Original Article

Enhanced learning and memory in GAT1 heterozygous mice

Jun Shi1, Youqing Cai1, Guoxiang Liu1, Neng Gong2, Zhenze Liu3, Tianle Xu2, Zhugang Wang4, and Jian Fei3,4*

1Laboratory of Molecular Cell Biology, Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, China
2Institute of Neuroscience, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, China
3School of Life Science and Technology, Tongji University, Shanghai 200092, China
4Shanghai Research Center for Model Organisms, Shanghai 201210, China
*Correspondence address. Tel: +86-21-65980334; Fax: +86-21-65982429; E-mail: jfei@tongji.edu.cn.

γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system. The termination of GABA transmission is through the action of a family of membrane proteins, called GABA transporters (GAT1–4). It is well established that GABA system is involved in the modulation of memory. Our previous study showed that homozygous GAT1−/− mice exhibited impaired hippocampus-dependent learning and memory. To evaluate the impact of endogenous reduced GABA reuptake on mice cognitive behaviors, the ability of learning and memory of heterozygous GAT1+/− mice was detected by the passive avoidance paradigm and Morris water maze. The hole board paradigm was also used to measure changes in anxiety-related behavior or exploratory behavior in such mice. As one form of synaptic plasticity, long-term potentiation was recorded in the mouse hippocampal CA1 area. We found that GAT1+/− mice displayed increased learning and memory, decreased anxiety-like behaviors, and highest synaptic plasticity compared with wild-type and homozygous GAT1−/− mice. Our results suggest that a moderate reduction in GAT1 activity causes the enhancement of learning and memory in mice.

Keywords GAT1; cognition; LTP; hippocampus; microarray

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Introduction

γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the vertebrate central nervous system. GABA functions through different GABA receptors: GABA_A, GABA_B, and GABA_C receptors [1], and the action is terminated by the reuptake of the released GABA in synaptic cleft through GABA transporters located on plasma membrane. Four distinct GABA transporter proteins (GAT1–4) have been identified and cloned [2]. Among these transporters, GAT1 is the major one and presents both at synaptic and extrasynaptic sites. It is particularly abundant in areas rich in GABAergic neurons, such as the cerebellum, hippocampus, neocortex, and retina [3,4].

It is well established that GABA system is involved in the modulation of memory [5]. GABA_A receptor agonist muscimol and GABA_B receptor agonist baclofen have been shown to enhance memory in some studies [6]. However, the effects of muscimol on Morris water maze learning are inconsistent [7,8]. There is also evidence that GABA_A receptor α5 subunit-selective inverse agonists enhance learning performance in rats [9,10]. Other findings suggest that activation of the benzodiazepine recognition sites can result in learning and memory impairments [11–13].

Another approach to stimulate the GABAergic tone can be achieved by inhibition of GABA reuptake. Clinical evidence suggests that GABA reuptake inhibitors, specifically tiagabine, improve performance in verbal memory when used as an anticonvulsant add-on therapy [14]. Similarly, analog of tiagabine, NNC-711 also exhibits cognition-enhancing properties [15]. However, there is contradictory evidence showing that tiagabine impaired spatial learning of rats in the Morris water maze [16].

The GAT1 gene knockout mice were generated in our laboratory previously. [3H]-GABA uptake assay showed that GAT1 heterozygous mice (GAT1+/−) displayed a moderate reduction of transporter activity compared with that of wild-type littermates. However, uptake activity is dramatically reduced to near baseline in GAT1 knockout mice (GAT1−/−) [17]. Our previous study showed that homozygous GAT1−/− mice exhibited impaired hippocampus-dependent learning and memory [18]. We also found that overexpression of GAT1 led to cognitive deterioration in transgenic mice [19]. Interestingly, the heterozygous and homozygous GAT1 knockout mice showed opposite behavioral response to some of the actions of ethanol, including...
locomotor stimulation, preference, and reward [17]. However, the learning and memory of heterozygous GAT1 mice have not been studied yet. In the present investigation, we used the GAT1+/− mice to evaluate the impact of endogenous reduced GABA reuptake on mice cognitive behaviors. We used passive avoidance paradigm to study learning and memory for a stress stimuli elicited by electric foot shock. The Morris water maze paradigm was used to measure spatial learning and memory. We also used the hole board paradigm to measure changes in anxiety-related behavior or exploratory behavior in mice.

