

## Original Article

# Gamma aminobutyric acid transporter subtype 1 gene knockout mice: a new model for attention deficit/hyperactivity disorder

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**Attention deficit/hyperactivity disorder (ADHD) is characterized by hyperactivity, impaired sustained attention, impulsivity, and is usually accompanied by varying degrees of learning difficulties and lack of motor coordination. However, the pathophysiology and etiology of ADHD remain inconclusive so far. Our previous studies have demonstrated that the gamma aminobutyric acid transporter subtype 1 (GAT1) gene knockout (ko) mouse (*gat1*<sup>-/-</sup>) is hyperactive and exhibited impaired memory performance in the Morris water maze. In the current study, we found that the *gat1*<sup>-/-</sup> mice showed low levels of attentional focusing and increased impulsivity. In addition, the *gat1*<sup>-/-</sup> mice displayed ataxia characterized by defects in motor coordination and balance skills. The hyperactivity in the ko mice was reduced by both methylphenidate and amphetamine. Collectively, these results suggest that GAT1 ko mouse is a new animal model for ADHD studying and GAT1 may be a new target to treat ADHD.**

**Keywords** GAT1; gene knockout mice; ADHD; learning problems; motor coordination

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## Introduction

Attention deficit/hyperactivity disorder (ADHD) is a developmental disorder that affects 3–6% of school age children [1]. Diagnosis is made on the basis of persistent and developmentally inappropriate levels of distractibility, lack of sustaining attention and task persistence, excessive motor activity and impulsive responding [2]. More than 50% of the children diagnosed with ADHD will have social adjustment difficulty or psychiatric disorders as adolescents and young adults [3].

ADHD is a highly heterogeneous disorder resulting from complex gene-gene and gene-environment interactions [4]. ADHD has been linked to polymorphism of a variety of

genes encoding components of monoamine neurotransmission, including the D4 and D5 dopamine receptor (DRD4 and DRD5) [5,6], the dopamine transporter (DAT), serotonin transporter, and synaptosomal-associated protein 25 (SNAP-25) [7,8]. DA is thought to have a primary role in the etiology of ADHD, based on the efficacy of psychostimulants (e.g. amphetamine and methylphenidate) for ADHD and the behavioral profile of the DAT knock out/down mice and rats with neonatal DA lesions [9–12]. The coloboma mouse, with mutations in the gene encoding SNAP-25, also exhibits the behaviors similar to human ADHD [13]. Clinical studies have demonstrated that a proportion of ADHD patients carry a polymorphism in the SNAP gene [14].

Gamma aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the central nerve system. Since ADHD can be viewed as a disorder of ‘disinhibition’, it is reasonable to suspect that GABA neurotransmission is implicated. Considering the modulation of dopamine transmission by GABA and the central role of dopamine in the etiology of ADHD [15–17], it is conceivable that abnormal DA neurotransmission could be due to changes in GABA transmission. Fan *et al.* [18] have demonstrated that SNAP-25 inhibits GAT-1 reuptake in the presence of syntaxin 1A. A previous study by Comings suggested that microsatellite polymorphism of the GABRA3 and GABRB3 genes contributes to the variance of ADHD score [1]. Recently, Masuo *et al.* found the reduced expression of GABA transporter gene *gat3* in rats with neonatal 6-OHDA lesions and a lesser known ADHD model of the wiggling (Wig) rats [19,20]. Taken together, these findings suggested that functional hyperactivity of GABA neurons may contribute to ADHD.

Action of GABA in synaptic cleft is mainly terminated by reuptake *via* GATs, located in the plasma membrane of both neurons and glial cells [21,22]. Molecular cloning studies have identified multiple subtypes of GABA transporters, including GABA transporter subtype 1 (GAT1), GAT2, GAT3, and GAT4. GAT1 is the major subtype in the brain

[23,24]. More than 75% of GABA reuptake could be attributed to GAT1 in the central nervous system [25].

In previous studies, we showed that the *gat1*<sup>-/-</sup> mice are hyperactive and with impaired learning ability [26–28]. In the current study, other behavioral traits relevant to ADHD (e.g. hyperactivity, impaired sustained attention, impulsivity, motor coordination, and balance skills) were systematically examined in the *gat1*<sup>-/-</sup> mice.

