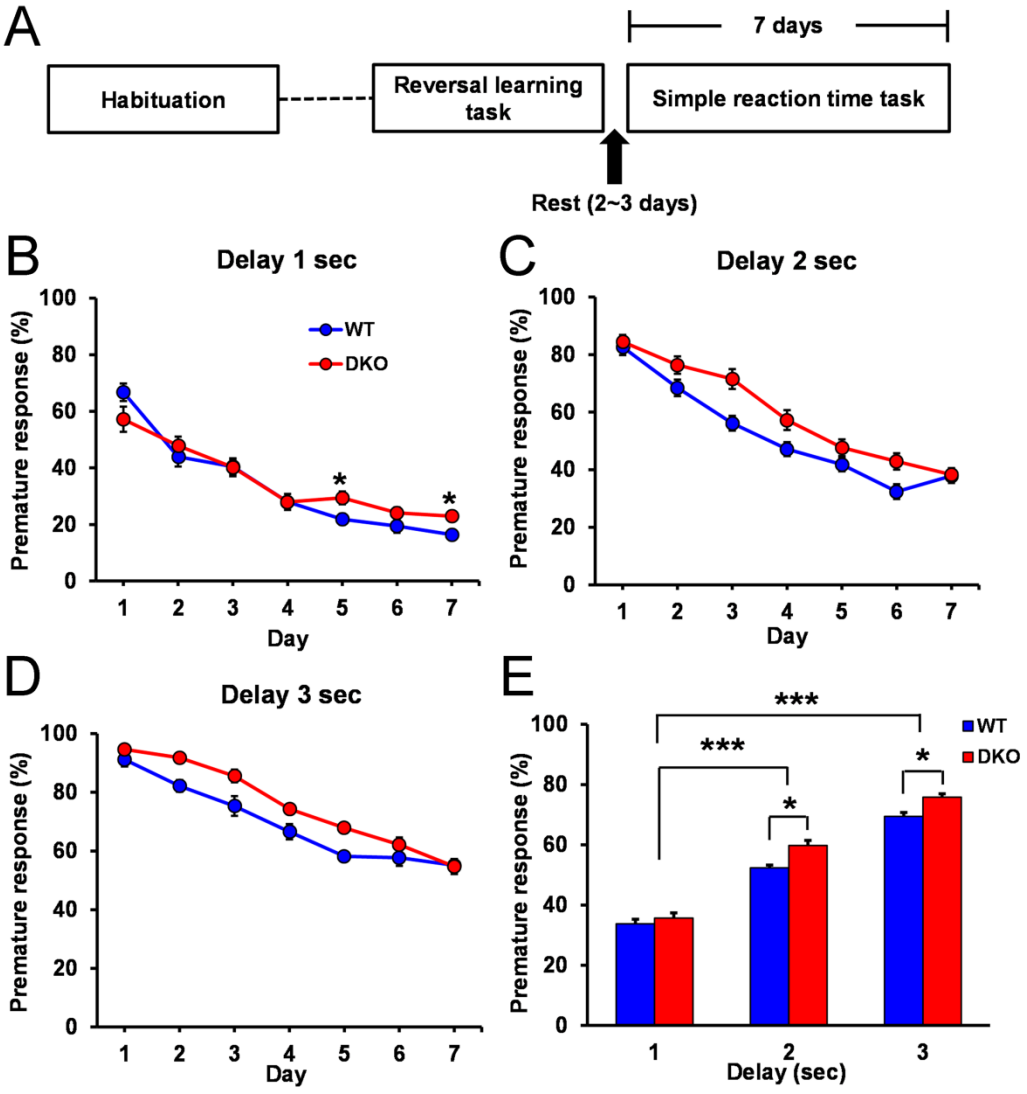

A
7 days
Habituation
Reversal learning task
Simple reaction time task
Rest (2~3 days)
B
Delay 1 sec
WT
DKO
Premature response (%)
Day
C
Delay 2 sec
Premature response (%)
Day
D
Delay 3 sec
Premature response (%)
Day
E
***
***
*
*
WT
DKO
Premature response (%)
Delay (sec)

