Sex-dependent sensitivity to positive allosteric modulation of GABA action in an APP knock-in mouse model of Alzheimer’s disease: Potential epigenetic regulation

James Auta a,1, Andrea Locci b,1, Alessandro Guidotti a, John M. Davis a, Hongxin Dong b,∗

a Center for Alcohol Research in Epigenetics, Psychiatric Institute, Department of Psychiatry, College of Medicine, University of Illinois at Chicago, Chicago, IL, USA
b Department of Psychiatry and Behavioral Sciences, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA

1. Introduction

Alzheimer’s disease (AD) is the most common neurodegenerative disorder characterized by progressive memory decline combined with certain neuropsychiatric symptoms, pathological changes such as neurofibrillary tangles and senile plaques, and preferential neuronal loss in hippocampus and prefrontal cortex (Kwakowsky et al., 2018a; Braak and Braak, 1991; Alzheimer’s Association, 2012). Years of extensive research into the mechanisms underlying AD have yielded several possible mechanisms including β-amyloid (Aβ) oligomer-induced neuronal death and synaptic dysfunction, glutamate-induced excitotoxicity (Muke and Selkoe, 2012), loss of cholinergic neurons, and reduced cortical choline acetyltransferase activity (Mufson et al., 2008).

There is growing evidence in support of important contributions of the primary inhibitor neurotransmitter, gamma-aminobutyric acid (GABA), to the pathogenesis of AD (Fritschy, 2008; Rudolph and Mohler, 2014a; Solas et al., 2015; Francis, 2005). In particular, postmortem studies have reported that AD patients have increased GABA levels in the cerebrospinal fluid (Li et al., 2016a) as well as decreased GABA levels in the most brain areas (Govindpani et al., 2017). However, inconsistent findings of GABA levels in the frontal, parietal and temporal cortices, and the hippocampal regions of AD postmortem brains have also been reported (Govindpani et al., 2017; Imbimbo and Giardina, 2011). In a preclinical study, Bell and colleagues found significant increase in
GABAergic presynaptic bouton density in a TgCRND8 mouse model of AD (Bell et al., 2003). Most significantly, administration of the GABA_A receptor antagonists, bicuculline and picrotoxin rescued memory deficits in adult APP/PS1 mice (Yoshiike et al., 2008). However, previous studies painted a complex, convoluted, and often inconsistent picture of the relationship between GABAergic system and AD. Given the importance of GABAergic neurotransmission in neuronal function and homeostasis, maintenance of excitatory/inhibitory balance, and learning and memory processes, alterations of GABAergic function could be a critical factor in AD pathogenesis. Therefore, a better understanding of the role of GABAergic remodeling in AD could help discovering new targets for the development of innovative therapeutic alternatives. In fact, there is increasing interest in developing novel therapeutic agents targeting the alterations of GABAergic neurotransmission that have been reported in AD (Kwakowsky et al., 2018b; Li et al., 2016b; Guzman et al., 2018).

Although mounting evidence linking dysregulated GABAergic neurotransmission and AD (Kwakowsky et al., 2018a, 2018b; Govindapani et al., 2017; Guzman et al., 2018), the molecular mechanisms and sex-related impact underlying this linkage remain elusive. In this study, we used a newly developed AD mouse model, namely APP^{NLGF} mice, which expresses a humanized Aβ$_{42}$ sequence harboring three familial AD mutations (Swedish (NL), Beyreuther/Iberian (F), and Artic (G)) to model Aβ amyloidosis without non-physiological overexpression of APP (Sasaguri et al., 2017a). We used this AD mouse model to examine the sex-dependent role of GABAergic neurotransmission in AD by focusing on the pharmacological effects of a positive allosteric modulator of GABA action at GABA_A receptors. Specifically, we evaluated the behavioral alterations elicited by acute administration of diazepam, a typical and full positive allosteric modulator of GABA action at GABA_A receptors (Guidotti et al., 2005), on locomotor activity, anxiety-like behavior, and memory function in male and female APP^{NLGF} mice. Of note, we used acute diazepam administration as a pharmacological tool to better understand the functional status of the GABAergic neurotransmission, rather than exploring a possible “therapeutic effect” of this drug in AD mice. Moreover, we evaluated the expression of specific GABA_A receptor subunits in the hippocampus of male and female APP^{NLGF} mice. We chose the hippocampus because it is one of the brain areas linked to memory deficits in AD and one of the most vulnerable areas exhibiting neuronal loss in both postmortem brains of AD subjects and preclinical models of AD (Braak and Braak, 1991; Serrano-Pozo et al., 2011). In addition, we examined the possible epigenetic mechanisms underlying differential expression of GABA_A receptor subunits in the hippocampus between male and female APP^{NLGF} mice, and most likely, the differential sensitivity to diazepam-induced behavioral effects in these mice.

2. Materials and methods

A total of 116 (12-month-old) male (n = 58) and female (n = 58) APP^{NLGF} mice [APP KM670/671NL (Swedish), APP 1716F (Iberian), APP E693G (Artic); modification: APP: knock-in; homozygous; C57BL/6J background] and their respective wildtype littermates were used for this study. APP^{NLGF} breeders were kindly provided by RIKEN (Japan). Genotyping was done at 21–24 days after birth by PCR with APP^{NLGF} primers (forward: 5’-CTCCTTGTGGCTGGCGGTCACAC-3’; reverse: 5’-CTATGTGGACCGAGAATGGTCAAG-3’). The offspring were then separated with their original littermates into cages based on sex. Heterozygous APP^{NLGF} mice were not used in the current study. Animals were housed in groups of 5 on a 12-hr light/dark cycle (lights on at 8:00 a.m.) with access to chow and water made available ad libitum. All experiments and procedures were carried out in accordance with the National Institute of Health Guidelines for the treatment of animals and the Current Guide for the Care and Use of Laboratory Animals under protocol approved by the Northwestern University Animal Care and Use Committee.

2.1. Drug treatment

Diazepam was obtained from Hoffman-La Roche (Nutley, NJ). The drug was dissolved in 5–10% of DMSO (depending on the desired final concentration) and subsequently diluted with vehicle containing 11% polyethylene glycol-400, 50% propylene glycol and 30% sterile water. Injection for vehicle and diazepam was 0.1 ml/10g body weight. Mice received intraperitoneal (i.p.) injection of vehicle or diazepam 30 min prior to behavioral testing or euthanasia.

