Developmental changes in GABA<sub>A</sub> tonic inhibition are compromised by multiple mechanisms in preadolescent dentate gyrus granule cells

Sudip Pandit<sup>#,†</sup>, Gyu Seung Lee<sup>#,¶</sup>, and Jin Bong Park*

Department of Physiology, School of Medicine and Brain Research Institute, Chungnam National University, Daejeon 35015, Korea

ABSTRACT The sustained tonic currents (I<sub>tonic</sub>) generated by γ-aminobutyric acid A receptors (GABA<sub>A</sub>Rs) are implicated in diverse age-dependent brain functions. While various mechanisms regulating I<sub>tonic</sub> in the hippocampus are known, their combined role in I<sub>tonic</sub> regulation is not well understood in different age groups. In this study, we demonstrated that a developmental increase in GABA transporter (GAT) expression, combined with gradual decrease in GABA<sub>A</sub>R<sub>α<sub>5</sub></sub> subunit, resulted in various I<sub>tonic</sub> in the dentate gyrus granule cells (DGGCs) of preadolescent rats. Both GAT-1 and GAT-3 expression gradually increased at infantile (P<sub>6-8</sub> and P<sub>13-15</sub>) and juvenile (P<sub>20-22</sub> and P<sub>27-29</sub>) stages, with stabilization observed thereafter in adolescents (P<sub>34-36</sub>) and young adults (P<sub>41-43</sub>). I<sub>tonic</sub> facilitation of a selective GAT-1 blocker (NO-711) was significantly less at P<sub>6-8</sub> than after P<sub>13-15</sub>. The facilitation of I<sub>tonic</sub> by SNAP-5114, a GAT-3 inhibitor, was negligible in the absence of exogenous GABA at all tested ages. In contrast, I<sub>tonic</sub> in the presence of a nonselective GAT blocker (nipecotic acid, NPA) gradually decreased with age during the preadolescent period, which was mimicked by I<sub>tonic</sub> changes in the presence of exogenous GABA. I<sub>tonic</sub> sensitivity to L-655,708, a GABA<sub>A</sub>R<sub>α<sub>5</sub></sub> subunit inverse agonist, gradually decreased during the preadolescent period in the presence of NPA or exogenous GABA. Finally, Western blot analysis showed that the expression of the GABA<sub>A</sub>R<sub>α<sub>5</sub></sub> subunit in the dentate gyrus gradually decreased with age. Collectively, our results suggested that the I<sub>tonic</sub> regulation of altered GATs is under the final tune of GABA<sub>A</sub>R<sub>α<sub>5</sub></sub> subunit activation in DGGCs at different ages.

INTRODUCTION

The activation of synaptic and extrasynaptic γ-aminobutyric acid A receptors (GABA<sub>A</sub>Rs) generates phasic and tonic forms of inhibition (tonic GABA<sub>A</sub> current, I<sub>tonic</sub>), respectively [1,2], and has a profound influence on the hippocampal neural circuitry. I<sub>tonic</sub> is particularly interesting in the context of different ages because extrasynaptic GABA<sub>A</sub>R signaling is implicated in brain physiology and rage of pathophysiolgies [3-7]. Changes in extracellular GABA concentrations alter the relative contribution of specific GABA<sub>A</sub>Rs to I<sub>tonic</sub> as different receptor populations are recruited [8]. GABA<sub>A</sub>Rs containing the α<sub>5</sub> subunit (α<sub>5</sub>·GABA<sub>A</sub>Rs) contribute to I<sub>tonic</sub> when the ambient GABA concentration increases, while at low ambient GABA concentrations the activation of δ subunit-containing receptors predominates [9]. In dentate gyrus granule cells (DGGCs), I<sub>tonic</sub> increases during initial postnatal
maturation [10,11], and further increases as adolescents mature into adulthood [12]. The age-dependent increase of I\textsubscript{tonic} in DGGCs may mirror the increased expression of δ-GABA\textsubscript{A}Rs in adults [13], which raises the question of whether and how a developmental change in α\textsubscript{t}-GABA\textsubscript{A}Rs alters I\textsubscript{tonic} at different ages.