Fig.7

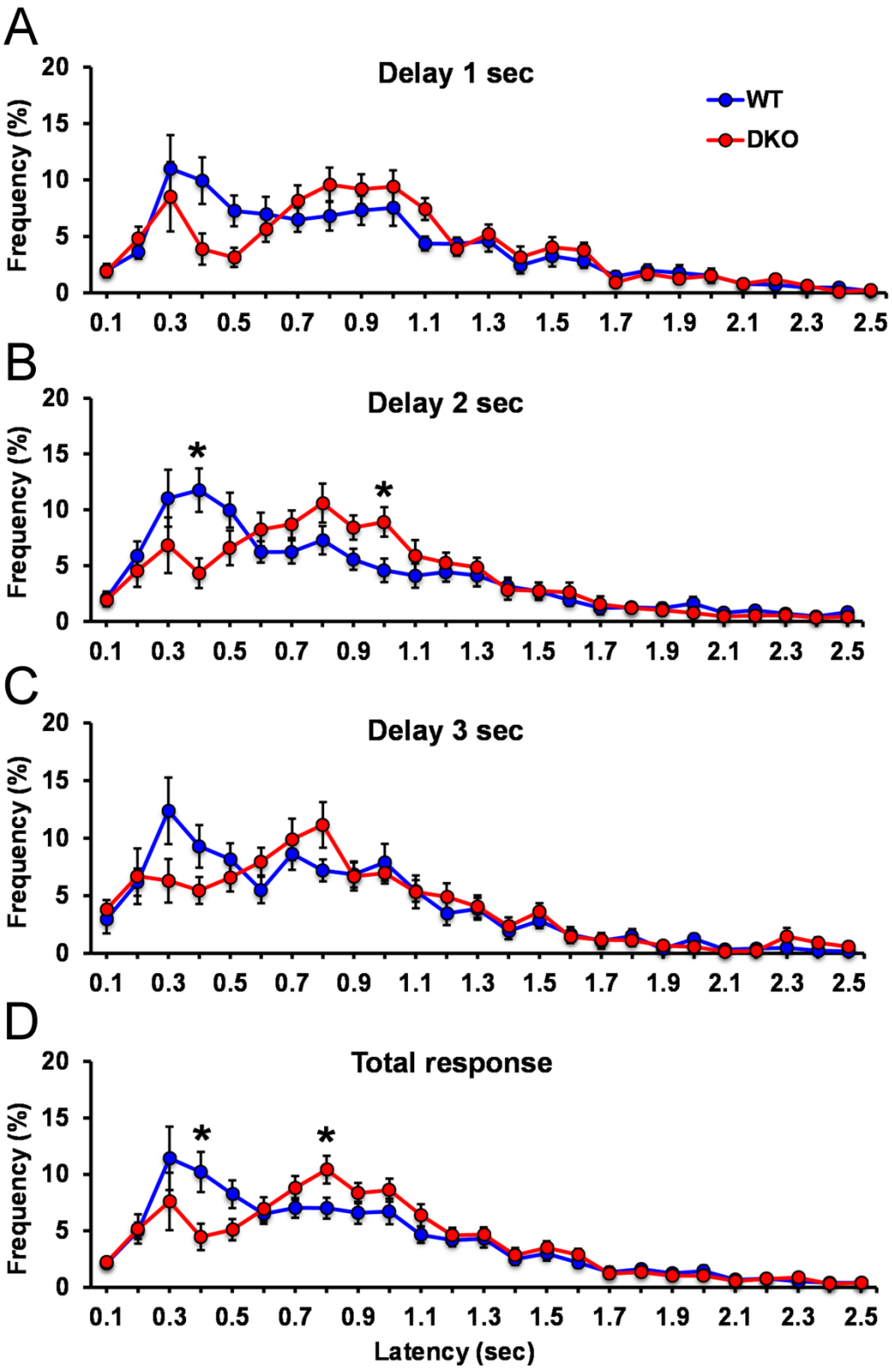

A
Delay 1 sec
WT
DKO
Frequency (%)
20
15
10
5
0
0.1 0.3 0.5 0.7 0.9 1.1 1.3 1.5 1.7 1.9 2.1 2.3 2.5
B
Delay 2 sec
*
*
C
Delay 3 sec
D
Total response
*
*
Latency (sec)