2.2. Behavioral studies

Mice were handled once daily for five consecutive days before initiating behavioral experiments to minimize the potential stress that might be caused by interaction with the experimenter. A series behavioral test separated by 5-days inter-test intervals to allow complete drug washout were conducted beginning at 12 months of age; all behavioral tasks began at 9 a.m. Prior to testing, mice were transferred from their normal housing to the testing room 1 h before experimental session to allow for pretest-acclimation and to minimize potential stress due to exposure to new environment. Importantly, between each session, the chambers, tools, and instruments were carefully cleaned with 70% alcohol to eliminate olfactory odors. As previously described (Locci et al., 2021), the following testing sequence was used: a) locomotor activity/open field; b) novel object recognition; c) Y-maze; d) light-dark box.

2.2.1. Open field (OF)

The open field test was used to evaluate general locomotor activity and to assess anxiety behavior and the functional status of GABAergic neurotransmission in adult APP^{NLGF} mice. A computerized automated system (Any-Maze) with an open field box chamber (50 cm × 50 cm × 40 cm) was used to evaluate locomotor activity as previously described (Locci and Pinna, 2019). Thirty minutes post vehicle or diazepam treatment, mice were placed in the center of the chambers. Horizontal sensor beam interruptions recorded as distance travelled in the open field box during 10 min trial sessions.

2.2.2. Light dark box (LD)

Mice were tested for anxiety-like behavior using the LD exploration/transition test (Rulesskaya and Voikar, 2014). The apparatus consists of a two-chamber metal grid floor shuttle box separated by a guillotine door. One compartment is dark, and the other is illuminated with a bright stimulus light (500 lux). Photobeams installed in each chamber detect the location and locomotor activity of the mice and report these activities in a Graphic State computer program (Colbourne), which controls the experimental processes. The testing equipment was housed within a sound-attenuating chamber, which contained a fan that provides 68 dB of background noise. On testing day, the animal was placed in the light chamber allowed to explore both the dark and light chambers for 10 min. Rodents prefer dark over light areas; however, when exposed to a novel environment, rodents tend to explore. These two conflicting “emotions” lead to observable anxiety-like behavior manifested as greater time spent in the safer, dark areas. The percent of time spent in the light and dark compartments represents anxiety-like or anxiogenic-like behavior.

2.2.3. Novel object recognition (NOR)

The Novel object recognition is a well-established behavioral benchmark for assessing recognition memory, the human correlate of declarative memory (Brodmann et al., 2009a). The NOR test was performed in an apparatus consisting of an evenly illuminated plexiglass box (25 cm × 25 cm × 25 cm). The procedure consisted of 3 phases over a 24-day period: habituation, training, and retention. During the habituation phase (Day 1), each mouse was placed in the empty box and allowed to freely explore the box for 10 min. For the training phase (day
2–24) phase, two identical objects were placed in the box and positioned 6 cm away from the walls on opposite corners and each mouse allowed to explore the identical objects for 10 min and then returned to its home cage. The retention or testing (day 24) phase began 1 h after completion of the training phase; one of the two familiar objects was replaced with a novel object and the mouse allowed to explore the familiar and novel objects for 10 min. Object exploration was defined as sniffing, licking, or touching the objects with forepaw, but not leaning against, turning around, standing, or sitting on the objects. Object exploration was later scored from video recordings by an experimenter who was blinded to the treatment groups. The proportion of time spent exploring the novel object served as the measure of recognition memory for the familiar object. The data were expressed as discrimination index (DI) which represents the percentage of the time a mouse spent exploring the novel object (compared to the time spent exploring the familiar object) as a proportion of the time spent exploring both objects during retention testing.

2.2.4. Y-maze

The Y-maze is a spontaneous alternation behavioral paradigm used to assess short term and/or spatial working memory by evaluating the “willfulness” of rodents to explore novel environments (Broadbent et al., 2009b). This spontaneous alternation Y-shaped apparatus consists of three opaque arms (5 cm wide × 21 cm long × 15.5 cm high) containing special cues positioned in the top inner part of each arm and oriented at 120° from each other. Mice are driven by an innate curiosity to explore previously unvisited areas; therefore, mice were allowed to explore the three arms of a maze placed on a stable table with an overhead video recorder. Mice were placed in the arm “A” (starting point, excluded by the analysis) facing the end of the arm and allowed to freely explore the apparatus for 5 min while a camera records their movements. A correct alternation is defined as discrete and successive entries into each open arm, including events where the animal directly progresses from one arm to the next in consecutive fashion (that is ABC, ACB, BAC, BCA, CAB, and CBA) without reentering two previously visited arms. The percentage of spontaneous alternation is expressed as the ratio of total successful alternations by the total number of possible alternations (i.e., the number of total entries minus two) multiplied by 100. The activity of each mouse was also recorded using an automated tracking system (Any-Maze).

2.3. Tissue collection, RNA extraction, and real-time polymerase chain reaction (PCR)

Five days after behavioral testing, mice were sacrificed by i.p. injection of Euthasol® solution followed by cervical dislocation and hippocampal tissue quickly dissected, frozen and stored at –80 °C. Only tissues belonging to vehicle-treated groups were used for relative quantitative real-time polymerase chain reaction (RT-qPCR), ChIP assay or Western blot. Total RNA was isolated from hippocampal tissue using TRIzol reagent and further purified using Qiagen RNeasy Kit and converted to cDNA using the Applied Biosystems (USA) High-Capacity Archive Kit (4368813). Relative qPCR was performed with the Applied Biosynthesis Real-Time PCR system using Maxima SYBR Green master mix and primers targeting two locations within Gabra1 and Gabra5 as previously described (Pandey et al., 2015; Gatta et al., 2021). Briefly, hippocampal tissues were weighed and fixed in methanol-free 1% formaldehyde by incubating on a rocking platform at room temperature for 15–20 min followed by homogenization in lysis buffer and DNA shearing by sonication. The resulting cross-linked DNA-chromatin complex was immunoprecipitated (IP) with anti-acetylated histone H3 or H3K4me3 antibody and antibody-chromatin complexes precipitated using protein A/G antibody-chromatin complexes precipitated using protein A/G Plus-Agarose (Santa Cruz Biotech, Inc., Dallas, Texas). The final precipitated and purified DNA was concentrated using Chlex 100 Resin (BioRad, DesPlaines, IL) and quantified using qPCR with Maxima SYBR Green master mix and primers targeting two locations within Gabra1 (Forward: 24 to –3, Reverse: +83 to +63 [site 1] and Forward: 451 to –430, Reverse: 343 to –324 [site 2]) and Gabra5 (Forward: 74 to –56, Reverse: +8 to +17 [site 1] and Forward: 183 to –165, Reverse: 86 to –65 [site 2]) GABA A receptor promoter, respectively. Input DNA was used as internal standard for normalization. After subtraction of input DNA Ct value from the Ct values of the respective samples, the ΔΔCt method (Livak and Schmittgen, 2001) was used to determine the fold change in levels of H3K4me3 H3-acetylated at the Gabra1 and Gabra5 promoters. The percentages of IP DNA were calculated using the following formula: % (IP/total input) = 2^(-ΔΔCt) (10% input: Ct (IP) – 100%). The sequences for the two sets of primer pair for Gabra1 and Gabra5 promoters are provided in Supplementary Table 2. Detailed information on the antibodies used for our experiments are reported in Supplementary Table 3.