GABA transporters (GATs) are members of a family of Na\textsuperscript{+}-dependent neurotransmitter reuptake proteins. To date, four different GATs (GAT-1, GAT-2, GAT-3, and Betain/GABA transporter type 1) have been described in rat brain. Of these, GAT-1 is a primary neuronal GAT, while GAT-3 is commonly associated with glial cells [14]. Accordingly these two GAT subtypes are responsible for controlling extracellular GABA released from vesicular and non-vesicular sources, respectively [15]. In the hippocampus, GAT-1 predominantly determines the GABA concentration surrounding neurons, while GAT-3 activity is apparent with increased extracellular GABA concentration, especially when GAT-1 is blocked [16]. However, GAT-1 expression is low at early postnatal age, with GAT-3 expression dominating in that period [17]. Overall, it remains unknown whether and how the interaction between GAT-1 and GAT-3 modulates I\textsubscript{tonic} during postnatal brain maturation. In this study, we investigated the combined role of GAT-1 and GAT-3 in I\textsubscript{tonic} regulation in DGGCs at different ages; the results suggested that I\textsubscript{tonic} mirrored the changes in expression of extrasynaptic GABA\textsubscript{A}Rs activated by elevated extracellular GABA, according to the interrelationship between neuronal and glial GATs.

**METHODS**

**Experimental animals**

Male Sprague-Dawley rats purchased from Samtako Bio (Kyung Gi-Do, Korea) were housed under a 12/12 h light/dark schedule with free access to food and water until used. Animals were grouped by postnatal day (P), as follows: infantile (P\textsubscript{7-9} and P\textsubscript{14-16}), juvenile (P\textsubscript{21-23} and P\textsubscript{28-30}), adolescence (P\textsubscript{36-37}), and young adulthood (P\textsubscript{42-44} and P\textsubscript{49-51}). Brains were rapidly extracted for electrophysiological recordings or Western blotting from animals euthanized by decapitation without anesthesia. All animal experimentation was conducted in compliance with the policies of Chungnam National University regarding the use and care of animals.

**Electrophysiological recordings and data analysis**

Patch-clamp recordings were obtained in acutely prepared coronal hippocampal slices from male rats, as described previously [6,18]. Briefly, slices were perfused with artificial cerebrospinal fluid (aCSF; in mM: NaCl 126, KCl 2.5, MgSO\textsubscript{4} 1, NaHCO\textsubscript{3} 26, Na\textsubscript{2}PO\textsubscript{4} 1.25, glucose 20, ascorbic acid 0.4, CaCl\textsubscript{2} 1, pyruvic acid 2; pH 7.3~7.4; saturated with 95%O\textsubscript{2}-5%CO\textsubscript{2} at a ~3 ml/min flow. Recordings were obtained at 32°C using an Axopatch 200B amplifier (Axon Instruments, Foster City, CA). The series resistance was motored throughout the experiments. Neurons localized in the outer half of the granule cell layer were selected to minimize the effects of neurogenesis [19]. Patch pipettes were filled with a high Cl\textsuperscript{−} containing solution (in mM): KCl 140, HEPES 10, Mg\textsuperscript{2+}-ATP 5, MgCl\textsubscript{2} 0.9, and EGTA 10. Current output was filtered at 2 kHz and digitized at 10 kHz (Digidata 1322A, pClamp 9 software, Axon Instruments). I\textsubscript{tonic} was defined as the difference between the holding current (I\textsubscript{holding}) before and after application of the GABA\textsubscript{A} receptor blocker bicuculline (20 µM). Drugs were added to the perfusing aCSF solution at known concentrations. All drugs except NO-711 (Tocris, UK) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