Long-term potentiation (LTP) is widely considered as one of the major cellular mechanisms that underlie learning and memory [20,21]. In the following experiments, we also investigated the synaptic plasticity in three genotypes of mice (GAT1+/+, GAT1+/−, and GAT1−/−) by field potential recordings performed in mouse hippocampal CA1 area. Further, we used microarray analysis to investigate the gene expression profile in the hippocampus for the purpose of exploring the changes in cognition-related gene expression. In this study, we show improved cognitive performance of GAT1+/− mice in passive avoidance and Morris water maze tests. We also find reduced anxiety-like behavior of GAT1+/− mice in the hole board test. These results confirm that GAT1 plays an important role in learning and memory, and indicate a relationship between anti-anxiety and cognitive enhancement. In addition, we demonstrate that GAT1+/− mice show an enhancement of theta-burst stimulation (TBS)-induced LTP.

Materials and Methods

Animals

GAT1 knockout mice were generated in our laboratory as described previously [17]. Homozygous (GAT1−/−, or Homo), heterozygous (GAT1+/−, or Het), and wild-type (GAT1+/+, or WT) littermates were derived from crossing GAT1 heterozygous mice. Only male mice were used in this study. Mice were maintained for 10–16 weeks under specific pathogen-free conditions before analysis started. Littermates were group-housed (3–5 mice per cage) in a temperature- and humidity-controlled room on a 12-h light/dark cycle (lights on at 07:00 AM) with water and food available ad libitum. All behavioral tests were performed in an isolated room. Naive mice were used in each experiment, and the age and weight of mice were matched. All animal experiments described in this article were approved by the Institutional Animal Care and Use Committee.

Passive avoidance

Passive avoidance test was conducted basically according to Ader et al. [22]. The passive avoidance apparatus (Zhenghua Bioinstrumentation, Huaibei, China) consisted of a white illuminated compartment and a dark compartment equipped with a metal grid floor available for foot electric shocks, the light compartment and the dark compartment were separated by a sliding door. On the training day, each mouse was placed in the illuminated compartment facing away from the dark compartment. After 30 s habituation, the door was raised and the initial latency to enter the dark compartment was recorded. Once the mouse entered completely into the dark compartment, the sliding door was closed and a foot shock (0.3 mA, 2 s) was delivered. The mouse was then removed and returned to its home cage. The testing trial was conducted on the next day following the same procedures but without any shock and the latency time was recorded to a maximum of 300 s.

Morris water maze

The Morris water maze task was based on the method described previously [23,24]. The water maze was located in the center of the test room, the experimental conditions (room extra cues, water temperature, room temperature, light) were constant throughout the experiment. The apparatus was a white circular steel swimming pool (1 m in diameter, 50 cm high) and filled with water maintained at 25 ± 2°C to a depth of 30 cm. Water was added with milk powder so as to hide the underwater platform. The pool was divided into four equal quadrants with four designated starting positions at the perimeter of each quadrant, and a white removable escape platform was set 1.5 cm below the water level in the center of one quadrant which was the target quadrant. The position of the platform was fixed throughout the training tasks. This task consisted of four training trials per day for 7 consecutive days, followed by probe trials on the fourth day and the eighth day. On each trial, a mouse was released into the water facing the pool wall, from one of the starting positions randomly. The mouse was allowed to search the platform for a maximum of 60 s. If the mouse failed to find the platform in 60 s, it was gently placed on the platform by the experimenter. Once the mouse had climbed or had been placed onto the platform, it was allowed to stay on it for 30 s before the next trial began. On the probe trial day, the platform was removed and the mouse swimming paths were recorded for 60 s. Swiming paths were monitored by a CCD video camera mounted above the center of the pool and data were later analyzed by an automated tracking system, Morris Maze Analyzer V1.1 (BGB, Shanghai, China).

Hole board test

The hole board test used for exploratory behavior was modified from that described by File and Wardill [25]. The hole board apparatus (Kinder Scientific, Poway, USA) consisted of a clear Plexiglas box and a white plastic panel with eight evenly spaced holes (each 2.5 cm in diameter.
arranged in a 3 × 3 configuration, center hole sealed) with built-in infrared sensors. The test was carried out between 09:00 AM and 02:00 PM. One hour before the test, the mice were transferred to the experimental room for acclimatization. The mouse was placed singly in the center of the hole board and allowed to freely explore for 10 min. After each trial the floor of the apparatus was cleaned with a solution containing neutral soap to remove traces of previous mouse. The number of head dips, the time spent head dipping, and total movement distance were recorded automatically by built-in infrared sensors.