## Materials and Methods

### Subjects

GAT1 knockout mice were generated as previously described [29]. Heterozygotes (*gat1*<sup>+/-</sup>) from chimeric mice were crossed with wild-type mice (C57BL/6J) for seven generations prior to inter-breeding to generate animal subjects for behavioral experiments. Mice were maintained in a specific-pathogen-free facility with a 12-h dark/light cycle (light on 07:00 AM) until 10–15 weeks of age before the experiments. Only male mice were used in behavioral testing. Mice were housed in the testing room for at least 1 week prior to the experiments. Animal behavior was observed in a separate room through a video-monitoring system. Observers were blind to the genotype and treatment. Naive mice were included in each experiment. The weight and date of birth were matched except the heterozygotes were 2 g heavier than the wild-type or the homozygotes. All animal experiments were approved by the Institutional Animal Care and Use Committee.

### Drugs

D-Amphetamine sulphate (Sigma-Aldrich, St Louis, USA) and methylphenidate hydrochloride (Suzhou Pharmaceutical Group, Suzhou, China) were dissolved in saline.

### Modified incentive runway task

The runway apparatus consisted of three compartments: a start box ( $15 \times 15 \times 18 \text{ cm}^3$ ), a goal box ( $15 \times 15 \times 18 \text{ cm}^3$ ), and an adjustable central runway ( $15\text{--}75 \times 15 \times 18 \text{ cm}^3$ ), separated by sliding doors. A video camera was used to track the trajectory of the mice. A dish in the goal box contained a piece of sweet breakfast cereal. Mice were habituated to the apparatus for three consecutive days (10 min/trial, three trials/day) before the start of training.

Mice were deprived of food for 20 h prior to each session. The training period lasted for 11 sessions on alternating days (22 days training period). Each session consisted of three trials. On the first three training sessions, mice were placed directly in the goal box and allowed to eat the reward for 3 min. On training Session 4, the runway was set at 15 cm away from the goal box. Mice were placed in the start box for 30 s with the door closed; then the door was opened, and the mouse was allowed to proceed into the runway. Mice

were gently pushed toward the goal box if not leaving the start box within 3 min. The length of the runway was increased to 30 cm in Session 5, 45 cm in Session 6, 60 cm in Session 7, and 75 cm in Sessions 8–14.

The training was followed by three testing sessions (12, 13, and 14), each consisting of one trial only. In the 13th and 14th sessions, a pink ping pong ball was placed in the middle of the runway.

Incentive runway behavior was videotaped for later analysis. Entry into the goal box was defined as all four limbs crossing the line between the runway and the goal box. Numbers of entry into, and time spent in the goal box was monitored. Numbers of forelimb or head contact with the ping pong ball were also recorded.

### Incentive passive avoidance

The passive avoidance box consisted of a small white illuminated compartment ( $16 \times 15 \times 23 \text{ cm}^3$ ) and a large black dark compartment ( $29 \times 19 \times 23 \text{ cm}^3$ ), separated by a trap door. Food was supplied in the dark compartment as a reward throughout the experiment. Mice were allowed to habituate in the testing environment for 2 days (5 min per day) before the testing. Mice were deprived of food for 12 h prior to each session. On the testing day, subjects were placed in the illuminated section and allowed to move freely in the chamber. The time spent prior to moving into the dark compartment (step-through latency) was measured. When the mouse had completely entered the dark compartment, the trap door was shut and a mild foot shock ( $30 \text{ V} \times 10 \text{ s}$ , 50 Hz) was delivered. The trap door was opened at the end of the shock period to allow return to the illuminated compartment. Mice were returned to the home cage for 2 min prior to a repeated session.

### Rotating rod task

During training period, each mouse received five daily training sessions with a constant rotation speed (8 rpm), each consisting of three trials (3 min/trial, if the mouse can stay on the rotarod more than 3 min, then 3 min were recorded), followed by two daily testing sessions, each consisting of only one trial, which lasted for 10 min. The rotation speed was gradually increased from 0 to 30 rpm at the trial completion. Time staying on the rod was recorded.

### Parallel bars test

Two parallel yellow plastified iron rods (length: 60 cm; diameter: 1 cm; distance between the rods: 3 cm) were diagonally placed on the top of an open black plexiglass box ( $50 \times 50 \times 50 \text{ cm}^3$ ). The home cage was placed underneath the end of the rods. Each mouse received five trials. The average time needed to traverse the rods was recorded. The average number of times a mouse dropped a hind paw below the rod level was registered as ‘number of slips’. For both

measures, performance across the five trials was calculated for each subject.