Fig.8

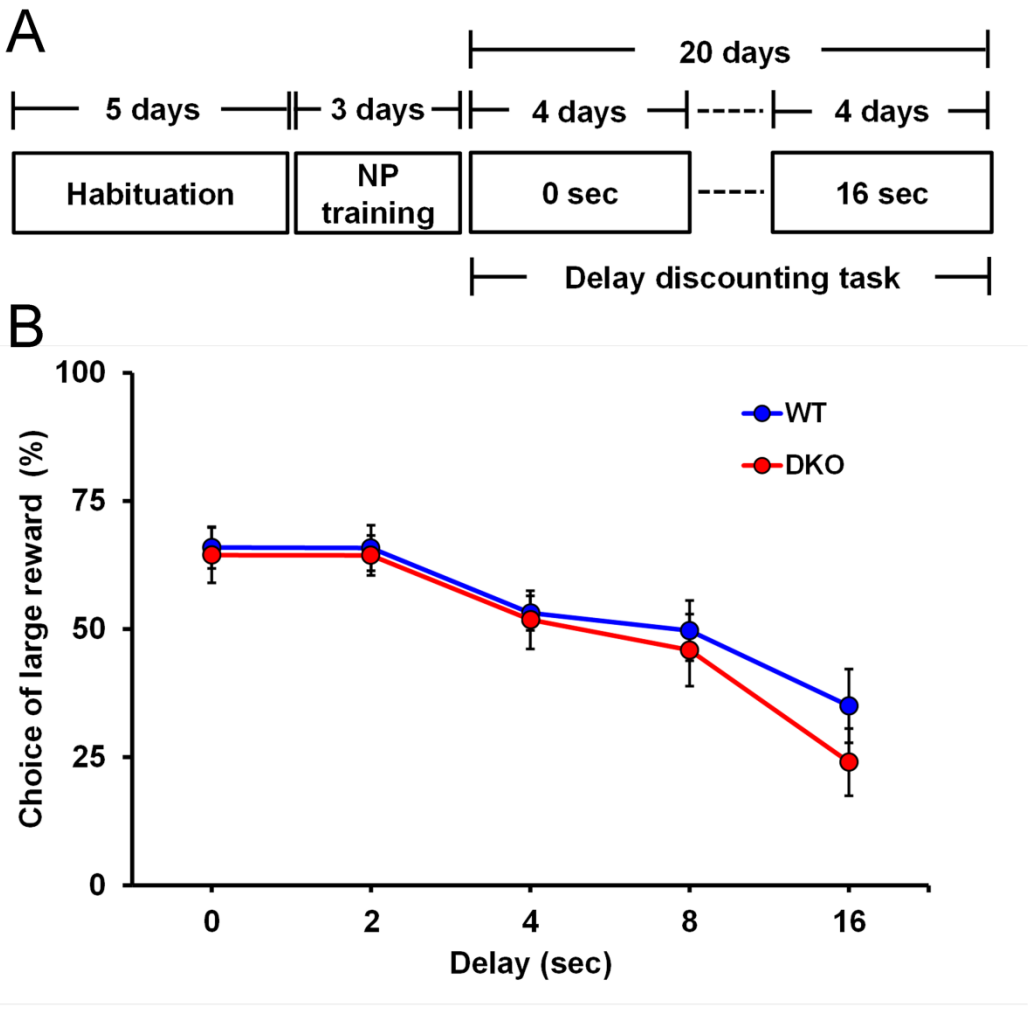

A
20 days
5 days
3 days
4 days
4 days
Habituation
NP training
0 sec
16 sec
Delay discounting task
B
100
75
50
25
0
Choice of large reward (%)
WT
DKO
0
2
4
8
16
Delay (sec)