Electrophysiology
Transverse hippocampal slices (300 μm thickness) were prepared from young adult male mice at the age of 2–3 months. Animals were killed by decapitation in accordance with institutional regulations. Slices were obtained using a vibratome (ATF-1000; Leica, Wetzlar, Germany) in ice-cold artificial cerebrospinal fluid (ACSF) and then kept at room temperature for at least 1.5 h before transferring to the recording chamber. The same ACSF was also used in recording, which contained 119 mM NaCl, 2.5 mM KCl, 2.5 mM CaCl$_2$, 1.25 mM KH$_2$PO$_4$, 1.3 mM MgSO$_4$, 26.2 mM NaHCO$_3$, and 11 mM d-glucose, saturated with 95% O$_2$/5% CO$_2$ (pH 7.4). Extracellular recordings were performed in the CA1 region of the hippocampus. The stimulating electrode (bipolar platinum-iridium electrode) was placed at the Schaffer collateral pathway in the stratum radiatum. The field excitatory postsynaptic potentials (fEPSPs) was placed at the Schaffer collateral pathway in the stratum stimulating electrode (bipolar platinum-iridium electrode) performed in the CA1 region of the hippocampus. The slope of fEPSPs was determined from Sigma (St Louis, USA) and filtered at 5 kHz. Data were acquired and analyzed using Clampfit 9.0 software (Axon Instruments). In the present study, the stimuli intensity was adjusted to evoke 50% of the maximum fEPSP. TBS was delivered to induce LTP, and consisted of five bursts (four pulses, 100 Hz) delivered at an interburst interval of 200 ms, and repeated once at 20 s. The slope of fEPSPs was determined by Clampfit 9.0 software. All reagents were purchased from Sigma (St Louis, USA).

Microarray analysis
The homemade SBC mouse Oligo microarray (Shanghai Biochip Co., Ltd Shanghai, China) containing 31,798 genes and expressed sequence tags was used. Briefly, total RNA was prepared from mouse hippocampus using TRIzol reagent (Invitrogen, Carlsbad, USA), and further purified with RNeasy mini kit (Qiagen, Hilden, Germany). Two micrograms total RNA was amplified using Low RNA Input Linear Amplification Kit (Agilent Technologies, Santa Clara, USA). RNA from three wild-type mice was labeled with Cy5 (GE Healthcare, Waukesha, USA), while the RNA from three GAT1$^{+/−}$ mice was labeled with Cy3 (GE Healthcare). The Cy5 and Cy3 probes were mixed and hybridized at 42°C for 16 h, and washed with standard protocol. The microarray slides were scanned using an Agilent Scanner (Agilent Technologies, Santa Clara, USA), and the image data were extracted using ImaGene 4.2 (Biodiscovery, Santa Monica, USA).

Statistical analysis
Data are represented as the mean ± SEM. Groups were compared using one-way analysis of variance followed by t-test. The criterion for significance was $P < 0.05$ in all statistical evaluations. All statistical analyses were performed using Origin 7.5 (OriginLab, Northampton, USA) or PASW Statistics 18.0 (IBM SPSS, Chicago, USA).

Results
Cognitive enhancement in GAT1$^{+/−}$ mice
Passive avoidance learning involves not only hippocampus-dependent contextual memory but also amygdala-dependent emotional memory. In the passive avoidance test, latency to the dark compartment did not differ in the three groups of genotypes in the training session (GAT1$^{+/+}$: 34.8 ± 4.9 s, $n = 9$; GAT1$^{+/−}$: 34.4 ± 3.9 s, $n = 10$; GAT1$^{−/−}$: 40.5 ± 5.6 s, $n = 8$) [Fig. 1(A)]. During the retention trial 24 h later, retention latency significantly increased in GAT1$^{+/−}$ mice (277.5 ± 11.2 s, $n = 10$) than in GAT1$^{+/+}$ mice (204.9 ± 18.3 s, $n = 9$), whereas retention latency significantly decreased in GAT1$^{−/−}$ mice (118.1 ± 20.5 s, $n = 8$) [Fig. 1(B)]. The percentages of mice reaching a latency of 300 s were 50% of GAT1$^{+/−}$, 11% of GAT1$^{+/+}$, and 0 of GAT1$^{−/−}$, respectively.

