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G protein-coupled receptor 158 modulates sensitivity to the sedative-hypnotic effect of ethanol in male mice

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Abstract

Background: Sensitivity to ethanol is used to assess a predisposition to recover from unconsciousness induced by excessive ethanol. The role of G protein-coupled receptor 158 (GPR158) in modulating sensitivity to the sedative-hypnotic effect of ethanol has not been investigated.

Methods: Loss of righting reflex (LORR) is a behavioral feature to indicate a state of hypnosis in rodents. In this study, *Gpr158*$^{-/-}$ mice and wild-type (WT) littermates (n = 8/genotype) were tested with the paradigms of LORR induced by a dose of 3.5 g/kg ethanol, open-field test (OFT) and blood ethanol concentration measurement. The OFT was used to examine the role of GPR158 in the ethanol effect on motor activity in *Gpr158*$^{-/-}$ mice (n = 6/genotype). Furthermore, *CamK2A-Cre;Gpr158*$^{fl/fl}$ (n = 9) and *Vgat-Cre;Gpr158*$^{fl/fl}$ mice (n = 10) went through LORR test and OFT compared to the controls (n= 9 and 8, respectively).

Results: *Gpr158* deficiency led to a prolonged LORR duration by 110.6% (t_{14} = -5.241, p = 0.0001), without altering spontaneous activity (t_{14} = -0.718, p = 0.485) or ethanol metabolism ($F_{1, 8}$ = 0.259, p = 0.625). *Gpr158* knockout did not affect the ethanol effect on locomotion ($F_{1, 10}$ = 0.262, p = 0.62). Furthermore, LORR duration became longer in the conditional knockouts of *Gpr158* within calcium/calmodulin dependent protein kinase II alpha-positive (CamK2A^{+}) neurons by 69% (t_{16} = -2.914, p = 0.01) and vesicular GABA transporter-positive (Vgat^{+}) neurons by 92% ($t_{9.802}$ = -2.519, p = 0.023), respectively, while locomotion was not altered in

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Camk2A-Cre;Gpr158$^{fl/fl}$ (t_{16} = 0.49, p = 0.631) or *Vgat-Cre;Gpr158*$^{fl/fl}$ mice (t_{16} = 0.035, p = 0.972).

Conclusions: This study reveals a critical role of neuronal GPR158 in shaping sensitivity to the sedative-hypnotic effect of ethanol, suggesting that GPR158 may be a potential target for treating alcohol use disorder.

Keywords

G protein-coupled receptor 158, ethanol, sensitivity, loss of righting reflex

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Introduction

Alcohol is consumed to facilitate socialization and relieve pressure in daily life. Harmful alcohol use accounts for approximately 3,000,000 deaths worldwide per year and is involved in the etiology of liver, esophagus and larynx cancers (Bagnardi et al., 2001; Turati et al., 2013; Anderson, 2021). Individual sensitivity to the sedative-hypnotic effect of ethanol can be defined as the capability to tolerate alcohol or the predisposition to be sedated, which is shaped by many factors such as psychological, environmental and biological factors (Schuckit, 2018; Popoola et al., 2018; Naassila et al., 2002; Aguayo et al., 2014; Thiele et al., 2000). The studies on ethanol sensitivity may facilitate uncovering the mechanism underlying harmful alcohol use and alcohol use disorder (AUD) in clinic, of which the neurobiological substrates are far from clear.

Ethanol is a depressant for the central nervous system (CNS). After being absorbed into the circulation, ethanol is firstly degraded into acetaldehyde by alcohol dehydrogenase (ADH) and then metabolized into acetate by acetaldehyde dehydrogenase (ALDH) in the liver (Aguayo et al., 2014; Guillot et al., 2019; Thiele et al., 2000). Ethanol can pass the blood-brain barrier (BBB) to interact with the CNS and influence the psychological state (Schuckit, 2018; Popoola et al., 2018; Thiele et al., 2000). Ethanol can disinhibit the dopaminergic neurons through acting on the mesocorticolimbic system including prefrontal cortex (PFC), ventral tegmental area (VTA) and nucleus accumbens (NAc), while ethanol at a high dose may

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unspecifically suppress cortical excitation and leads to a sedative state (Pal et al., 2018; San Martin et al., 2021; Vore et al., 2021). Thus, sensitivity to ethanol is determined by both ethanol metabolism and individual vulnerability of the CNS to ethanol (Schuckit, 1984; Aguayo et al., 2014; Chen et al., 2021).