**Western blotting**

All proteins from the dissected dentate gyrus (DGs) were lysed with 1× passive lysis buffer (Cell Signaling Technology, Danvers, MA, USA) and quantified using a Coomassie Protein assay kit (Bio-Rad, Hercules, CA, USA). Approximately 50 µg of protein was electrophoresed on a 10% sodium dodecyl sulfate polyacrylamide gel (SDS–PAGE) and transferred onto nitrocellulose membranes. The blots were blocked with 1× Tris buffered saline (TBS)-TWEEN 20 containing 3% bovine serum albumin (BSA) +2% heparan sulfate (HS) for 1 h at room temperature (5% TTBS; Gibco, USA). The blots were then incubated at 4°C with primary antibodies against GABA\textsubscript{A}R α\textsubscript{t}, subunit, GAT-1, and GAT-3 (1:1,000; Millipore, USA) in 5% TTBS, respectively. The next day, the blots were incubated with a horseradish peroxidase-conjugated goat anti-rabbit secondary antibody (1:2,000; Santa Cruz Biotechnology, USA). An enhanced chemiluminescence detection kit (ECL; Pierce, USA) was used to visualize antibody binding, and the intensity of the bands was measured using Image J software 1.42q (NIH, USA).

**Statistical analysis**

Numerical data are presented as means±standard error of the mean (SEM). Student’s t-tests and analysis of variance, followed by post-hoc tests, were used as appropriate.

**RESULTS**

Electrophysiological recordings were obtained from a total of 114 DGGCs. In addition to blocking synaptic transmission, the GABA\textsubscript{A}R antagonist, bicuculline (BIC, 20 µM), induced a variable outward shift in the holding current (I\textsubscript{holding}) in DGGCs.
Increased expression of GAT-1 and GAT-3 in preadolescence

Altered expression of neuronal and glial GATs could contribute to $I_{\text{tonic}}$ changes by altering the extracellular GABA concentration at different ages. To understand the age-dependent changes in GAT expression, we directly compared the expression of GAT-1 and GAT-3 in the DGs at different ages. Western blot analysis showed that the expression of GAT-1 and GAT-3 in the DGs gradually increased at P7, P14, and P21, and thereafter stabilized at P28, P35, and P42 (Fig. 1).

Effect of GAT-1 and GAT-3 blockers on $I_{\text{tonic}}$ according to age

Changes in the expression and/or relative contribution of GAT-1 and/or GAT-3 may cause $I_{\text{tonic}}$ changes at different ages. To discriminate the functional role of neuronal and glial GATs in $I_{\text{tonic}}$, we measured and compared the $I_{\text{tonic}}$ of DGGCs in the presence of selective GAT-1 and GAT-3 blockers at different ages.

Bath application of NO-711 (5 µM), a selective GAT-1 blocker [20], failed to induce consistent changes in $I_{\text{holding}}$ at P6-8, while it induced a similar inward shift in $I_{\text{holding}}$ (INO-711) at P13-15 (21.8±4.4 pA, n=7), P20-22 (20.8±6.1 pA, n=6), P27-29 (21.4±3.6 pA, n=5), and P34-36 (27.3±3.4 pA, n=7) (Fig. 2A). In a subset of experiments, we measured INO-711 in the presence of 1 µM GABA to confirm that GAT-1 activity facilitates $I_{\text{tonic}}$ at the early infantile age. GABA (1 µM)-induced $I_{\text{holding}}$ shift were decreased with age (P6-8, 16.3±4.2 pA, P20-22, 13.2±1.0 pA, n=5 and P34-36, 21.9±0.9 pA, n=5). An additional 100 µM SNAP-5114 efficiently induced an inward shift in $I_{\text{holding}}$ at P6-9, while it failed to cause significant changes in $I_{\text{holding}}$ at P20-22 and P34-36 in the presence of 1 µM GABA (Fig. 2D). These results support the idea that GAT3 activity is apparent with an increased extracellular GABA concentration in the hippocampus [16].

Nipecotic acid-induced $I_{\text{tonic}}$ in preadolescence

To investigate the combined role of GAT-1 and GAT-3 in developmental $I_{\text{tonic}}$ changes, we measured and compared the $I_{\text{tonic}}$ of DGGCs in the presence of the nonselective GAT blocker, nipecotic acid (NPA), at different ages.

In contrast to selective GAT-1 or GAT-3 blockade, NPA (100 µM) induced a significant inward shift in $I_{\text{holding}}$ of DGGCs (INPA), blocked by BIC, at all tested age groups. Interestingly, INPA gradually decreased during the infantile and juvenile periods (P6-8, 16.3±4.2 pA, P20-22, 7.6±1.7 pA, and P34-36, 3.3±1.2 pA, n=5–6). Additional application of NO-711 (GABA+NPA) caused a significant inward shift in $I_{\text{holding}}$ even at P6-9, which was still much smaller than at P20-22 and P34-36 (p<0.01 in both cases; Fig. 2B).