### Locomotor activity

The open field was an arena ( $40 \times 42 \times 30 \text{ cm}^3$ ) with clear plexiglass walls and floor, brightly illuminated by overhead fluorescent lighting. Motor activity was videotaped using a camera fixed above the floor and analyzed with a video-tracking system. Mice were habituated for 30 min prior to each session. Mice (initially 10–15 weeks of age) were used repeatedly to determine responses to stimulants: each mouse received a vehicle session, followed by testing for amphetamine or methylphenidate, at increasing doses. Doses of amphetamine and methylphenidate were 0, 2, 8 and 0, 0.2, 1, 8 mg/kg, respectively. Sessions were separated by 1 week. Activity measures commenced immediately after the mice were put into the open field and lasted for 90 min (30 min prior to treatment; 60 min after treatment). In amphetamine treatment experiment, the traveled distance data were collected from 10 to 50 min after amphetamine treatment. In methylphenidate treatment experiment, traveled distance data were collected from 20 to 50 min after methylphenidate treatment.

### Statistical analysis

Data of repeated measures (e.g. runway test, rotarod task, and open-field) were analyzed using a two-way analysis of variance [ANOVA; genotype  $\times$  time (sessions)] followed by Bonferroni correction. The remaining data were analyzed using a one-way ANOVA followed by the Tukey's test.  $P < 0.05$  denoted the presence of a statistically significant difference. Wild-type, heterozygous, and homozygous GAT1-deficient mice are designated as +/+, +/-, and -/-. Data are presented as mean  $\pm$  SE.

## Results

### Runway task

Latency to enter the runway was not different across the three genotypes [Fig. 1(A)]. But the run time (time from leaving the start box to entering the goal box) was longer in the *gat1*<sup>-/-</sup> mice than in the controls ( $P < 0.001$ ) [Fig. 1(B)]. The distance traveled was also longer in the *gat1*<sup>-/-</sup> mice than in controls when the runway was 75-cm long (session 8~12) ( $P < 0.05$ ) [Fig. 1(C)].

In comparison to the wild-type controls, the *gat1*<sup>-/-</sup> mice had higher number of entry into the goal box ( $P < 0.001$ ) [Fig. 1(D)], longer total distance traveled ( $P < 0.001$ ) [Fig. 1(E)], and shorter total time in the goal box ( $P < 0.001$ ) [Fig. 1(F)]. *gat1*<sup>+/-</sup> mice exhibited an intermediate profile.

With the introduction of the ping pong ball in Session 13, the wild-type controls made more entries into the goal box

[Fig. 2(B)], traveled longer distance in the runway [Fig. 2(D)], and stayed for shorter period of time in the goal box than that in Session 12 [Fig. 2(C)], but the differences did not reach statistical significance. These changes became less apparent in the next session, Session 14. However, all of index in the *gat1*<sup>-/-</sup> mice were relatively stable through Sessions 12–14 (Fig. 2), except that the *gat1*<sup>-/-</sup> mice showed higher frequency of touching the ball than that of wild type in Sessions 13 and 14 [Fig. 2(A)].

### Incentive passive avoidance task

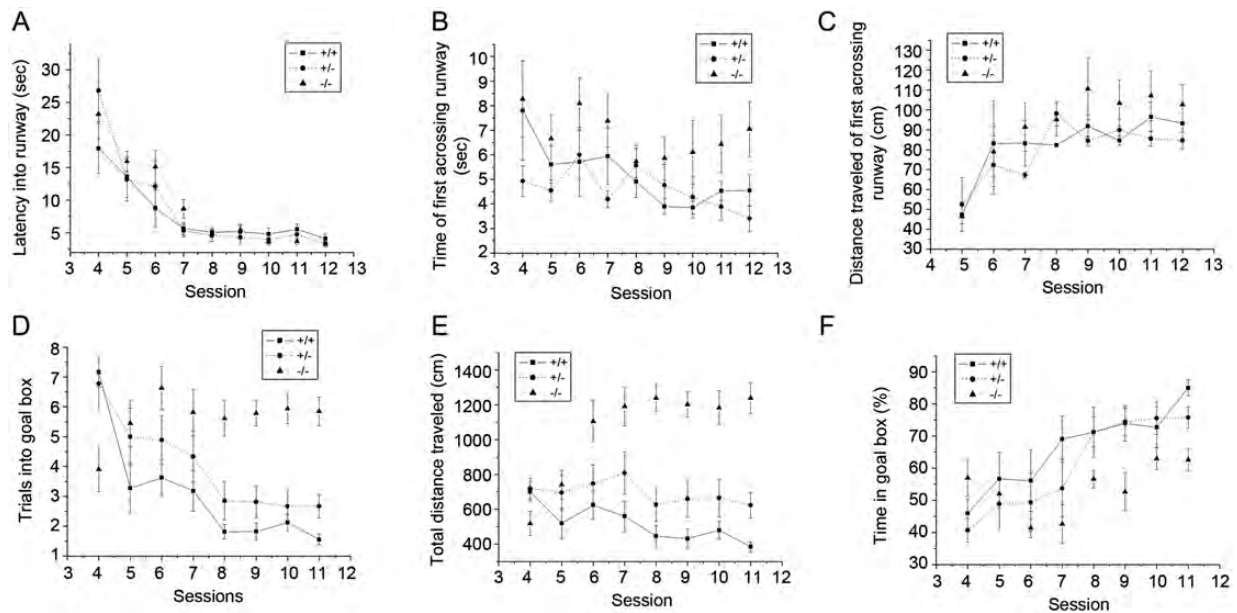
Before the foot-shock, the latency to enter the dark compartment was longer in the *gat1*<sup>-/-</sup> mice than in the wild-type controls (-/- vs. +/+;  $20.4 \pm 4.6$  vs.  $6.1 \pm 2.1$  s;  $P < 0.05$ ). Exposure to foot-shock increased the latency in later sessions in all three genotypes (+/+; from  $6.1 \pm 2.1$  to  $192 \pm 37$  s,  $P < 0.001$ ; +/-; from  $12.8 \pm 5.5$  to  $249 \pm 27$  s;  $P < 0.001$ ; -/-; from  $20.4 \pm 4.6$  to  $73 \pm 24$  s;  $P < 0.05$ ). The latency after foot-shock exposure was significantly shorter in the *gat1*<sup>-/-</sup> mice than in the wild-type mice (-/- vs. +/+;  $73 \pm 24$  vs.  $192 \pm 37$  s;  $P < 0.01$ ) (Fig. 3).