G protein-coupled receptor 158 (GPR158) is a receptor densely expressed in the neurons distributed in cortex and hippocampus (Sutton et al., 2018; Condomitti et al., 2018). It has been proved that GPR158 plays a role in stress-induced depression and age-related memory loss by affecting synaptic transmission and synaptic differentiation in the PFC and hippocampus (Sutton et al., 2018; Condomitti et al., 2018; Cetereisi et al., 2019; Itakura et al., 2019). Stress contributes to alcohol dependency due to the perceived comforting properties of ethanol (Spanagel et al., 2014; Broccoli et al., 2018). Besides, GPR158 has been reported to participate in transducing the downstream signaling of osteocalcin (OCN) in modulating memory and anxiety behaviors partially via brain-derived neurotrophic factors (BDNF) and inositol 1,4,5-trisphosphate (IP3) signaling pathways (Kosmidis et al., 2018; Patterson-Buckendah et al., 2011). Plasma OCN is highly sensitive to stress and can regulate expression of the adrenal genes which may further affect alcohol drinking and intoxication, while alcohol consumption decreases serum OCN level in human and rodents (Khrimian et al., 2017; Nielsen et., 1990). Thus, we speculate that GPR158 may play a role in modulating sensitivity to the effect of ethanol.

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In this study, we focused on clarifying the role of GPR158 in the sedative-hypnotic effect of ethanol in the tests of loss of righting reflex (LORR), locomotor activity and ethanol metabolism. To further elucidate the mechanism, *CamK2A-Cre;Gpr158*$^{fl/fl}$ and *Vgat-Cre;Gpr158*$^{fl/fl}$ mice were used to evaluate the function of GPR158 within pyramidal and GABAergic neurons in modulating sensitivity to the sedative-hypnotic effect of ethanol. The experimental design is shown in Figure 1.

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Materials and Methods

Animals and Drugs

Genetically modified mice and WT littermates were housed in a pathogen-free SPFII facility with constant temperature (23±1°C) and controlled humidity (50±10%) on a 12h/12h light/dark cycle (light on at 07:00) in the Center of Laboratory Animal Science, Southern University of Science and Technology (SUSTech), China. Male mice were aged at 8-10 weeks and approximately 20g at the beginning of the behavioral tests. *Gpr158*$^{-/-}$ (*Gpr158*$^{tm1(KOMP)Vlcg}$) and *Gpr158*$^{fl/fl}$ mice were imported from Columbia University (NY, USA). In *Gpr158*$^{-/-}$ mice, the first two exons of *Gpr158* were replaced with a LacZ cassette (Sutton et al., 2018; Chang et al., 2023). *CamK2A-Cre* and *Vgat-Cre* mice were bought from Shanghai Model Organisms Company, China. Western blot results validated that the expression of GPR158 was significantly decreased in *Gpr158*$^{-/-}$ mice using the mPFC tissue (Figure 2A). Ai14 mice (Jackson laboratory, Bar Harbor, ME) were crossed with the Cre-expressing mice to confirm that Cre was expressed in our target cells. Sibling WT and *Gpr158*$^{fl/fl}$ littermates were used as controls for the global and conditional *Gpr158* knockouts, respectively. Mice were maintained in a group of 4 to 5 in clear plastic cages with free access to drinking water and standard food. All experiments were conducted according to the NIH Guide for the Care and Use of Laboratory Animals (NIH publications no. 80-23, revised 1996), and the procedures were approved by the Animal Care Committee in the SUSTech, China.