Morris water maze task was performed to assess the hippocampus-dependent formation and retrieval of spatial memory. Escape latencies in the training session and probe trial measures were recorded. GAT1$^{+/−}$ mice showed significant improvement of spatial learning and memory both in the training session and in probe trial (Fig. 2). During the training session, GAT1$^{+/−}$ mice spent significantly less time to reach the platform compared with GAT1$^{+/+}$ mice on Day 2 and Day 3 (GAT1$^{+/−}$: 12.1 ± 1.7 s; GAT1$^{+/+}$: 20.5 ± 2.6 s; GAT1$^{−/−}$: 28.5 ± 3.6 s; $n = 12–13$ for each genotype) [Fig. 2(A)]. These results indicated that GAT1$^{+/−}$ mice had learned to find the platform in the shortest time. GAT1$^{−/−}$ mice spent significantly longer time compared with GAT1$^{+/+}$ mice throughout the training session, indicating impairment of spatial learning and memory. During the two probe trials, GAT1$^{+/−}$ mice also showed significantly more times of crossing the platform location on the 4th day (GAT1$^{+/−}$: 4.0 ± 0.3; GAT1$^{+/+}$: 5.7 ± 0.4; GAT1$^{−/−}$: 2.1 ± 0.4; $n = 12–13$ for each
genotype) and on the 8th day ($GAT1^{+/+}$: 4.4 ± 0.4; $GAT1^{+/−}$: 6 ± 0.6; $GAT1^{−/−}$: 2.7 ± 0.6) [Fig. 2(B,C)]. In addition, $GAT1^{+/−}$ mice showed higher preference for the target quadrant during the trial on the 4th day ($GAT1^{+/+}$: 31.0% ± 3.6%; $GAT1^{+/−}$: 39.3% ± 2.2%; $GAT1^{−/−}$: 28.0% ± 1.8%; n = 12–13 for each genotype) [Fig. 2(D)], but showed no significant difference in preference for the target quadrant compared with the $GAT1^{+/+}$ mice during
The hole board test has been used widely to assess anxiety, emotionality, or responses to stress in animals [26–28]. An increase in head-dipping behavior in the hole board test reflects an anxiolytic-like state [28]. In the present study, GAT1<sup>+/−</sup> mice displayed increased number of head-dipping behaviors (GAT1<sup>+/−</sup>: 43.92 ± 3.92, n = 9; GAT1<sup>+/+</sup>: 59.8 ± 4.62, n = 13; GAT1<sup>−/−</sup>: 29.91 ± 2.91, n = 12), but no significant change of exploring time (GAT1<sup>+/+</sup>: 28.8 ± 5.06 s, n = 9; GAT1<sup>−/−</sup>: 31.5 ± 2.77 s, n = 13; GAT1<sup>+/−</sup>: 14.9 ± 2.99 s, n = 12) compared with GAT1<sup>+/+</sup> mice [Fig. 3(A,B)]. In addition, the motor activity was also increased in GAT1<sup>−/−</sup> mice compared with GAT1<sup>+/+</sup> mice (GAT1<sup>+/+</sup>: 1366 ± 82 cm, n = 9; GAT1<sup>+/−</sup>: 1546 ± 38 cm, n = 13; GAT1<sup>−/−</sup>: 1676 ± 102 cm, n = 12) [Fig. 3(C)]. GAT1<sup>−/−</sup> mice also displayed increased motor activity, but displayed decreased number of head-dipping behaviors and exploring time compared with GAT1<sup>+/+</sup> mice. These results suggest a reduction in anxiety-like behavior in GAT1<sup>−/−</sup> mice. Interestingly, GAT1<sup>−/−</sup> mice showed contradictory behaviors in this paradigm.

**Enhancement of TBS-induced LTP in GAT1<sup>+/−</sup> mice**

Next, we investigated the synaptic plasticity in GAT1<sup>−/−</sup>, GAT1<sup>+/−</sup>, and GAT1<sup>+/+</sup> mice. Field potential recordings were performed in mouse hippocampal CA1 area. Hippocampal LTP can be successfully induced by TBS [five bursts (four pulses at 100 Hz) delivered at 5 Hz and repeated once at 20 s]. We found that GAT1<sup>−/−</sup> mice showed a significant impairment of TBS-induced LTP (GAT1<sup>+/+</sup>: 131% ± 5% of the baseline level, n = 12; GAT1<sup>−/−</sup>: 107% ± 2% of the baseline level, n = 13) [Fig. 4(A)], but GAT1<sup>+/−</sup> mice showed an enhancement of TBS-induced LTP (GAT1<sup>−/−</sup>, 146% ± 5% of the baseline level, n = 16) compared with GAT1<sup>+/+</sup> mice. These results suggest that LTP in the CA1 region of the hippocampus was altered by changing activity of GAT1 and indicate enhanced hippocampal synaptic plasticity in GAT1<sup>−/−</sup> mice.
Whole genome gene expression profile in the hippocampus of GAT1<sup>+/−</sup> mice