### Motor coordination

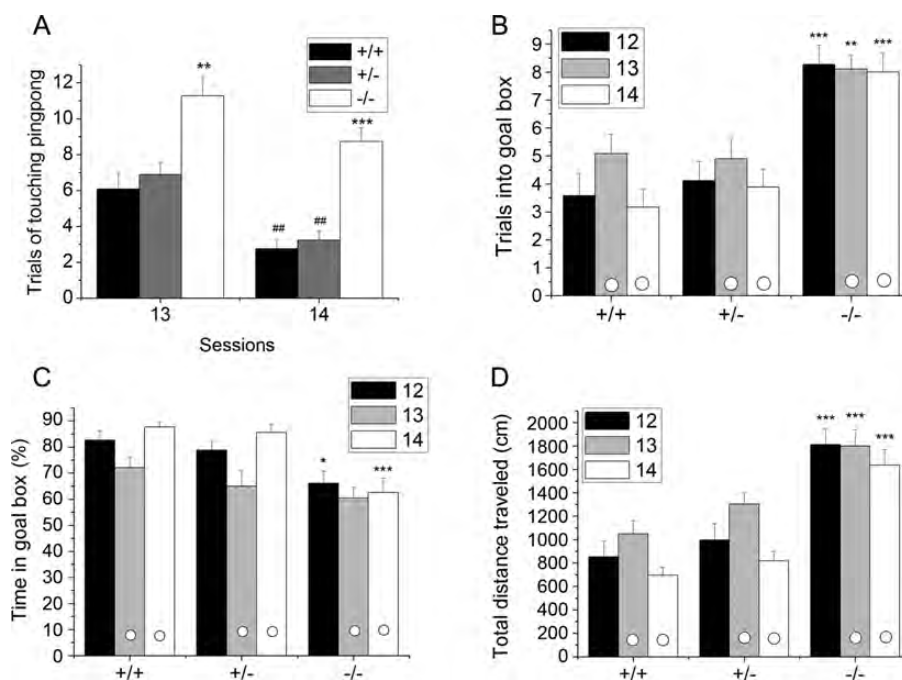
In comparison to the wild-type controls, latency to fall-off in the rotating rod task was significantly shorter in the *gat1*<sup>-/-</sup> mice in both the learning ( $P < 0.001$ ) and testing phases ( $P < 0.001$ ) [Fig. 4(A)]. The mutant mice also displayed impaired performance in parallel bars test [Fig. 4(B,C)]. The time needed to traverse the plastified parallel bars was significantly longer in the *gat1*<sup>-/-</sup> mice (-/- vs. +/+;  $15 \pm 1.2$  s vs.  $5.7 \pm 0.8$  s;  $P < 0.001$ ) [Fig. 4(B)] than in the wild-type mice. The *gat1*<sup>-/-</sup> mice also made more slips (-/- vs. +/+;  $3.5 \pm 0.5$  vs.  $0 \pm 0$ ;  $P < 0.001$ ) [Fig. 4(C)]. In the glossy parallel bars test, all wild-type mice ( $n = 8$ ) but none of the *gat1*<sup>-/-</sup> mice ( $n = 11$ ) succeeded in crossing the bars.