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Gpr158$^{-/-}$, CamK2A-Cre;*Gpr158*$^{fl/fl}$ and *Vgat-Cre;Gpr158*$^{fl/fl}$ mice were used in this study. Considering the potential effect from estrous fluctuation, only male mice were tested in this study. The gene *Gpr158* was globally deleted in a *Gpr158*$^{-/-}$ mouse on a C57BL/6J background (Sutton et al., 2018; Chang et al., 2023). Conditional null *Gpr158* mice were generated by crossing the male *Gpr158*$^{fl/fl}$ mice with the female mice carrying a Cre recombinase driven by the promoter of the gene expressing calcium/calmodulin-dependent protein kinase II alpha (*CamK2A*) or vesicular GABA transporter (*Vgat*). For the genotyping of *Gpr158*$^{-/-}$, we used the following primers, GTGTAGCCTCTGCCCACTTC (KO-wt-F), CCTTTCTGTGCTTTCCTTGC (KO-wt-R), CTGCTGGGGATGTAACCTGT (KO-tg-F) and ATCTCTCCTCTGCAGGACCA (KO-lacZ-R), while the band size is at 312 bp for the global knockouts and 463 bp for the WTs. For the genotyping of *Gpr158*$^{fl/fl}$, we used the following primers, AGGCTTGCATCCAATTGACAAAAC (Flox-F), CAAGCTTCGACCCCTTTCCTACTAC (Flox-R) and CTTTGGACCTTAGAGACAGATTAC (Delta 1), while the band size is at 577 bp for the floxed mice and 494 bp for the WTs. For the genotyping of *CamK2A-Cre*, we used the following primers GTTCTCCGTTTGCACTCAGG (cKO-F) and CAGGTTCTTGCGAACCTCAT (cKO-R), while the band size is at 500 bp for the transgenic mice. For the genotyping of *Vgat-Cre*, we used the following primers CTTCGTCATCGGCGGCATCTG (vKO-COM), CAGGGCGATGTGGAATAGAAA (vKO-wt-R) and CCAAAAGACGGCAATATGGT (vKO-tg-R), while the band size is at 200 bp for the transgenic mice and 323 for the WTs. The representative images for

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genotyping are shown in Figure 2B, C, D and E, and only the homozygous transgenic mice were used in this study.

Ethanol, bought from Beijing Chemical Factory (EINEC number: 200-578-6; Beijing, China), was prepared in saline (Kelun Pharmaceuticeutical Company, Chengdu, China) and administered intraperitoneally (*i.p.*) in a volume of 1 mL/kg bodyweight.

Loss of Righting Reflex

LORR is a paradigm designed to assess sensitivity to the sedative-hypnotic effect of ethanol and other anesthetic agents (Harris et al., 1995; Bilbao et al., 2019). A LORR test was performed bewteen10:00 and 13:00 in the light phase of the circadian cycle, and light intensity was set at 350 lux during the test. A mouse was administered intraperitoneally with an injection of 3.5 g/kg ethanol (20%, v/v). Time point of the injection was recorded as TP1, while the time point when a mouse could not right itself within 30 seconds is set as the onset of LORR as TP2, and mice were laid in a V-shape trough in a supine posture during LORR (Crabbe et al., 2012). When a mouse righted itself 3 times in 1 min, it was thought to regain the righting reflex (TP3). The latency to LORR and LORR duration were calculated as (1) Latency = TP2 - TP1; (2) LORR duration = TP3 - TP2.

Open Field Test (OFT)

A paradigm of the OFT was used to evaluate spontaneous activity in rodents (Wei et

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al., 2020). OFT was performed 3 d after the LORR test to minimize potential influences from alcohol intoxication. Prior to the test, mice had 30 min to get habituated to the environment. An open box at the size 40 cm long × 40 cm wide × 40 cm high was used, and the floor area was divided into 16 squares, of which the central 4 squares were designated as the Center (20 cm × 20 cm). The open-field test was performed in the duration of 10:00-13:00, and the light intensity was adjusted to a low level at 15 lux. Distance traveled and time spent in the Center were recorded for 10 min by Noldus EthoVision XT software (Leesburg, USA).