Gene expression profile was analyzed using the Whole Mouse Genome Oligo Microarray. The results showed a similar overall gene expression profile between GAT1<sup>+/+</sup> mice and GAT1<sup>+/−</sup> mice. Data showed that there was no gene whose expression ratio was beyond the range of 0.5–2.0, which indicates the differential expression (Supplementary Table S1).

Discussion

In the present study, we employed two tasks to investigate cognition behaviors of three genotypes of GAT1 gene knockout mice. We found improved cognitive performance of GAT1<sup>+/−</sup> mice in passive avoidance and Morris water maze tests. In passive avoidance test, GAT1<sup>+/−</sup> mice exhibited significantly increased retention latency compared with wild-type mice, whereas GAT1<sup>−/−</sup> mice exhibited significantly decreased retention latency. In Morris water maze test, GAT1<sup>+/−</sup> mice also exhibited an improved performance in the water maze model of spatial learning and memory. These results indicate enhanced hippocampus-dependent memory in GAT1 heterozygous mice. This conclusion was also confirmed by the data of LTP experiment.

Previous studies showed that increased GABA levels have distinct influence on process of learning and memory. Schmitt and Hiemke [16] found that inhibition of GABA reuptake caused cognitive impairment through augment GABAergic inhibition. In other studies, GABA reuptake inhibitor exhibited contrary effect. O’Connell et al. [15] showed NNC-711 exhibited significant cognition-enhancing actions, and administration of NNC-711 significantly reduced escape latencies in the water maze paradigm. This result is in accordance with observations in our research on GAT1<sup>+/−</sup> mice. GABA analog, gabapentin, which is thought to increase GABA levels was also found to enhance cognitive performance in mice [29,30]. Our study is the first to demonstrate cognitive enhancement in a genetic mouse model of decreased GABA reuptake activity of GAT1.

From the functional perspective, GABA reuptake inhibitors display a marked difference with GABA receptor drugs. GABA receptor agonists have action sites on certain subunits of GABA receptors, they can cause various effects depending on property, location, distribution, and density of their binding subunits. GABA reuptake inhibitors have an effect on GABAergic system by increasing GABA level in synaptic cleft, thus have a comprehensive impact on GABAergic system activity. In O’Connell’s research, the cognition-enhancing effects of NNC-711 exhibited bell-shaped dose–response effects [15]. GAT1<sup>+/−</sup> mice used in the present study had a moderate down-regulated GABA reuptake function [17], the effect of cognitive enhancement of GAT1<sup>+/−</sup> resembles dose-dependent cognition-enhancing effects of NNC-711. These results indicate that certain degree of activation of GABAergic transmission may enhance the learning and memory process.

A clear link between the GABAergic system and anxiety has long been established [31–33]. There was also evidence that anxiolytic drugs acting at GABAA receptors can make an impact on cognition function [13]. Passive avoidance is a classical fear-motivated test to assess memory function. Increased retention latency in GAT1<sup>+/−</sup> mice showed an enhancement of emotional memory encoding that depends on amygdala and hippocampus and their interactions. In the hole board test, an increase in head-dipping behavior reflects an anxiolytic-like state in GAT1<sup>+/−</sup> mice, and in previous research by our lab, we found reduced anxiety and depression-like behaviors in GAT1<sup>+/−</sup> mice [34]. Interestingly, performance of mice of these two genotypes in the hole board paradigm was different. Both groups exhibited significantly increased locomotor activity, whereas the number of head-dipping behaviors in GAT1<sup>+/−</sup> mice was less than that in wild-type mice, which reveals functional lesion on exploratory behavior. In consideration of significantly impaired function of cognition in GAT1<sup>+/−</sup> mice, it can be perceived that different extent of activation of GABAergic transmission may result in distinct effects on the anxiety process as well as on the cognition process.