### Motor activity

The *gat1*<sup>-/-</sup> mice displayed more motor activity during the initial open-field exposure (motor activity of the first 10 min were compared) in the first week ( $P < 0.001$ ) and the re-exposure to the open-field in the second ( $P < 0.001$ ) or third weeks ( $P < 0.001$ ) [Fig. 5(A)]. The *gat1*<sup>-/-</sup> mice were also more active after receiving saline ( $P < 0.01$ , the motor activity during the 10th–50th minute after injection were compared) [Fig. 5(B)]. Amphetamine sulfate (2 mg/kg) significantly reduced motor activity (motor activity during the 10th–50th minute after injection were compared) in both the *gat1*<sup>-/-</sup> and wild-type mice [Fig. 5(B)], while at a higher dose of 8 mg/kg, amphetamine markedly increased the locomotor activity in both groups [Fig. 5(B)]. As same as amphetamine, methylphenidate at lower concentration (0.2 and 1 mg/kg) reduced the locomotor activity in

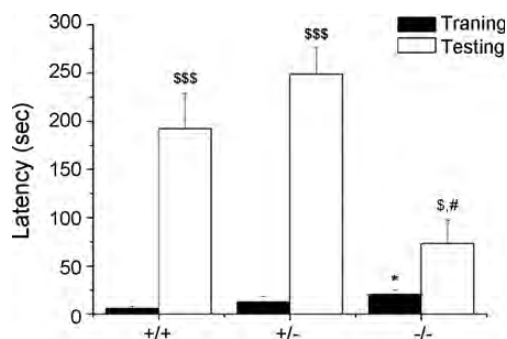


**Figure 1** *gat1*<sup>-/-</sup> mice showed poorer attentional focusing in incentive runway test ( $N_{+/+} = 12$ ,  $N_{+/-} = 9$ ,  $N_{-/-} = 12$ ) in training period. Latency to enter the runway was not different across the three genotypes (A). The *gat1*<sup>-/-</sup> mice spent more time (B) [ $P < 0.001$ ,  $-/-$  vs.  $+/+$ ;  $P < 0.001$ ,  $-/-$  vs.  $+/-$ ; two-way ANOVA followed by Bonferroni post-tests (genotype  $\times$  session)] and traveled more distance (C) in the runway when mice initially crossed the runway compared with wild-type controls. The *gat1*<sup>-/-</sup> mice made more traveled distance at their first crossing through the runway (75 cm) in session 8 to 12 [ $P < 0.05$ ,  $-/-$  vs.  $+/+$ ; two-way ANOVA followed by Bonferroni post-tests (genotype  $\times$  session)]. The *gat1*<sup>-/-</sup> mice made more entries into the goal box (D) [ $P < 0.001$ ,  $-/-$  vs.  $+/+$ ;  $P < 0.05$ ,  $+/-$  vs.  $+/+$ ; two-way ANOVA followed by Bonferroni post-tests (genotype  $\times$  session)], traveled more distance (E) [ $P < 0.001$ ,  $-/-$  vs.  $+/+$ ;  $P < 0.001$ ,  $+/-$  vs.  $+/+$ ; two-way ANOVA followed by Bonferroni post-tests (genotype  $\times$  session)] and spent less time in the goal box (F) [ $P < 0.001$ ,  $-/-$  vs.  $+/+$ ; two-way ANOVA followed by Bonferroni post-tests (genotype  $\times$  session)].