Fig.9

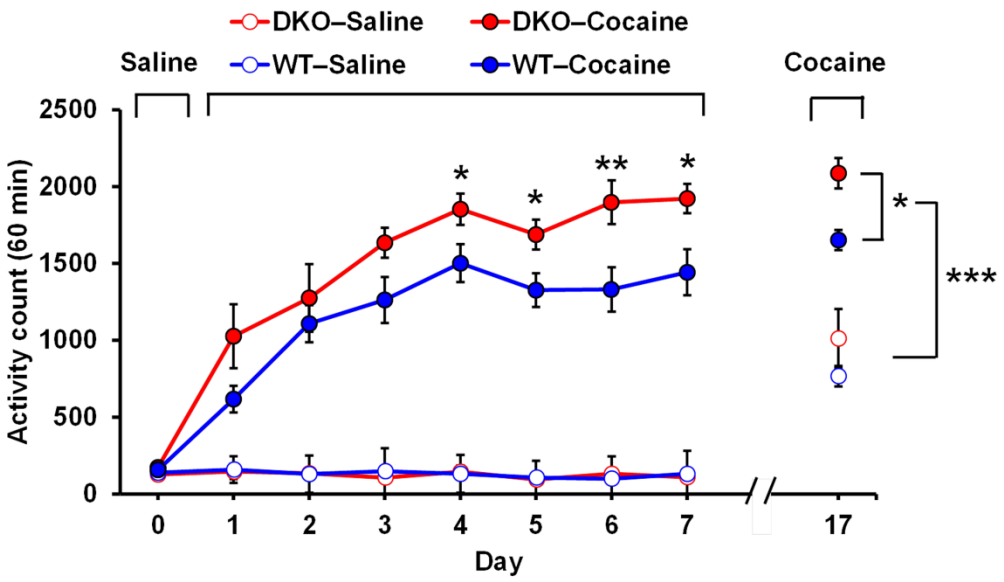

DKO–Saline
DKO–Cocaine
WT–Saline
WT–Cocaine
Saline
Cocaine
Activity count (60 min)
2500
2000
1500
1000
500
0
0
1
2
3
4
5
6
7
17
Day
*
*
**
*
*
***