To investigate the functional significance of changes in GAT-3 expression, a selective GAT-3 blocker, SNAP-5114, was used [14]. Because SNAP-5114 (100 and 300 µM) alone failed to elicit changes in $I_{\text{holding}}$, we sequentially applied NO-711 and NO-711+SNAP-5114. In the presence of NO-711, 300 µM SNAP-5114 induced a significant inward shift in $I_{\text{holding}}$ (INSNAP-5114) at P6-8, P20-22, and P34-36. INSNAP-5114 was significantly smaller at P6-8 than at P20-22, and P34-36 (p<0.05 in both cases; Fig. 2C). Even in the presence of NO-711, 100 µM SNAP-5114 did not induce significant changes in $I_{\text{holding}}$ at all tested age groups (Fig. 2C). Thus, in a subset of experiments, we assessed INSNAP-5114 in the presence of 1 µM GABA at different ages. GABA induced significant $I_{\text{holding}}$ changes at P6-8 (22.6±3.5 pA, n=6), while it induced minimal changes in $I_{\text{holding}}$ at P20-22 (1.3±1.0 pA, n=5) and P34-36 (1.9±0.9 pA, n=5). An additional 100 µM SNAP-5114 efficiently induced an inward shift in $I_{\text{holding}}$ at P6-9, while it failed to cause significant changes in $I_{\text{holding}}$ at P20-22 and P34-36 in the presence of 1 µM GABA (Fig. 2D). These results support the idea that GAT3 activity is apparent with an increased extracellular GABA concentration in the hippocampus [16].

Fig. 1. Expression of GABA transporter-1 (GAT-1) and GABA transporter-3 (GAT-3) in the dentate gyrus at different ages. Western blot analysis showing neuronal GAT-1 (A) and glial GAT-3 expression (B) in the dentate gyrus at different postnatal days (P). The expression was normalized to the level detected at P7, and compared with the expression at each age group (n=5), shown by the bar graphs. **p<0.01 compared with P7 expression.
we directly compared INPA inhibition of L-655,708, an inverse agonist at the benzodiazepine binding site of $\alpha_5$-GABA$_A$Rs [21]. The bath application of L-655,708 (5 $\mu$M) efficiently blocked INPA at all tested ages (p<0.01 in all cases; Fig. 3A). Interestingly, in agreement with gradual INPA attenuation with age, L-655,708-sensitive INPA also gradually decreased during preadolescence (Fig. 3B). As a result, the portion of L-655,708-sensitive total INPA did not differ by age.

Age-dependent decrease in the GABA$_A$R $\alpha_5$ subunit in preadolescence

To understand the functional changes in GABA$_A$Rs according to age, we directly compared $I_{\text{tonic}}$ activated by exogenous GABA (5 $\mu$M) and their sensitivity to L-655,708 at different ages. $I_{\text{tonic}}$ gradually decreases as infants mature into adolescence. The large $I_{\text{NPA}}$ that characterized infantile periods (P6-8 and P13-15)
Developmental ionic changes of DGGCs

Gradually decreased at the juvenile (P20-22, and P27-29) and adolescent (P34-36) stages, and thereafter stabilized in young adults (P41-43) (Fig. 4A and B). The bath application of L-655,708 (5 μM) partially blocked \( I_{\text{ionic}} \) in the presence of GABA at all tested ages (p<0.01 in all cases; Fig. 4A). In agreement with \( I_{\text{ionic}} \) attenuation, L-655,708-sensitive \( I_{\text{ionic}} \) gradually decreased in preadolescence (Fig. 4A and B). We observed a tendency for the portion of L-655,708-sensitive total \( I_{\text{ionic}} \) to decrease with age, although this did not reach statistical significance.

In further experiments, we directly compared the expression of the GABA\(_{\alpha 5}\) receptor \( \alpha_5 \) subunit in DGs in the different age groups (Fig. 4C and D). Although we detected very low level of GABA\(_{\alpha 5}\) subunit immune reactivity at P7 and P14 in DGs, Western blot analysis showed that the expression of the GABA\(_{\alpha 5}\) subunit gradually decreased in the juvenile (P21 and P28) and adolescent (P35) periods, and thereafter stabilized in the young adults (P42). The degree of GABA\(_{\alpha 5}\) subunit expression was not further changed at P49 and P56 (data not shown).