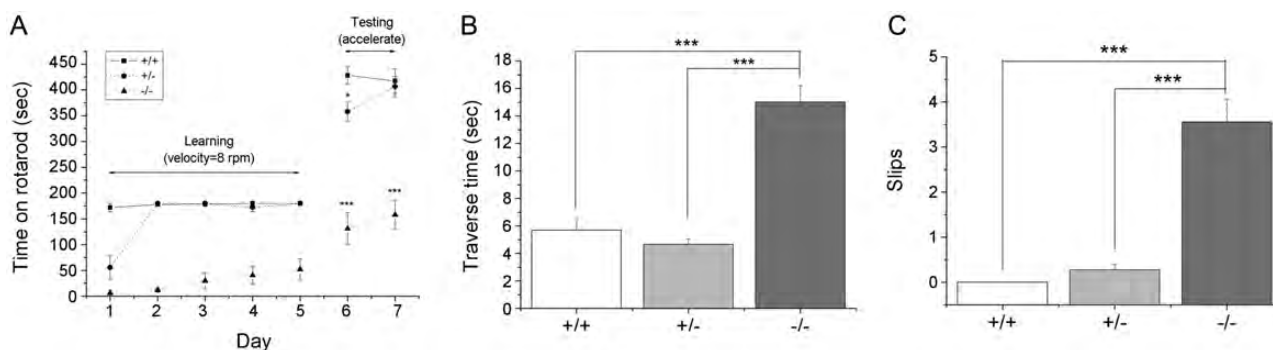


**Figure 2** *gat1*<sup>-/-</sup> mice showed poorer attentional focusing in incentive runway test in testing period ( $N_{+/+} = 12$ ,  $N_{+/-} = 9$ ,  $N_{-/-} = 12$ ) (A) The frequency of touching pingpong is higher in the *gat1*<sup>-/-</sup> mice than in the *gat1*<sup>+/+</sup> and *gat1*<sup>+/-</sup> mice in Sessions 13 and 14. The *gat1*<sup>-/-</sup> mice make more entries into the goal box (B), spent less time in the goal box (C) and made longer traveled distance (D) in Sessions 13 and 14 compared with the *gat1*<sup>+/-</sup> mice. \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$  *gat1*<sup>-/-</sup> vs. *gat1*<sup>+/+</sup> mice on the same session (one-way ANOVA), ## $P < 0.01$  *gat1*<sup>-/-</sup>: Session 14 vs. Session 13; *gat1*<sup>+/-</sup>: Session 14 vs. Session 13 (one-way ANOVA).

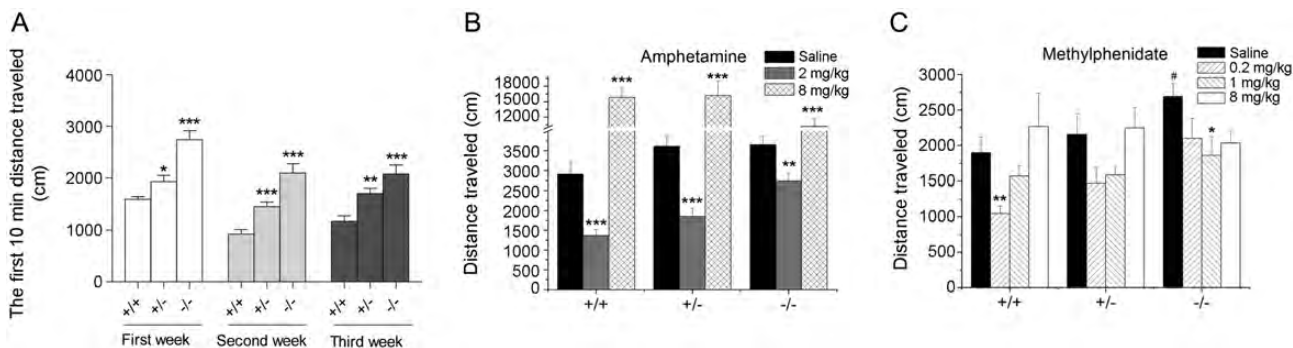




**Figure 3** *gat1*<sup>-/-</sup> mice showed altered impulsivity in incentive passive avoidance task ( $N_{+/+} = 11$ ,  $N_{+/-} = 11$ ,  $N_{-/-} = 13$ ) Before foot-shock, *gat1*<sup>-/-</sup> mice needed longer time into the dark compartment than the wild-type mice. Two minutes after the foot-shock, all the genotypes showed a prolonged time into the dark section; however, the *gat1*<sup>-/-</sup> mice needed less time than the wild-type mice. \* $P < 0.05$ , vs. wild-type mice in training; # $P < 0.05$  vs. wild-type mice in testing;  $^{\$}P < 0.05$ ,  $^{$$$}P < 0.001$ , the same genotype mice in testing vs. mice in training (one-way ANOVA).



**Figure 4** *gat1*<sup>-/-</sup> mice showed motor coordination defects on rotating rod test and balance defects on parallel bars test (A) The latency to fall was quantified by the rotarod test on the uniform velocity rod (8 rpm) (learning periods) [ $P < 0.001$ ,  $-/-$  vs.  $+/+$ , two-way ANOVA followed by Bonferroni post-tests (genotype  $\times$  session)], as measured during three trials, and on the accelerating rod (testing periods) ( $P < 0.001$ ,  $-/-$  vs.  $+/+$ , in Days 6 and 7, one-way ANOVA with Tukey's post-tests), as measured during one trial ( $N_{+/+} = 9$ ,  $N_{+/-} = 8$ ,  $N_{-/-} = 11$ ). (B) Mean time that mice need to travel through the 60 cm parallel bars. (C) The mean number of slips that mice show while traversing parallel bars ( $N_{+/+} = 9$ ,  $N_{+/-} = 8$ ,  $N_{-/-} = 11$ ). \* $P < 0.05$ , \*\*\* $P < 0.001$  (one-way ANOVA).