Blood Ethanol Concentration (BEC) Measurement

BEC was detected at different time points after an injection of 3.5 g/kg ethanol in *Gpr158*$^{-/-}$ mice and WT littermates. Blood samples (30 μL) were taken from tail vein at 30, 60, 120, 240 and 480 min after the injection. Serum was used to measure BECs with EnzyChromTM ethanol detection kits (Lesenku Biological Technology Co., Ltd, Guangzhou, China) and MS-480 Clinical Chemistry Analyzer (Meikang Shengde Biological Technology Co., Ltd, Ningbo, China) with the NADH enzyme spectrophotometric method.

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Statistical Analysis

Student's independent samples *t* test was implemented when the data from 2 groups were compared, while one-way analysis of variance (ANOVA) was adopted when more than 2 groups were compared followed by LSD tests for *post hoc* comparisons using IBM SPSS software version 22.0 (Armonk, NY). Repeated measures ANOVA was used if the requirements were fulfilled. Data were expressed as the mean ± SEM. The level of significance was set as $p < 0.05$.

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Results

Gpr158 deficiency prolonged LORR duration, but not the latency to LORR or locomotor activity

In the test, we did not find significant difference in latency to LORR between *Gpr158*$^{-/-}$ mice and the control WTs [t_{14} = -0.454, p = 0.657; WT vs KO: 3.50 ± 1.04 min vs 2.60 ± 0.38 min] as shown in Figure 3A. However, *Gpr158*$^{-/-}$ mice had a significantly longer LORR duration [t_{14} = -5.241, p = 0.0001], twice the LORR duration in WT littermates [WT vs KO: 38.4 ± 5.83 min vs 80.88 ± 5.15 min; n = 8/genotype] as shown in Figure 3B.

In the OFT, no difference was found in distance traveled [t_{14} = -0.718, p = 0.485], moving velocity [t_{14} = -0.825, p = 0.627] or time spent in the Center [t_{14} = -0.812, p = 0.431] between *Gpr158*$^{-/-}$ mice and WT littermates, as shown in Figure 3C, D and E.

Gpr158 deficiency did not affect blood ethanol concentrations

A new group of *Gpr158*$^{-/-}$ mice and WT littermates (n = 5/genotype) were used in this test. BECs were measured at 30, 60, 120, 240 and 480 min after an ethanol injection at a dose of 3.5 g/kg. It is found that a global deletion of *Gpr158* did not affect the patterns of ethanol metabolism [$F_{1, 8}$ = 0.259, p = 0.625] analyzed with one-way ANOVA, as shown in Figure 4.

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Gpr158 deficiency did not affect sensitivity to the effect of ethanol on locomotor activity

In this test, a new batch of *Gpr158*$^{-/-}$ mice (n=6) and WT littermates (n = 6) went through a paradigm of open field to evaluate genotype difference in ethanol-induced changes of motor activity. Here, a low dose of 0.5 g/kg and a high dose of 2 g/kg ethanol were adopted, while the mouse was put into open field immediately after an ethanol injection. The time point to measure locomotor activity is based on the reported result that the peak brain ethanol concentration appears in the first 10 min after the injection (Smolen and Smolen, 1989). In this test, baseline locomotion was first measured in mice injected with vehicle, and 3 days later, the test to evaluate the effect of 0.5 g/kg ethanol was performed in the same batch of mice. Then, after a 3-d washout period, the test to assess the effect of 2 g/kg ethanol on motor activity was carried out. Repeated measures ANOVA results showed that there were no genotype effects in locomotion [$F_{1, 10} = 0.262$, $p = 0.62$], moving speed [$F_{1, 10} = 0.119$, $p = 0.737$] or time spent in the center [$F_{1, 10} = 3.324$, $p = 0.737$] as shown in Figure 5. Of interest, the baseline locomotion did differ from that at the doses of 0.5 g/kg ($p = 0.004$) and 2 g/kg ($p = 0.018$), respectively, while baseline time spent in the center of open field was not different from that under the treatment of 0.5 g/kg ($p = 0.509$) and 2 g/kg ($p = 0.067$), when the main effects were compared using LSD *post hoc* tests following repeated measures ANOVA. Furthermore, 2 g/kg ethanol induced higher moving speed compared to the baseline ($p = 0.014$), while 0.5 g/kg did not ($p = 0.633$). From the results, we concluded that *Gpr158* deficiency did not affect sensitivity to the effect