Fig.10

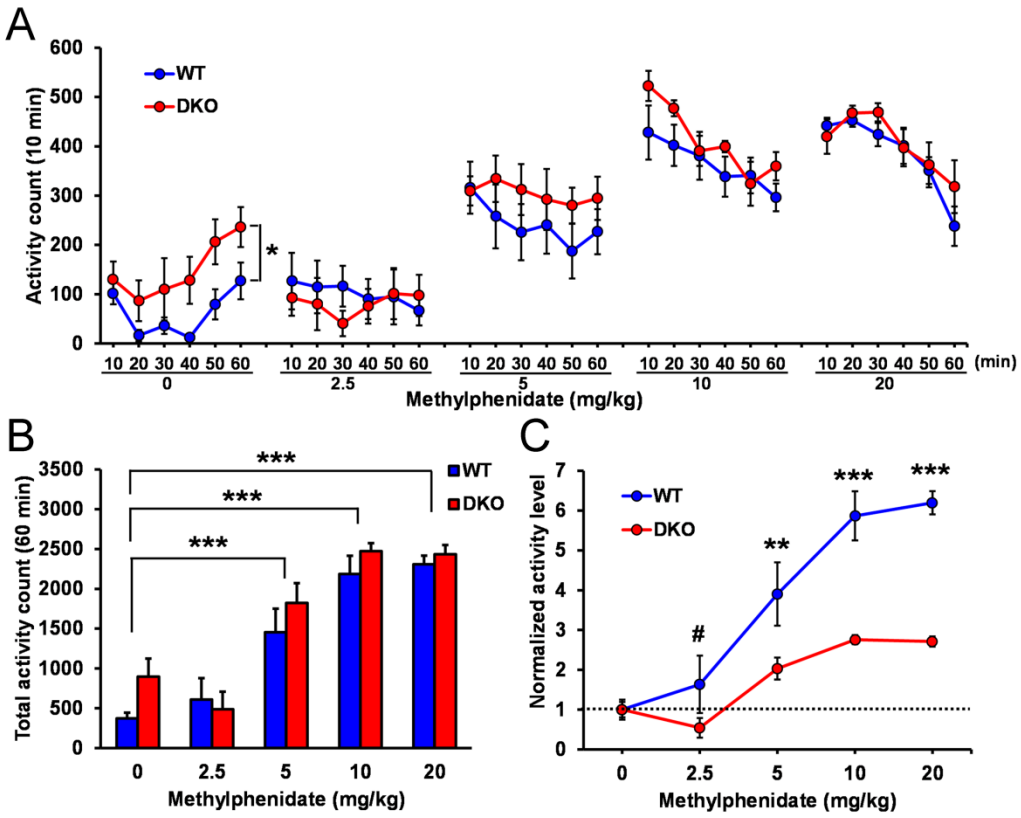
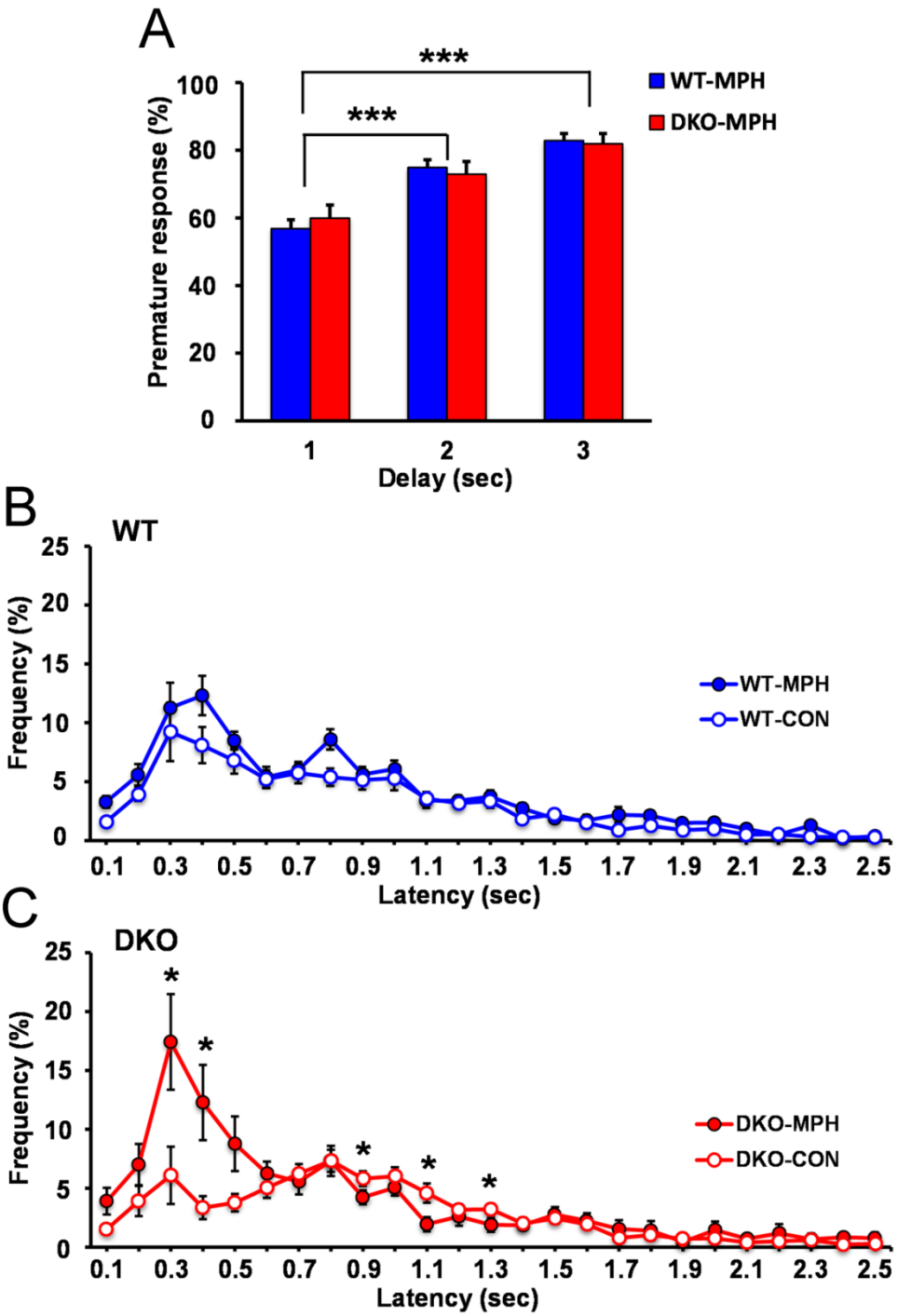

A
WT
DKO
Activity count (10 min)
600
500
400
300
200
100
0
*
10 20 30 40 50 60
0
2.5
5
10
20
(min)
Methylphenidate (mg/kg)
B
Total activity count (60 min)
3500
3000
2500
2000
1500
1000
500
0
***
***
***
WT
DKO
0
2.5
5
10
20
Methylphenidate (mg/kg)
C
Normalized activity level
7
6
5
4
3
2
1
0
WT
DKO
#
**
***
***
0
2.5
5
10
20
Methylphenidate (mg/kg)

Fig.11


A
***
***
WT-MPH
DKO-MPH
Premature response (%)
100
80
60
40
20
0
1
2
3
Delay (sec)
B
WT
Frequency (%)
25
20
15
10
5
0
WT-MPH
WT-CON
0.1 0.3 0.5 0.7 0.9 1.1 1.3 1.5 1.7 1.9 2.1 2.3 2.5
Latency (sec)
C
DKO
Frequency (%)
DKO-MPH
DKO-CON
Latency (sec)