**Figure 5** *gat1*<sup>-/-</sup> mice showed hyperactivity and effects of amphetamine ( $N_{+/+} = 9$ ,  $N_{+/-} = 9$ ,  $N_{-/-} = 9$ ), methylphenidate ( $N_{+/+} = 8$ ,  $N_{+/-} = 9$ ,  $N_{-/-} = 9$ ) on the three genotype mice (A) The *gat1*<sup>-/-</sup> mice showed more distance traveled than the *gat1*<sup>+/+</sup> mice in novel environments and the re-exposure to the open-field in the second and the third weeks [\* $P < 0.05$ , \*\* $P < 0.01$  and \*\*\* $P < 0.001$  vs. wild-type mice (one-way ANOVA)]. (B) Lower concentration of amphetamine reduces the distance traveled of the mice, and the higher concentration treatment increases the mice's distance traveled. (C) Lower concentration of methylphenidate reduces the distance traveled of the mice. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$  vs. the same phenotype treated with saline (one-way ANOVA). # $P < 0.05$  vs. wild-type mice treated with the same drug (one-way ANOVA).

both genotypes. However, at a higher dose of 8 mg/kg, methylphenidate increased the locomotor activity in the wild-type mice but reduced the locomotor activity in the *gat1*<sup>-/-</sup> mice [Fig. 5(C)].

## Discussion

In this paper, we showed that the *gat1*<sup>-/-</sup> mice have phenotypes of hyperactivity, impaired sustained attention, impulsivity, lack of motor coordination. These characters together with the learning deficiency of the mice which we reported before indicated that the *gat1*<sup>-/-</sup> mouse could be used as a model of ADHD.

A deficit in habituation often leads to hyperactivity, although hyperactivity is not necessarily caused by a habituation deficit [10,30]. We found that the *gat1*<sup>-/-</sup> mice showed higher locomotor activity than the wild-type mice when they were re-exposed to the same environment three

times (the interval is 1 week) [Fig. 5(A)], suggesting that hyperactivity in *gat1*<sup>-/-</sup> mice is not due to decreased habituation to novel environment.

Consistent with the therapeutic effects of stimulants for ADHD, the hyperactivity in the *gat1*<sup>-/-</sup> mice was ameliorated by both amphetamine and methylphenidate. Amphetamine acts at the presynaptic terminal to promote catecholamine release [31]. And methylphenidate can significantly increase extracellular dopamine concentration by enhancing the release of dopamine and blocking the DAT [32]. At low doses, both amphetamine and methylphenidate decreased locomotor activity in both *gat1*<sup>-/-</sup> and control mice. At a high dose, both drugs increased motor activity in the controls. However, in *gat1*<sup>-/-</sup> mice, amphetamine increased motor activity but methylphenidate not. It may be because of the 8 mg/kg concentration is still not high enough to cause hyperactivity for the *gat1*<sup>-/-</sup> mice. The different behavioral responses to the indirect acting dopamine agonists amphetamine and methylphenidate in the *gat1*<sup>-/-</sup> mice likely reflect an abnormal synaptic signal transmission resulting from the GAT1 gene absence. There is convincing evidence to suggest that the activity of dopamine neurons is decreased in ADHD [33]. Dopamine receptors are found in GABA neurons and modulate the GABAergic transmission [34]. The firing pattern of dopaminergic neurons can be also effectively modulated by GABAergic inputs [35]. It was reported that GAT1 deficiency leads to enhanced extracellular GABA levels resulting in an over-activation of GABA<sub>A</sub> receptors responsible for a post-synaptic tonic conductance [25], and it was also found that the GAT1-deficient mice lacked a pre-synaptic GABA<sub>B</sub> receptor tone [25]. We speculate that over-activation of postsynaptic GABA<sub>A</sub> receptors inhibits dopaminergic neurons in the *gat1*<sup>-/-</sup> mice, and which finally leads to higher motor activity in the *gat1*<sup>-/-</sup> mice than in the wild-type controls. The motor inhibiting effects of amphetamine and methylphenidate at low doses in the *gat1*<sup>-/-</sup> mice are consistent with the response of ADHD patients to stimulants. But the magnitude of the stimulant effects was smaller in the *gat1*<sup>-/-</sup> mice than in the wild-type mice, indicating the activity of dopaminergic system may be decreased following by the hyper-GABAergic system in *gat1*<sup>-/-</sup> mice.