**DISCUSSION**

The main findings of the present study were as follows: 1) age-dependent increase in the \( I_{\text{NO-711}} \) and \( I_{\text{SNAP-514}} \) of DGGCs at infantile stages were partially consistent with the gradual increase in GAT-1 and GAT-3 expression in infantile and juvenile DGs; 2) the age-dependent decrease in \( I_{\text{SNAP}} \) was compromised as GABA\(_{\alpha 5}\) subunit expression gradually decreased during preadolescence.

---

**Fig. 3. Effects of nipecotic acid (NPA) on \( I_{\text{ionic}} \) according to age.** (A) Representative current traces before and during the sequential application of NPA (100 μM), a nonselective GAT blocker, and NPA+L-655,708, an inverse agonist of the GABA\(_{\alpha 5}\) receptor \( \alpha_5 \) subunit. Note that the effects of NPA, which were blocked by BIC, were larger at P6-8 than in older age. (B) Mean changes in the inward \( I_{\text{holding}} \) shift after NPA (INPA), and the outward \( I_{\text{holding}} \) shift following additional application of L-655,708 (IL,655-708), are summarized. Summarized data are shown as means±SEM (n=6-7). *p<0.05, **p<0.01 compared with P6-8.

**Fig. 4. Age-dependent decrease of GABA\(_{\alpha 5}\) subunit expression in preadolescent rats.** (A) Representative current traces before and after the sequential application of GABA (5 μM) and GABA+L-655,708, an inverse agonist of the GABA\(_{\alpha 5}\) receptor \( \alpha_5 \) subunit. (B) Mean changes in the inward \( I_{\text{holding}} \) shift by GABA and outward \( I_{\text{holding}} \) shift following additional application of L-655,708 (IL,655-708) are summarized. Summarized data are shown as means±SEM (n=6-7). *p<0.05, **p<0.01, ***p<0.001 compared with P6-8. (C) Representative Western blot analysis showing GABA\(_{\alpha 5}\) subunit expression at different ages. (D) Summarized GABA\(_{\alpha 5}\) subunit expression at different ages. The protein expression was normalized to the level detected at P21. Summarized data are shown as means±SEM (n=4). *p<0.05, **p<0.01, ***p<0.001 compared with P21.
Together, these findings suggest that, in addition to regulation of the ambient GABA concentration according to GAT activity, the change in age-dependent \( I_{\text{tonic}} \) mirrored the altered expression and/or composition of extrasynaptic \( \alpha_5 \)-GABA\(_A\)Rs during preadolescence.

## Greater role of GAT-1 versus GAT-3 in regulating the \( I_{\text{tonic}} \) of DGGCs

GATs embedded in axon terminal membranes and/or astrocyte plasma membranes regulate ambient GABA levels. In many neural systems, GAT-1 antagonists alone result in a smaller \( I_{\text{tonic}} \) versus that observed when both GAT-1 and GAT-3 are blocked [22,23]. This was explained by the involvement of both GAT-1 and GAT-3 transporters in regulating extracellular GABA concentrations around neurons. Similarly, \( I_{\text{tonic}} \) was much larger than \( I_{\text{NO-711}} \) in infantile and juvenile DGGCs in the present study. Interpretation of this difference is complicated because, as opposed to NO-711, NPA is a GAT substrate. In addition, NPA could result in heteroexchange for GABA by GATs [24]. However, given that the concentrations of NPA and NO-711 used in this study were, respectively, about five and ten times that of the \( I_{\text{IC}_{50}} \) used for GAT-1 blockade [14], a simple interpretation could be that NPA blocked GAT-3 more efficiently than NO-711 during the infantile stage. However, our results showed that the GAT-1 blocker alone, and the GAT-1 blocker with additional application of SNAP-5114 (100 \( \mu \)M), resulted in a similar average \( I_{\text{tonic}} \) of 20 pA in DGGCs after the late infantile periods; these results contradict the idea that GAT-3 actively contributed to the \( I_{\text{tonic}} \) of DGGCs. In the present study, SNAP-5114 facilitated \( I_{\text{tonic}} \) at the concentration of 300 \( \mu \)M, which was near to the \( I_{\text{IC}_{50}} \) for GAT-1 blockade [14], further confounding the role of GAT-3 in the \( I_{\text{tonic}} \) of DGGCs. Combined with the fact that both GAT-1 and GAT-3 expression increased until the late juvenile periods, these results appear to support a major role of GAT-1 in the GABA uptake regulating \( I_{\text{tonic}} \) in the adult hippocampus [16]. In general, our results suggest that GAT-1 and GAT-3 play a primary and adjunctive role, respectively, in regulating \( I_{\text{tonic}} \) of DGGCs in preadolescence.