2.6. Statistical analysis

All data are represented as mean ± SEM and were analyzed using levels were calculated as Ct (Livak and Schmittgen, 2001; Schmittgen and Livak, 2008).

2.4. Western blot (WB)

We performed WB analysis to investigate whether the changes in α1 and α5 GABAA receptor subunit mRNA expression observed were accompanied with changes in protein expression. As we previously described (Locci and Pinna, 2019; Locci et al., 2020), hippocampal tissues were homogenized in a mixture of ice-cold RIPA buffer (Sigma-Aldrich) and protease inhibitor cocktail solution (Fisher Scientific), centrifuged at 20,000 g for 10 min at 4 °C and supernatant collected for total protein concentration determination using Pierce® bicinchoninic acid (BCA) assay kit (Fisher Scientific). Protein lysates were re-suspended in Laemmli reducing buffer and 20 μg of each sample loaded and resolved by electrophoresis in 10% Criterion TGX Stain-Free Precast Gels at 100 V for 1.5 h (BioRad). Membranes were blocked using 5% milk and probed by overnight incubation at 4 °C with specific primary antibodies against α1 (1:200; 12410-1-AP, Proteintech) and α5 (1:500; NB300-195, Novus) GABA A receptor subunits, respectively. Blots were detected by Gel Doc EQ System Universal Hood II (Biorad) and quantified using ImageJ software (https://imagej.nih.gov/ij/download.html). The expression levels for the respective proteins were normalized with β-actin (1:1000; sc-47778, Santa Cruz Biotechnology). In addition, we confirmed that β-actin expression was not impacted by sex by conducting a parallel experiment using Gapdh as primary antibody (1:1000; sc-32233, Santa Cruz Biotechnology).

2.5. Chromatin immunoprecipitation (ChIP) assay

To examine the role of epigenetic mechanisms in the differential expression of α1 and α5 GABAA receptor subunits in hippocampal of male and female APP N/LGd and wildtype mice we measured the levels of histone acetylation and methylation at α1 (Gabra1) and α5 (Gabra5) GABAA receptor subunit gene promoters. We used specific antibodies against anti-acetylated histone H3 (Millipore Corp USA, catalog # 06–599) and tri-methyl histone H3 lysine4 (H3K4me3) (Cell Signaling, catalog # 9751S) for ChIP assay as previously described (Pandey et al., 2015; Gatta et al., 2021). Briefly, hippocampal tissues were weighed and fixed in methanol-free 1% formaldehyde by incubating on a rocking platform at room temperature for 15–20 min followed by homogenization and lysis buffer and DNA shearing by sonication. The resulting cross-linked DNA-chromatin complex was immunoprecipitated (IP) with anti-acetylated histone H3 or H3K4me3 antibody and antibody-chromatin complexes precipitated using protein A/G Plus-Agarose (Santa Cruz Biotech, Inc., Dallas, Texas). The final precipitated and purified DNA was concentrated using Chlex 100 Resin (BioRad, DesPlaines, IL) and quantified using qPCR with Maxima SYBR Green master mix and primers targeting two locations within Gabra1 (Forward: 24 to –3, Reverse: +83 to +63 [site 1] and Forward: 451 to –430, Reverse: 343 to –324 [site 2]) and Gabra5 (Forward: 74 to –56, Reverse: +8 to +17 [site 1] and Forward: 183 to –165, Reverse: 86 to –65 [site 2]) GABA A receptor promoter, respectively. Input DNA was used as internal standard for normalization. After subtraction of input DNA Ct value from the Ct values of the respective samples, the ΔΔCt method (Livak and Schmittgen, 2001) was used to determine the fold change in levels of H3K4me3 H3-acetylated at the Gabra1 and Gabra5 promoters. The percentages of IP DNA were calculated using the following formula: % (IP/total input) = 2^(-ΔΔCt) (10% input: Ct (IP) – 100%). The sequences for the two sets of primer pair for Gabra1 and Gabra5 promoters are provided in Supplementary Table 2. Detailed information on the antibodies used for our experiments are reported in Supplementary Table 3.
mice, behavioral data, differences between mean values were determined using 3-way ANOVA followed by post-hoc comparison using Tukey’s test when statistical significance for ANOVA was reached. Whereas differences between mean values for biochemical measurements were determined using 2-way ANOVA followed by post-hoc comparison with Tukey’s test when significance for ANOVA was reached. Level of statistical significance for all experiments was set at \( p < 0.05 \).

3. Results

3.1. Male APP\(^{NLGF}\) mice exhibit greater sensitivity to diazepam-induced impairment of locomotor activity

To evaluate the functional status of GABAergic neurotransmission in APP\(^{NLGF}\) mice, we compared the effects of three doses (0.2, 1, and 3 mg/kg) of diazepam, a positive allosteric modulator of GABA action at a variety of GABA\(_A\) receptor subtypes (Guidotti et al., 2005), on locomotor activity in male and female APP\(^{NLGF}\) mice and their wildtype counterparts. It is noteworthy that acute or chronic administration of this dose-range of diazepam does not increase GABA\(_A\) receptor subunit expression in the hippocampus (Impagnatiello et al., 1996). Three-way ANOVA revealed that 3 mg/kg of diazepam markedly suppressed locomotor activity in both wildtype and APP\(^{NLGF}\) mice (Fig. 1, F \([1,48] = 206, p < 0.0001\) ). There was no significant interaction between drug - sex - genotype (F \([1,48] = 0.0823, p = 0.3686\) ). We also found that at 1 mg/kg, diazepam significantly suppressed locomotor activity (Fig. 1, F \([1,48] = 37.42, p < 0.0001\) ); specifically, post hoc test showed significant effect between diazepam-treated wildtype male mice vs. diazepam-treated APP\(^{NLGF}\) male mice \((p < 0.0278)\) and diazepam-treated APP\(^{NLGF}\) male mice vs. diazepam-treated APP\(^{NLGF}\) female mice \((p < 0.0279)\). In addition, there was significant interaction between drug - sex - genotype (F \([1,48] = 6.507, p = 0.0140\) ) and between drug and genotype (F \([1,48] = 16.60, p = 0.0002\) ). Although three-way ANOVA analyses revealed statistically significant drug effect at 0.2 mg/kg of diazepam (Fig. 1, F\([1,48] = 23.67, p < 0.0001\) ), there was no significant interaction between drug-sex-genotype (F \([1,48] = 1.035, p = 0.3141\) ). However, there was significant effect of genotype (F \([1,48] = 15.93, p = 0.0002\) ) and a significant interaction between drug-genotype (F \([1,48] = 7.374, p = 0.0092\) ). All together, these data suggest that APP\(^{NLGF}\) male and female mice have significantly higher basal locomotor activity than their wildtype counterparts. Most importantly, male APP\(^{NLGF}\) mice showed greater sensitivity to the effect of diazepam than the female APP\(^{NLGF}\) and their wildtype counterparts. Based on these observations, we chose 0.2 mg/kg of diazepam, which produced minimal suppression of locomotor activity and similar effects in male and female APP\(^{NLGF}\) and wildtype mice, to further investigate the effects of diazepam on anxiolytic-like behavior, recognition, and spatial working memory paradigms.