GAT1<sup>+/−</sup> mice showed not only enhanced cognitive abilities in behavioral tests, but also provided a model for studying mechanism of learning and memory. The results of electrophysiological experiment in GAT1<sup>+/−</sup> mice provide much evidence for understanding the physiological mechanism of effect of GAT1 on cognition function. Synaptic plasticity is generally viewed as a cellular mechanism for learning and memory [21,35]. To assess the functional changes in GAT1<sup>+/−</sup> mice, we investigated the synaptic plasticity in GAT1<sup>+/+</sup>, GAT1<sup>+/−</sup>, and GAT1<sup>−/−</sup> mice. Field potential recordings were performed in mouse hippocampal CA1 area. We found that GAT1<sup>+/−</sup> mice showed an enhancement of TBS-induced LTP compared with wild-type littermates, whereas GAT1<sup>−/−</sup> mice showed a significant impairment of TBS-induced LTP. This experimental result is in accordance with the enhancing cognitive performance in GAT1<sup>+/−</sup> mice in preceding tests.

Jensen et al. [36] concluded that GAT1 deficiency led to enhanced extracellular GABA levels, resulting in overactivation of GABAA receptors responsible for a postsynaptic tonic conductance. Chronically elevated GABA levels also downregulated phasic GABA release and reduced presynaptic signaling via GABAB receptors, thus causing an enhanced tonic and a diminished phasic inhibition [36].
a previous study using the same strain of \textit{GAT1}^{-/-} mice as used in our current research, Gong et al. [18] demonstrated that GAT1 disruption specifically impaired TBS-induced LTP. They showed that GAT1 disruption specifically altered the temporal pattern of hippocampal theta oscillations by reducing the oscillation frequency, and these specific changes in the theta network activity and synaptic plasticity were accompanied by severe impairment of hippocampus-dependent learning and memory. Furthermore, the result of significant suppression of burst-induced fEPSPs by GAT1 disruption showed that the excitatory action of subsequent bursts was inhibited by prolonged GABA action. Interestingly, it appears that the interburst interval is critical for the GABAergic modulation of LTP. The multiple burst stimulation-induced LTP in the wild-type mice showed a bell-shaped dependence on the frequency of stimulation ranging from 3 to 7 Hz, with the maximum LTP at 5 Hz, and this bell-shaped dependence could be abolished by GAT1 deletion. Noticeably, this observed optimal range agrees well with the physiological theta frequency which plays an important role in learning and memory [37], and can be mimicked by LTP induced by TBS [38].

GABA reuptake via GAT1 is essential for modulating the rhythm of theta activity, GAT1 activity does not affect the physiological expression of theta oscillation activity, but modulates the precise frequency of this oscillation [18]. In wild-type mice, the time course of GABA release and reuptake cycle which present a bell-shaped effect generates a moderate inhibition at subsequent excitatory action. However, disruption of reuptake in \textit{GAT1}^{-/-} mice resulted in prolonged GABA-mediated tonic inhibition, and the time course of this tonic inhibition was beyond the bell-shaped effect range. Differently, \textit{GAT1}^{+/+} mice might present a fine prolonged GABA-mediated tonic inhibition, and the time course of this inhibition may be even more effective but less redundant in function than that in wild-type mice. This shifting time course is supposed to alter hippocampal rhythmic activities which may be important for memory formation [37], and to play a crucial role in the presence of cognitive enhancement in \textit{GAT1}^{+/+} mice.

Microarray analysis of hippocampus showed that there was no significant difference in gene expression profile between \textit{GAT1}^{+/+} and \textit{GAT1}^{+/+} mice. However, mild changes were found in some genes involved in GABAergic and glutamatergic transmission, synaptic plasticity, and cognitive formation. Other evidences also indicated that neither genes expression nor synaptic structure changed significantly in the hippocampus of \textit{GAT1} knockout mice [18,36]. These data indicate that behavioral and electrophysiological changes in \textit{GAT1}^{-/-} mice may mainly depend on the process of neurotransmission. However, we still cannot exclude the effect of the slight change of gene expression profile in hippocampus of \textit{GAT1}^{+/+} mouse on its learning and memory enhancement.

In the present study, we found that \textit{GAT1}^{+/+} mice displayed improved cognitive ability and decreased anxiety-like behaviors compared with wild-type mice. We also found that \textit{GAT1}^{+/+} mice did not show any evidence of behavioral abnormalities in executed tests or significant changes in hippocampal gene expression. These results indicate that the strain of \textit{GAT1} knockout heterozygous mice can be used as a model of steady and moderately altered GABA-glutamate balance for investigation of GABA-related neural function and mechanism.

**Supplementary Data**

Supplementary data are available at \textit{ABBS} online.

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**References**

Cognitive enhancement in GAT1 heterozygous mice