As with other neurotransmitter transporters, GATs can also act in reverse mode and thus release GABA from cells. Indeed, GABA can be secreted from cells by the reversed transport direction of GATs, particularly during early postnatal stages [25,26]. Thus, it is possible that GABA release via reversed GAT activity is integral in maintaining GABA levels that activate \( I_{\text{tonic}} \) [27] in the infantile and early juvenile stages. However, our finding that GAT-1 and GAT-3 blockers always enhanced the \( I_{\text{tonic}} \) of DGGCs suggest that the two transporters operate synergistically to promote GABA uptake, which was seen at all tested ages in our experiments.

### Role of \( \alpha_5 \)-GABA\(_A\)Rs in age-dependent \( I_{\text{tonic}} \) attenuation during preadolescence

Both the \( \alpha_5 \) and \( \delta \) subunit are key mediating components of the \( I_{\text{tonic}} \) of DGGCs [28]. Alterations in GABA concentrations affect the relative contribution of specific GABA\(_A\)Rs to \( I_{\text{tonic}} \) as different receptor populations are recruited [8]. In the present study, BIC uncovered basal \( I_{\text{tonic}} \) shown by the outward \( I_{\text{holding}} \) shift went over the initial level, especially when the inward shift in \( I_{\text{holding}} \) by exogenous GABA and GAT blockers was less than ~30 pA. \( \alpha_5 \)-GABA\(_A\)Rs contribute to \( I_{\text{tonic}} \) when ambient GABA concentrations increase, while at low ambient GABA concentrations the activation of \( \delta \)-GABA\(_A\)Rs predominates [9]. In the present study, \( I_{\text{OPA}} \) was larger than \( I_{\text{NO-711}} \) during preadolescence, which could be explained by an ambient GABA concentration sufficient to recruit additional \( \alpha_5 \)-GABA\(_A\)Rs in the presence of NPA, but not in NO-711. Our results showed that the portion of L-655,708-sensitive \( I_{\text{OPA}} \) ranged from ~27% to ~32%; this is consistent with previous findings showing that \( I_{\text{tonic}} \) is mediated by \( \alpha_5 \)-GABA\(_A\)Rs and is responsible for ~29% of the total \( I_{\text{tonic}} \) in DGGCs [28]. Thus, \( \alpha_5 \)-GABA\(_A\)Rs mediated \( I_{\text{OPA}} \) at all tested ages. Combined with the results whereby \( I_{\text{OPA}} \) and GABA\(_A\)R \( \alpha_5 \) subunit expression gradually decreased with age, our results suggest that the age-dependent \( I_{\text{tonic}} \) decrease mirrors the functional decrease of \( \alpha_5 \)-GABA\(_A\)Rs rather than changes in GATs activity, during preadolescence.

However, there may be an as-yet undiscovered, non \( \alpha_5 \)-containing GABA\(_A\)Rs responsible for the large \( I_{\text{OPA}} \) observed during preadolescences. Indeed, in the present study, GABA\(_A\)R \( \alpha_5 \) subunit immunoreactivity was not detectable in DGs at infantile stages. It is also notable that the small amplitude of \( I_{\text{NO-711}} \) prevented us from comparing the sensitivity of \( I_{\text{OPA}} \) and that of \( I_{\text{NO-711}} \) to L-655,708 in DGGCs. Regarding GABA\(_A\)Rs activated in the presence of NPA, it is also noteworthy that NPA can directly activate GABA\(_A\)R-like channels [29]. However, to the best of our knowledge, there is no information on the composition of GABA\(_A\)R\_s directly activated by NPA. However, it is still of interest that GABA\(_A\)R \( \alpha_5 \) subunit expression gradually decreased with the postnatal development in various brain regions.