3.2. A low dose of diazepam induced anxiolytic-like effect and worsened recognition memory impairment in male APP\(^{NLGF}\) mice

In the LD test for anxiety-like behavior (Fig. 2A), 0.2 mg/kg of diazepam produced significant increase of time spent in the light compartment in both wildtype and APP\(^{NLGF}\) male and female mice (F \([1,48] = 34.45, p < 0.0001\) ). In addition, post hoc comparison indicated that at baseline, male \((p = 0.0305)\) but not the female \((p = 0.1228)\) APP\(^{NLGF}\) mice spent significantly \((p = 0.0305)\) less time in the light compartment when compared to the wild type counterparts. Although this dose of diazepam did not produce statistically significant increase of time spent in the light compartment in male and female wild type mice, it produced significant anxiolytic-like effects in male \((p < 0.0001)\) and female \((p = 0.0002)\) APP\(^{NLGF}\) mice. However, diazepam was more efficacious in male than in female APP\(^{NLGF}\) mice (diazepam-treated male APP\(^{NLGF}\) mice vs. diazepam-treated female APP\(^{NLGF}\) mice, \(p = 0.0005\) ). Furthermore, three-way ANOVA analysis revealed significant effect of sex (F \([1,48] = 9.770, p = 0.003\) ), interactions of drug-sex (F \([1,48] = 9.606, p < 0.0032\) ), and drug - sex - genotype (F \([1,48] = 3.4746, p < 0.0001\) ) but no significant effect of genotype (F \([1,48] = 2.606, p = 0.1130\) ) or interaction of drug - sex-genotype (F \([1,48] = 3.746, p = 0.0588\) ). However, the number of crossings between light and dark compartment did not differ between all the experimental groups (Supplementary Fig. 1).

The result of the NOR test indicated that 0.2 mg/kg of diazepam resulted in a significant decrease of the discrimination index for the novel object in both wild type and APP\(^{NLGF}\) mice \((F[1,48] = 22.85, p < 0.0001)\) (Fig. 2B). Interestingly, we also found a highly significant interaction for genotype (F \([1,48] = 208.2, p < 0.0001\) ), suggesting memory deficits in both male and female APP\(^{NLGF}\) mice at this age. Most importantly, post hoc comparison revealed that diazepam administration significantly decreased discrimination index in male \((p = 0.0027)\) but not female \((p > 0.999)\) wild type mice. Furthermore, post hoc comparison also indicated that while diazepam significantly decreased discrimination index in male \((p = 0.0003)\) APP\(^{NLGF}\) mice, it had no effect \((p = 0.96)\) in female APP\(^{NLGF}\) mice. Three-way ANOVA analysis revealed significant effect for sex (F \([1,48] = 9.7580, p < 0.003\) ), interactions between drug - sex (F \([1,48] = 9.606, p < 0.0001)\), and of drug - sex - genotype (F \([1,48] = 3.4746, p < 0.0001)\) but no there were significant interactions between drug - sex-genotype, sex-genotype, or drug - sex-genotype.

In contrast to the LD and the NOR tests, three-way ANOVA analysis indicated no significant drug effect (F \([1,48] = 0.7894, p = 0.3787)\) on the Y maze test for spatial memory (Fig. 2C) but a significant effect of sex (F \([1,48] = 4.321, p = 0.0490)\) and genotype (F \([1,48] = 9.7580, p < 0.0001)\). Post hoc comparison revealed a significant difference \((p = 0.1222)\) between percent spontaneous alternation for diazepam-treated male APP\(^{NLGF}\) mice and diazepam-treated female APP\(^{NLGF}\) mice. Although this dose of diazepam failed to elicit spatial alternation memory deficits, the data revealed spatial alternation memory deficits in
the vehicle-treated male and female APP<sup>NLG</sup> mice when compared to their respective vehicle-treated wild type counterparts. Taken together, the behavioral data demonstrate that both male and female APP<sup>NLG</sup> mice showed memory impairment but not anxiety-like behavior at 12 months of age. However, a low dose of diazepam (0.2 mg/kg) which did not suppress locomotor activity or impaired spatial working memory, induced stronger anxiolytic-like effects in male than female APP<sup>NLG</sup> mice. Importantly, diazepam was able to significantly worsen recognition memory in male but not in female APP<sup>NLG</sup> mice, confirming a sex-biased pharmacological effect.

3.3. The expression of α<sub>1</sub> and α<sub>5</sub> GABA<sub>A</sub> receptor subunits is significantly higher in male App<sup>NLG</sup> mice

To investigate the molecular mechanisms underlying enhanced sensitivity of male App<sup>NLG</sup> mice to diazepam-induced anxiolytic-like effects and impairment of recognition memory, we studied the expression of α<sub>1</sub> and α<sub>5</sub> GABA<sub>A</sub> receptor subunits in hippocampus of the same cohort of male and female App<sup>NLG</sup> and wild type mice used in the behavioral experiments. We chose the hippocampus because it is the most studied brain area that has been shown to regulate the cellular aspects of synaptic plasticity, long-term potentiation, and long-term depression which underlie the cognitive, learning, and memory deficits that have been reported in AD subjects and preclinical models of AD (LaFerla and Green, 2012; Puzzo et al., 2015). Additionally, previous studies showed that α<sub>1</sub>-containing GABA<sub>A</sub> receptors are responsible for the sedative, ataxic, and cognitive impairment associated with routinely prescribed benzodiazepine recognition site ligands which act as full positive allosteric modulators of GABA action (Rudolph et al., 1999). In contrast, the α<sub>5</sub>-containing GABA<sub>A</sub> receptors which are abundantly expressed in hippocampus, regulate tonic GABA-currents, learning, and memory functions (Caraiscos et al., 2004).
Two-way ANOVA analysis for α1 GABA_A receptor subunit mRNA expression in hippocampus indicated statistically significant (F [1,26] = 14.23, p = 0.0008) differences between groups, significant effect of sex (F [1,26] = 6.896, p = 0.014) but not genotype (Fig. 3A) on α1 GABA_A mRNA expression. In addition, post hoc comparison revealed that α1 GABA_A mRNA expression was significantly lower in female APP^NLGF than male APP^NLGF (p = 0.001) and female wild type (p = 0.048) mice (Fig. 3A). The results for α5 GABA_A receptor subunit mRNA expression revealed no significant interaction between groups (Fig. 3B).