The runway task is a traditional means to investigate motivational impact of incentive stimulus. Some simply changes in the runway produce shifts in the animal's motivational strength to seek the incentive, and therefore it may be an appropriate tool for investigating the distractibility [36–38]. Agmo *et al.* have successfully established a procedure to measure rat's distractibility based on the runway task. They connected an additional runway ending in an empty box once the training was finished and the time spent investigating this additional runway is the measure of distractibility [36]. Clifton *et al.* changed the walls in the centre of the

runway after the training period to distract the subjects and used this task to investigate the effect of testosterone on attentional processes [38]. Here, we added a novel object in the centre of the runway to distract the subjects once the training was finished. In this modified incentive runway task, after the mice have been deprived of food for 20 h, the wild-type mice spent most of time in the goal box, but the *gat1*<sup>-/-</sup> mice spent more time in the runway, travelled longer distance in the runway, and made more transits between the runway and goal box runway than the wild-type controls, which means that *gat1*<sup>-/-</sup> mice spent less time in the goal box than the wild-type control where they can get the food reward. These results suggested *gat1*<sup>-/-</sup> mice showed less motivational strength to seek the incentive than the wild-type control. Introducing a novel subject in the task leads to increase the wild-type mice's transits, total distances travelled and reduce the time in the goal box. Effects of the novel subject on the *gat1*<sup>-/-</sup> mice were less pronounced, suggesting that the *gat1*<sup>-/-</sup> mice were lower levels of attentional focusing on the novelty than the wild-type controls. Taken together, the GAT1 knockout mice displayed an abnormal performance to the food reward and to the novelty in a modified incentive runway test, which may due to the low levels of attentional focusing in the *gat1*<sup>-/-</sup> mice.

Passive avoidance is not only a learning task, but also a good task to investigate psychological mechanisms underlying impulsivity [39,40]. In incentive passive avoidance, the mouse learns to refrain from stepping through a door to a dark compartment, where it seems apparently safer and rewards available but they have been previously punished. The latency to crossing into the punished compartment serves as an index of the ability to avoid, and allows impulsivity to be assessed in a short time (2 min later). According to the theory proposed by Patterson and Newman [41], failure to interrupt ongoing behavior following aversive events in mixed-incentive, passive avoidance situations form a key element of disinhibited behavior and impulsivity. When an impulsive human subject encounters a punishment during a dominant approach response set, he/she is less able to stop and reflect on why he/she was punished. Our results show, all genotypes of mice prolonged latency time to be back the dark room, which means the memory of punishment was set up in all genotypes of mice, however, the latency is significant shorter in the *gat1*<sup>-/-</sup> mice than controls, indicating that the *gat1*<sup>-/-</sup> mice showed a high impulsive proneness.

School-aged children with ADHD often have comorbid learning problems. Likewise, children with learning problems often have comorbid ADHD. The estimate of the overlap ranges from 10%–50% [42–44]. Also, many ADHD patients have motor problems severe enough to be diagnosed as developmental coordination disorder [45–47]. However, the underlying molecular mechanism is yet to be ascertained and the GABAergic system may be the key factor underlying the

relationship among these disorders. We reported previously that impaired learning was found in the *gat1*<sup>-/-</sup> mice. In this study we further investigated the motor coordination of *gat1*<sup>-/-</sup> mice using the rotarod test and parallel bars tasks. As a result, the *gat1*<sup>-/-</sup> mice did show motor coordination deficits and diminished balance skills.

Close interconnection between dopaminergic, noradrenergic, and serotonergic systems has been found in ADHD. Changes in any one system can alter the function of the other monoaminergic systems or nonmonoaminergic systems (primarily glutamate and GABA) and then alter the underlying neural circuits that control behavior. In our previous work, the *gat1*<sup>-/-</sup> mice exhibited measurable insensitive to fluoxetine (a selective serotonin reuptake inhibitor), amitriptyline (a 5-HTT and norepinephrine transporter agonist), buspirone (a serotonin1A receptor partial agonist, also an antagonist effect on D(2)-dopamine and the  $\alpha$ 2-adrenergic receptors), diazepam (a BDZ agonist), and tiagabine (a GAT1 antagonist) [26], which suggested that the serotonergic and adrenergic systems may be modified in the *gat1*<sup>-/-</sup> mice. The modified dopaminergic, serotonergic, and adrenergic systems following the GABAergic system altered may together contribute to the etiology of ADHD in the *gat1*<sup>-/-</sup> mice.

In conclusion, we provide the evidence that GAT1 gene function is involved in ADHD. The *gat1*<sup>-/-</sup> mice appear to be an animal model that exhibits some of the behavioral and pharmacological characteristics of ADHD. Due to ADHD is considered as a complex psychiatric and polygenic disorder, the disruption of GAT1 gene may just mimic a subset of its symptoms. Nevertheless, the study of these mutant mice may provide insights into the mechanisms and may facilitate the discovery of novel therapeutics for the treatment of ADHD.

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