### Functional consequences

Overall, our results showed that GATs blockades elevated ambient GABA level sufficiently to harmonize with \( \alpha_5 \)-GABA\(_A\)Rs, resulting in an age-dependent \( I_{\text{tonic}} \) decrease in preadolescent brains. Combined with the fact that GABA\(_A\)R \( \alpha_5 \) subunit expression in the hippocampus is closely related to learning and memory in young adults [30,31], selective pharmacological modulators, such as \( \alpha_5 \)-GABA\(_A\)R selective inverse agonists, may be effective in increasing cognitive performance in memory disorders [32]. Future studies are warranted to elucidate the pathophysiology of \( \alpha_5 \)-GABA\(_A\)Rs generating \( I_{\text{tonic}} \) combined with GAT blockades in the developing brains of preadolescents.
ACKNOWLEDGEMENTS

This work was supported by Chungnam National University and National Research Foundation of Korea funded by the Ministry of Education, Science and Technology (NRF-2015R1D1A1A02059430).

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

1. Farrant M, Nusser Z. Variations on an inhibitory theme: phasic and tonic activation of GABA(A) receptors. Nat Rev Neurosci. 2005;6:215-229.
2. Semyanov A, Walker MC, Kullmann DM, Silver RA. Tonically active GABA A receptors: modulating gain and maintaining the tone. Trends Neurosci. 2004;27:262-269.
3. Brickley SG, Mody I. Extrasynaptic GABA(A) receptors: their function in the CNS and implications for disease. Neuron. 2012;73:23-34.
4. Hines RM, Davies PA, Moss SJ, Maguire J. Functional regulation of GABAA receptors in nervous system pathologies. Curr Opin Neurol. 2012;25:522-528.
5. Jang HJ, Cho KH, Park SW, Kim MJ, Yoon SH, Rhie DJ. The development of phasic and tonic inhibition in the rat visual cortex. Korean J Physiol Pharmacol. 2010;14:399-405.
6. Pandit S, Jeong JA, Jo JY, Cho HS, Kim DW, Kim JM, Ryu PD, Lee SY, Kim HW, Jeon BH, Park JB. Dual mechanisms diminishing tonic GABAA inhibition of dentate gyrus granule cells in Noda epileptic rats. J Neurophysiol. 2013;110:95-102.
7. Pandit S, Jo JY, Lee SU, Lee YJ, Lee SY, Ryu PD, Lee JU, Kim HW, Jeon BH, Park JB. Enhanced astroglial GABA uptake attenuates tonic GABAA inhibition of the presym pathetic hypothalamic paraventricular nucleus neurons in heart failure. J Neurophysiol. 2015;114:914-926.
8. Semyanov A, Walker MC, Kullmann DM. GABA uptake regulates cortical excitability via cell type-specific tonic inhibition. Nat Neurosci. 2003;6:484-490.
9. Scimemi A, Semyanov A, Sperk G, Kullmann DM, Walker MC. Multiple and plastic receptors mediate tonic GABAA receptor currents in the hippocampus. J Neurosci. 2005;25:10016-10024.
10. Holter NI, Zylla MM, Zuber N, Bruehl C, Drughun A. Tonic GABAergic control of mouse dentate granule cells during postnatal development. Eur J Neurosci. 2010;32:1300-1309.
11. Lee CY, Liou HH. GABAergic tonic inhibition is regulated by developmental age and epilepsy in the dentate gyrus. Neuroreport. 2013;24:515-519.
12. Fleming RL, Wilson WA, Swartzwelder HS. Magnitude and ethanol sensitivity of tonic GABAA receptor-mediated inhibition in dentate gyrus changes from adolescence to adulthood. J Neurophysiol. 2007;97:3806-3811.
13. Bright DP, Smart TG. Methods for recording and measuring tonic GABAA receptor-mediated inhibition. Front Neural Circuits. 2013;7:193.
14. Borden LA. GABA transporter heterogeneity: pharmacology and cellular localization. Neurochem Int. 1996;29:335-356.