For levels of α1 GABA_A receptor subunit protein expression (Fig. 3C), two-way ANOVA analysis revealed statistically significant effect of genotype (F [1,26] = 6.44, p = 0.0198) but not sex for α1 GABA_A protein expression. Post hoc comparison revealed significantly higher expression (p = 0.04) of α1 GABA_A protein in APP^NLGF male when compared to male wild type, near statistically significant difference (p = 0.0512) when compared to female wild type mice, but no significant difference when compared to APP^NLGF female mice (Fig. 3C). We also found significant effects of sex (F [1,26] = 4.674, p = 0.04) and genotype (F [1,26] = 6.44, p = 0.002) for α5 GABA_A receptor subunit protein expression (Fig. 3D). Furthermore, post hoc comparison revealed significantly higher α5 GABA_A protein expression in APP^NLGF male mice when compared to male (p = 0.01) and female (p = 0.002) wild type, and near statistically significant difference (p = 0.07) when compared to their APP^NLGF female counterparts (Fig. 3D). All together these data indicate that α1 and α5 GABA_A protein expression was higher in male APP^NLGF than in female APP^NLGF mice.

3.4. H3K4me3 epigenetic mark occupancy at Gabra1 and Gabra5 promoters correspond with the transcript levels for these receptors in hippocampus of male APP^NLGF mice

We investigated the role of epigenetic mechanisms on the increased α1 and α5 GABA_A receptor subunits transcript levels observed in the hippocampus of male APP^NLGF mice. To this end, we studied acetylated histone H3 and tri-methyl histone H3 lysine4 (H3K4me3) occupancy at Gabra1 and Gabra5 promoters using specific antibodies against...
acetylated histone H3 and tri-methyl histone H3 lysine4 (H3K4me3) for ChIP assay (Fig. 4). Two-way ANOVA analysis indicated no statistically significant differences between groups for acetylated-H3 occupancy at site 1 (F [1,24] = 0.7310, p = 0.4439), and site 2 (F [1,24] = 0.6309) of Gabra1 promoter, and at site 1 (F [1,24] = 0.32369, p = 0.5116) and site 2 (F [1,24] = 0.3041, p = 0.5864) of Gabra1 promoter, respectively. Post hoc comparison revealed that H3K4me3 occupancy at site 1 of the Gabra1 promoter was significantly higher in male (p = 0.0007) APPNLGF mice when compared to female APPNLGF mice, female wild type (p = 0.0015) and male wild type (p = 0.0066) mice (Fig. 4E). For the occupancy of H3K4me3 at site 2 of the Gabra1 promoter, post hoc analysis revealed statistically significant higher occupancy in male (p = 0.0002) APPNLGF mice when compared to female APPNLGF mice, female wild type (p = 0.0009) and male wild type (p = 0.0033) mice (Fig. 4F).

Two-way ANOVA analysis of H3K4me3 occupancy at Gabra5 promoter revealed statistically significant differences at site 1 (F [1,22] = 4.420, p = 0.0467) but not at site 2 (F [1,23] = 0.1211, p = 0.7310) of Gabra5 promoter (Fig. 4G and H, respectively). Post hoc comparison indicated that H3K4me3 occupancy at site 1 of Gabra5 promoter was significantly higher in male (p = 0.028) APPNLGF mice when compared to female APPNLGF mice, female wild type (p = 0.037) and male wild type (p = 0.0286) mice (Fig. 4G). These data indicate that the expression of α1 and α5 GABAA receptor subunit transcripts observed in male APPNLGF mice is regulated by H3K4me3 epigenetic mark, however the occupancy of this epigenetic mark is more remarkable at Gabra1 than at Gabra5 promoter. All together our ChIP data demonstrate that the expression of α1 and α5 GABAA receptor subunit transcripts in hippocampus of male APPNLGF mice, is regulated by activating H3K4me3 epigenetic mark.

3.5. Downregulation of H3K4me3 demethylases in hippocampus of APPNLGF mice

Histone methyltransferases (Kmts) and demethylases (Kdms) are epigenetic regulators of H3K4me3 which is associated with gene activation (Santos-Rosa et al., 2002). Thus, H3K4 methylation could be enhanced and repressed by increased catalytic activity of Kmts and Kdms, respectively. To determine the controlling influence of Kdms and Kmts on H3K4 methylation and consequently the transcription of α1 and α5 GABAA receptor subunits, we measured hippocampal expression of Kdm1a, Kdm1b, and Kmt2b in the hippocampus of mice because changes in expression levels for these enzymes have been shown to contribute to cognitive and synaptic deficits in AD mouse models (Collins et al., 2019). We found that Kdm1a expression was significantly lower in males than female wildtype mice (p = 0.0012) in the hippocampus. Moreover, both male (p = 0.05) and female (p = 0.0002) APPNLGF mice showed statistically significant decrease (F [1,26] = 20.26, p = 0.0001) Kdm1a expression as compared to their respective wildtype control mice (Fig. 5A). Additionally, Kdm1b expression was significantly lower in female (p = 0.02) APPNLGF mice as compared to wildtype controls (Fig. 5B). However, we did not find sex differences in male and female
for both Kdm1a and Kdm1b expression in APPNLGF mice. Also, no significant differences for the expression of Kmt2b in hippocampus of wild type and APPNLGF mice (Fig. 5C). These data suggest that decreased hippocampal expression of Kdm1a and Kdm1b but not Kmt2b may lead to increased H3K4 methylation and consequently increased occupancy of this epigenetic mark at Gabra1 and Gabra5 promoters thereby activating the expression of α1 and α5 GABA_A receptor subunit transcripts in hippocampus of male APPNLGF mice.

4. Discussion

The main focus of this study was to examine the sex-dependent role of GABA-mediated neurotransmission and the impact of these changes on memory and behavior in an APP knock-in (APPNLGF) mouse model of AD. Accordingly, for the first time, we showed enhanced sensitivity of male APPNLGF mice to diazepam-induced anxiolytic-like effects as well as impairment of recognition memory when compared to female APPNLGF and their wild type counterparts. Indeed, we showed that a low dose of diazepam, which does not induce anxiolytic-like effects in wild type mice, induces stronger anxiolytic-like effects in male than female APPNLGF mice. Of note, the same low dose of diazepam significantly impairs recognition memory only in male APPNLGF mice. It is unlikely that these sex-specific behavioral effects of acute diazepam challenge are due to pharmacokinetic differences since it has been reported that diazepam is more rapidly metabolized in males than female rats because of higher hepatic enzymatic activity in male rats (Reilly et al., 1990). Notably, the effects of diazepam are long-lasting because it is metabolized into desmethyldiazepam and oxazepam, which are both pharmacologically active and are used as drug entities (Bertilsson et al., 1990). Moreover, it has been reported that female C57BL/6NCrlBR mice are more sensitive to the sedative effects of acute diazepam challenge than their male counterparts (Podhorna et al., 2002). Additionally, we demonstrated that the sex differences in response to diazepam-mediated behavioral effects is due to increased H3Kme3 occupancy at Gabra1 and Gabra5 promoters, which in turn leads to the increased expression of α1 and α5 GABA_A receptor subunit transcripts in hippocampus of male APPNLGF mice. Lastly, the epigenetic reprogramming of hippocampal H3K4me3 found in our study may, at least in part, be due to dysfunction of Kdm1a and Kdm1b demethylases catalytic activity which resulted in increased H3K4me3-mediated gene activation. Interestingly, we found that Kdm1a expression was higher in wildtype female mice, suggesting that a sex-dependent function of Kdm1a might exist. Indeed, previous studies suggest that Kdm1 is essential for zygotic genome activation and ovarian cancer cells migration (Ancelin et al., 2016; Shao et al., 2015). However, the possible existence of sex-dependent function of Kdm1a in brain needs further investigation. Although we found sex difference in baseline expression of Kdm1a, we did not observe sex differences in Kdm1a and Kdm1b deficits APPNLGF mice, suggesting that these demethylases may regulate different epigenetic processes and/or targets in male and female APPNLGF mice. In summary, our data suggests enhanced GABA-mediated inhibitory neurotransmission particularly in APPNLGF mice given a low dose of diazepam which fail to produce significant behavioral alteration in wild type mice, elicits anxiolytic-like effects in both male and female APPNLGF mice. Most significantly, we demonstrated that the sex differences in the sensitivity to diazepam-mediated effects may in part, be attributed to enhanced hippocampal α1 and α5-containing GABA_A receptors-mediated inhibited neurotransmission in male APPNLGF mice.

It is well known that alteration of GABAergic neurotransmission, the primary inhibitory neurotransmission in brain, plays a major role in a number of neuropsychiatric disorders, including AD (Li et al., 2016b; Rudolph and Mohler, 2014b; Schoushoebe et al., 2014). In addition, it has been suggested that facilitation of memory decline associated with Aβ accumulation, may be due to upregulation of GABA-mediated inhibitory neurotransmission in the brain (Sasaguri et al., 2017b). Furthermore, it has been reported that the upregulation of GABAergic neurotransmission observed in both clinical pathology and preclinical models of AD is due to a compensatory response to the aberrant excitatory neurotransmission found in hippocampus of APP or PS1 mouse model of AD (Palop et al., 2007). In addition, Kwakowsky and colleagues reported prominent increases in the expression of α1 and α5 GABA_A receptor subunits in all layers of the CA3 region and in CA1 region, respectively, of AD patients (Kwakowsky et al., 2018a). Moreover, Yoshiike et al. (2008) reported that hippocampal LTP and memory deficits observed in both adult (9–15 months) transgenic APP/PS1 and old (19–25 months) non-transgenic mice can be rescued by bicuculline, a competitive inhibitor of the GABA recognition site on GABA_A receptor complex and picrotoxin, a non-competitive antagonist of GABA action on the GABA_A receptor respectively. Taken together, these data suggests that the upregulation of α1 and α5 GABA_A receptor subunits in hippocampus of APPNLGF mice may underlie the increased sensitivity to diazepam-mediated behavioral effects.

In contrast to histone acetylation, it has been reported that perturbations of histone methylation repress or activate gene transcription...
depending on the methylation status of specific histone residues (Wood, 2018). For example, methylation of histone H3 lysine 4 leads to activation of gene transcription while methylation of histone H3 lysine 9 is associated with repression of gene transcription (Rea et al., 2000; Kuzmichev et al., 2002). More specifically, it has been reported that increased histone methylation negatively impacts cognitive function in AD (Collins et al., 2019). The important role of H3K4me3 in learning, memory (Gupta et al., 2010) and synaptic transmission (Cheung et al., 2010), has raised interest on the role of H3K4me3 in neurodegenerative disease, including AD. In a recent study, Cao, and colleagues (Cao et al., 2020) reported significant increases of H3K4me3 epigenetic mark in disease, including AD. The important role of H3K4me in learning, increased histone methylation negatively impacts cognitive function in AD, mice model. Our findings of significant increases in H3K4me3 occupancy at Gabra1 and Gabra5 promoter in male APPNLGF mice which associated with memory and behavioral changes were consistent with these observations. Given evidence suggesting that H3K4me3 is enriched at the transcriptional active sites of genes (Guenther et al., 2005), we hypothesize that the increase of hippocampal H3K4me3 occupancy at Gabra1 and Gabra5 promoters in male APPNLGF mice leads to increased expression of α1 and α5 GABA receptorsubunit transcripts and may consequently be responsible for the increase sensitivity to diazepam-induced behavioral effects in these mice. However, further studies using lentiviral approach to overexpress Kdm1a and/or Kdm1b in hippocampus or behavioral studies with negative allosteric modulators of α1- (β-carboline-3-carboxylate-t-buty1 ester, β-CCt) and α5-(MRK-016) containing GABA receptors will be needed in order to validate our hypothesis. H3K4me3 could be regulated by many factors including Kdms; we found that increased occupancy of H3K4me3 at Gabra1 and Gabra5 promoters is associated with decreased Kdm1a and Kdm1b expression in male but not in female APPNLGF mice. Based on our results, we propose that the decrease Kdm1a and Kdm1b expression contributes to H3K4me3-mediated enhanced of α1 and α5 GABA receptor subunit transcription in male APPNLGF mice, whereas the changes in these demethylases observed in female APPNLGF mice may regulate epigenetic marks that occupy promoters of different target genes. This speculation is based on our preliminary findings indicating preferential upregulation of inflammatory markers (Glap, Nlrp3, Cd11b, and Trlr2) in female APPNLGF mice (Supplementary Fig. 2) and the observation that increased H3K4me3 epigenetic mark has also been linked with the regulation of immune functions (Gjoneska et al., 2015). However, further studies will be needed to determine if the promoters of these inflammatory markers are also preferentially regulated by H3K4me3 epigenetic marks in female than in male APPNLGF mice.