15. Song I, Volyenski K, Brenner T, Uskokov A, Semyanov A, different transporter systems regulate extracellular GABA from vesicular and non-vesicular sources. Front Cell Neurosci. 2013;7:23.
16. Kersanté F, Rowley SC, Pavlov I, Gutiérrez-Mecinas M, Semyanov A, Reul JM, Walker MC, Linthorst AC. A functional role for both-aminobutyric acid (GABA) transporter-1 and GABA transporter-3 in the modulation of extracellular GABA and GABAergic tonic conductances in the rat hippocampus. J Physiol. 2013;591:2429-2441.
17. Evans JE, Frosdolm A, Rotter A. Embryonic and postnatal expression of four gamma-aminobutyric acid transporter mRNAs in the mouse brain and leptomeninges. J Comp Neurol. 1996;376:431-446.
18. Pai YH, Lim CS, Park KA, Cho HS, Lee GS, Shin YS, Kim HW, Jeon BH, Yoon SH, Park JB. Facilitation of AMPA receptor-mediated steady-state current by extrasynaptic NMDA receptors in suprapeptidic magnocellular neurosecretory cells. Korean J Physiol Pharmacol. 2016;20:425-432.
19. Schmidt-Hieber C, Jonas P, Bischoberger J. Enhanced synaptic plasticity in newly generated granule cells of the adult hippocampus. Nature. 2004;429:184-187.
20. Suzdak PD, Frederiksen K, Andersen KE, Sørensen PO, Knutsen IJ, Nielsen EB. NNC-711, a novel potent and selective gamma-aminobutyric acid uptake inhibitor: pharmacological characterization. Eur J Pharmacol. 1992;224:189-198.
21. Quirk K, Blurton P, Fletcher S, Leeson P, Tang F, Mellilo D, Ragan CL, McKernan RM. [3H]L-655,708, a novel ligand selective for the benzodiazepine site of GABAA receptors which contain the alpha 5 subunit. Neuropharmacology. 1996;35:1331-1335.
22. Gao H, Smith BN. Tonic GABAA receptor-mediated inhibition in the rat dorsal motor nucleus of the vagus. J Neurophysiol. 2018;109:904-914.
23. Keros S, Hablitz JJ. Subtype-specific GABA transporter antagonists synergistically modulate phasic and tonic GABAA conductances in rat neocortex. J Neurophysiol. 2005;94:2073-2085.
24. Solis JM, Nicoll RA. Postsynaptic action of endogenous GABA released by nipecotic acid in the hippocampus. Neursci Lerr. 1992;47:16-20.
25. Demarque M, Represa A, Becq H, Khalilov I, Ben-Ari Y, Aniksztejn L. Paracrine intercellular communication by a Ca2+- and SNARE-independent release of GABA and glutamate prior to synapse formation. Neuron. 2002;36:1051-1061.
26. Taylor J, Gordon-Weeks PR. Calcium-independent gamma-aminobutyric acid release from growth cones: role of gamma-aminobutyric acid transport. J Neurochem. 1991;56:273-280.
27. Richerson GB, Wu Y. Dynamic equilibrium of neurotransmitter transporters: not just for reuptake anymore. J Neurophysiol. 2003;90:1363-1374.
28. Glykys J, Mann EO, Mody I. Which GABA(A) receptor subunits are necessary for tonic inhibition in the hippocampus? J Neurosci. 2008;28:1421-1426.
29. Barrett-Jolley R. Nipecotic acid directly activates GABA(A)-like ion channels. Br J Pharmacol. 2001;133:673-678.
30. Gill KM, Grace AA. The role of a5 GABAA receptor agonists in the
31. Prut L, Prenosil G, Willadt S, Vogt K, Fritschi JM, Crestani F. A reduction in hippocampal GABA A receptor alpha5 subunits disrupts the memory for location of objects in mice. Genes Brain Behav. 2010;9:478-488.

32. Atack JR. Preclinical and clinical pharmacology of the GABA A receptor alpha5 subtype-selective inverse agonist alpha5IA. Pharmacol Ther. 2010;125:11-26.