5. Conclusions

Our study provided first evidence showing sex-dependent sensitivity to the action of a positive allosteric modulator of GABA action at GABA receptors in a knock-in (APPNLGF) mouse model of Alzheimer’s disease. Additionally, our data showed sex-dependent epigenetic regulation in the expression of GABA receptorsubunit transcripts in APPNLGF mice. Most importantly, our results suggest that perturbations of GABA neurotransmitter systems or epigenetic mechanisms may underlie the sex difference in memory deficits of AD. Future studies will continue this line of work by examining sex differences in GABA-mediated post-synaptic inhibitory currents and the modulatory effects of diazepam using electrophysiological recordings, and the epigenetic regulation on the of GABA receptorsubunit expression and functioning in other brain areas such as prefrontal cortex of APPNLGF mice. Additionally, the specific genes that are regulated by Kdm-H3K4me3 pathways differently between male and females in the mouse model of AD need to be determined.

Funding and disclosure

This work was supported by National Institute of Health grants RF1AG057884 and R01AG062249 to Dr. Hongxin Dong. The authors have no competing interest to disclose.

Authors statement

All the authors of this manuscript declare:

- no conflicts of interest;
- the data is not published and submitted elsewhere;
- all the procedures reported in this manuscript have been approved;
- the final version of the manuscript has been approved by all authors;
- This study was supported by RF1AG057884 and R56AG053491 to Hongxin Dong.

CRediT author contribution statement

James Auta: Conceptualization, Investigation, Data curation, Validation, Writing – original draft, preparation, Visualization. Andrea Locci: Conceptualization, Investigation, Data curation, Validation, Writing – original draft, preparation, Visualization, Writing – review & editing, Visualization. Alessandro Guidotti: Conceptualization, Writing – review & editing. John M. Davis: Writing – review & editing. Hongxin Dong: Conceptualization, Funding acquisition, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crneur.2021.100025.

References

Alzheimer’s Association. 2012. Alzheimer’s disease facts and figures. Alzheimer’s Dementia 8, 131–168.
Ancelin, K., Syx, L., Borensztein, M., Ranisavljevic, R., Vasiliev, I., 2016. Maternal LSD1/KDMIA is an essential regulator of chromatin and transcription landscapes during zygotic genome activation. Elite 5, 08851. https://doi.org/10.7554/eLife.08851.
Bell, K.F.S., de Kort, G.J.L., Stegerda, S., Shigemoto, R., Ribeiro-da-Silva, A., Cuello, A. C., 2003. Structural involvement of glutamatergic presynaptic boutons in a transgenic mouse model expressing early onset amyloid pathology. Neurosci. Lett. 353, 143–147.
Bertilsson, L., Baillie, T.A., Reviriego, J., 1990. Factors influencing the metabolism of diazepam. Pharmacol. Ther. 45, 85–91.
Braak, H., Braak, E., 1991. Neuropathological staging of Alzheimer-related changes. Acta Neuropathol. 82, 239–259.
Broadbent, N.J., Gaskin, S., Squire, L.R., Clark, 2009a. Object recognition memory and the rodent hippocampus. Learn. Mem. 17 (1), 5–11.
Broadbent, N.J., Gaskin, S., Squire, L.R., Clark, 2009b. Spatial memory, recognition memory, and the hippocampus. Proc. Natl. Acad. Sci. U.S.A. 101 (40), 14515–14520.
Cao, Q., Wang, W., Williams, J.B., Yang, F., Wang, Z., Yan, Z., 2020. Targeting histone K4 trimethylation for treatment of cognitive and synaptic deficits in mouse models of Alzheimer’s disease. Sci. Adv. 6 (50), eabe0906.
Carascos, V.B., Elliot, E.M., You-Ten, K.E., Cheng, Y.V., et al., 2004. Tonic inhibition in mouse hippocampal CA1 pyramidal neurons is mediated by α5-subunit-containing g-aminobutyric acid type A receptors. Proc. Natl. Acad. Sci. U.S.A. 101 (10), 3662–3667.
Cheung, I., Shulha, H.P., Jiang, Y., Matevosian, A., Wang, J., et al., 2010. Developmental regulation and individual differences of neuronal H3K4me3 epigenomes in the prefrontal cortex. Proc. Natl.Acad. Sci USA 107, 8824–8829.
Collins, B.E., Greer, C.B., Coleman, B.C., Sweatt, J.D., 2019. Histone H3 lysine K4 methylation and its role in learning and memory. Epigenet. Chromatin 12 (1), 7. https://doi.org/10.1186/s13072-018-0251-8.
Frances, P.T., 2005. The interplay of neurotransmitters in Alzheimer disease. CNS Spectr. 10, 6–9.
Fritschi, J.M., 2008. Epilepsy, E1 balance and GABAergic receptor plasticity. Front. Mol. Neurosci. 1, 5.
Gatta, E., Grayson, D.R., Aua, J., Saudagar, V., Dong, E., Chen, Y., 2021. Genome-wide methylation in alcohol used disorder subjects: implications for an epigenetic regulation of the cortico-limbic glucocorticoid receptor (NR3C1). Mol. Psychiatriy. 26, 1029–1041.
Gjoneska, E., Pfenning, A.R., Mathys, H., Quon, G., Kundaje, A., et al., 2015. Conserved epigenomic signals in mice and humans reveal intrinsic basis of Alzheimer’s disease. Nature 518, 356–359.
Govindapani, K., Guzman, B.C.G., Vinnakota, K., Walvdovg, H.J., Faull, R.L., et al., 2017. Towards a better understanding of GABAergic remodeling in Alzheimer’s disease. Int. Mol. Sci. 18 (8), 1813. https://doi.org/10.3390/ijms18081813.
Guenther, M.G., Jenner, R.G., Chevalier, B., Nakamura, T., Croce, C.M., et al., 2005. Chromatin structure by site-specific histone H3 methyltransferases. Nature 436, 783–790.
Guidotti, A., Aua, J., Davis, J.M., Dong, E., Grayson, D.R., et al., 2005. GABAergic dysfunction in schizophrenia: new treatment strategies on the horizon. Psychopharmacology 180, 191–205.
Gupta, S., Kim, S.Y., Artis, S., Molne, D.I., Schumacher, A., et al., 2010. Histone methylation regulates memory formation. J. Neurosci. 30, 3589–3599.
Kuzmichev, A., Nishioka, K., Erdjument-Bromage, H., Tempst, P., Reiberg, D., 2002. Histone methyltransferase activity associated with human multiprotein complex containing the enhancer Zeste protein. Genes Dev. 16, 2893–2906.
Kwakowsky, A., Calvo-Flores, G.B., Govindpani, K., Guzman, B.C.G., Vinnakota, C., Waldvogel, H.J., et al., 2018. The GABAergic system as a therapeutic target for Alzheimer’s disease. J. Neurochem. 145, 374–411.
LaFerla, F.M., Green, K.N., 2012. Animal models of Alzheimer disease hippocampus, subiculum, entorhinal cortex, and superior temporal gyrus. J. Neurochem. 122, 1555–1570.
Lim, Y., Sun, H., Chen, Z., Xu, H., Bu, G., Zheng, H., 2016a. Implications of GAABergic neurotransmission in Alzheimer’s disease. Front. Aging Neurosci. 8 https://doi.org/10.3389/fnagi.2016.00031.
Li, Y., Sun, H., Chen, Z., Xu, H., Bu, G., Zheng, H., 2016b. Implications of GAABergic neurotransmission in Alzheimer’s disease. Front. Aging Neurosci. 8, 31.
Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-delta delta CT) method. Methods 25, 402–408.
Loci, A., Pinna, G., 2019. Stimulation of peroxisome proliferator-activated receptor-a by N-palmitoylthanolamine engages allopregnanolone biosynthesis to modulate emotional behavior. Biol. Psychiat. 85 (12), 1036–1045.
Loci, A., Yan, Y., Rodriguez, G., Dong, H., 2020. Sex differences in CRF1, CRF, and CRFRBP expression in C57Bl/6J mouse brain across lifespan and in response to acute stress. J. Neurochem. 130 (5), 711–721. https://doi.org/10.1111/jnc.15157.
Locci, A., Orelend, H., Rodriguez, G., Gottlieb, M., McClarty, B., et al., 2021. Comparison of memory, affective behavior, and neuropathology in APP695 knock-in mice to 5xFAD and APP/PS1 mice. Behav. Brain Res. 404, 115192.
Mufson, E.J., Counts, S.E., Perez, S.E., Ginsberg, S.D., 2008. Cholinergic system during the progression of Alzheimer’s disease: therapeutic implication. Expert Rev. Neurother. 8 (11), 1703–1718.
Muke, L., Sekelo, D.J., 2012. Neurototoxicity of amyloid β-protein: synaptic and network dysfunction. Cold Spring Harb. Perspect. Med. 2, a006388.
Palop, J.J., Chinc, J., Roberson, E.D., Wang, J., Thwin, M.T., et al., 2007. Aberrant excitatory neuronal activity and compensatory remodeling of inhibitory hippocampal circuits in mouse models of Alzheimer’s disease. Neuron 55, 697–711.
Pandey, S.C., Saharkar, A.J., Tang, L., Zhang, H., 2015. Potential role of aldosterone exposure-induced amyloidoid histone modifications in anxiety and alcohol intake during adulthood. Neurobiol. Dis. 82, 607–619.
Podhorna, J., McCabe, S., Brown, E.R., 2002. Male and female C57Bl/6 mice respond differently to diazepam challenge in avoidance learning tasks. Pharmacol. Biochem. Behav. 72, 13–21.
Puzzo, D., Gulisano, W., Palmeri, A., Annacio, O., 2015. Rodent models of Alzheimer’s disease drug discovery. Expert Opin. Drug Discov. 10, 703–711.
Rea, S., Eisenhaber, F., O’Carroll, D., Strahl, B.D., Sun, Z., et al., 2000. Regulation of chromatin structure by site-specific histone H3 methyltransferases. Nature 406, 593–596.
Rudolph, U., Mash, S., 1994. Benzodiazepine receptor subtypes: therapeutic potential in Down syndrome, affective disorders, schizophrenia, and autism. Annu. Rev. Pharmacol. Toxicol. 54, 483–507.
Rudolph, U., Mohler, H., 2014a. GABA receptor subtypes: therapeutic potential in Down syndrome, affective disorders, schizophrenia, and autism. Annu. Rev. Pharmacol. Toxicol. 54, 483–507.
Rudolph, U., Crestani, F., Benke, D., Brung, I., Benson, J.A., et al., 1999. Benzodiazepine actions mediated by specific γ-aminobutyric acid receptor subtype. Nature 401, 796–800.
Santos-Rosa, H., Schneider, R., Bennett, A.J., Sherriff, J., Bernstein, B.E., et al., 2002. Active genes are tri-methylated at K4 of histone H3. Nature 419, 407–411.
Sasaguri, H., Nishio, P., Hashimoto, S., Nagata, K., Saito, T., Strooper, B.D., 2017a. APP mouse models for Alzheimer’s disease preclinical studies. EMBO J. 36, 2473–2487.
Sasaguri, H., Nishio, P., Hashimoto, S., Nagata, K., Saito, T., Strooper, B.D., 2017b. APP mouse models for Alzheimer’s disease preclinical studies. EMBO J. 36, 2473–2487.
Schmittgen, T.D., Livak, K.J., 2008. Analyzing real-time PCR data by comparative CT method. Nat. Protoc. 3, 1101–1108.
Schousboe, A., Madsen, K.K., Barker-Halikzi, M.L., White, H.S., 2014. The GABA synapse as a target for antiepileptic drugs: a historical overview focused on GABA transporters. Neurochem. Res. 39, 1980–1987.
Serrano-Pozo, A., Frosch, M.P., Masliah, E., Hyman, B.T., 2011. Neuropathological alterations in Alzheimer’s disease. Cold Spring Harb. Perspect. Med. 2, a006320.
Shao, G., Wang, J., Li, Y., Liu, X., Xie, X., et al., 2015. Lysine-specific demethylase 1 mediates epidermal growth factor signaling to promote cell migration via ovarian cancer cells. Sci. Rep. 5, 15344. https://doi.org/10.1038/srep15344.
Solas, M., Puerta, E., Ramirez, M.J., 2015. Treatment options in Alzheimer’s disease: the GABA story. Curr. Pharmaceut. Des. 21, 4960–4971.
Wood, I.C., 2018. The contribution and therapeutic potential of epigenetic modifications in Alzheimer’s disease. Front. Neurosci. 12, 649. https://doi.org/10.3389/fnins.2018.006189.