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of ethanol on motor activity or anxiety level in mice.

Conditional *Gpr158* knockout within pyramidal neurons increased LORR duration, but not latency to LORR or locomotor activity

In this test, *CamK2A-Cre;Gpr158*$^{fl/fl}$ mice and the controls (n = 9/genotype) were subject to LORR test. They did not show any difference in latency to LORR [t_{16} = 0.412, p = 0.412; control vs KO: 4.00 ± 0.60 min vs 5.33 ± 1.46 min] as illustrated in Figure 6A. However, *CamK2A-Cre;Gpr158*$^{fl/fl}$ mice had a prolonged LORR duration [t_{16} = -2.914, p = 0.01] increased by 69%, compared to that in the controls [controls: KO: 56.11 ± 11.4 min vs 94.78 ± 6.79 min] as shown in Figure 6B.

In the OFT, there was no genotype difference in distance traveled [t_{16} = 0.49, p = 0.631], moving velocity [t_{16} = 0.332, p = 0.744] or time spent in the Center [t_{16} = 1.727, p = 0.103] between mice with a conditional knockout of *Gpr158* within pyramidal neurons and the controls, as shown in Figure 6C, D and E.

Conditional *Gpr158* knockout within inhibitory GABAergic neurons prolonged LORR duration and reduced latency to LORR, but not affecting locomotor activity

In this test, *Vgat-Cre;Gpr158*$^{fl/fl}$mice (n = 10) and the controls (n = 8) went through a LORR test. *Vgat-Cre;Gpr158*$^{fl/fl}$ mice showed a shorter latency to LORR [t_{16} = 2.644, p = 0.018; controls vs cKO: 3.00 ± 0.33 min vs 1.88 ± 0.23 min], as shown in Figure

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7A. Furthermore, *Vgat-Cre;Gpr158*$^{fl/fl}$ mice had a prolonged LORR duration [$t_{9.802}$ = -2.519, p = 0.023] increased by 92%, compared to that in the control group [controls vs cKO: 50.10 ± 8.08 min vs 96.38 ± 17.99 min], as shown in Figure 7B.

In the OFT, *Vgat-Cre;Gpr158*$^{fl/fl}$ mice did not show different distance traveled [t_{16} = 0.035, p = 0.972], moving velocity [t_{16} = 0.096, p = 0.925] or time spent in the Center [t_{16} = -0.01, p = 0.992] compared to the controls, shown in Figure 7C, D and E.

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Discussions

Sensitivity to ethanol reflects the threshold to become sedated in response to the inhibition of CNS activity (Ozburn et al., 2013; Childs et al., 2019; Chen et al., 2021). Individual sensitivity to ethanol is determined by the interaction between the pharmacological effect of ethanol and individual responses to ethanol (Even-Chen et al., 2017, 2022; Ye et al., 2021). Our study was designed to clarify the mechanism underlying sensitivity to the sedative-hypnotic effect of ethanol in mice. In this study, ethanol-induced LORR duration was prolonged by 110.6%, while the patterns of ethanol metabolism or ethanol-induced locomotion were not changed in *Gpr158*$^{-/-}$ mice to WT littermates, suggesting that GPR158 plays a specific role in shaping sensitivity to ethanol in mice (Natividad et al., 2018; Huang et al., 2022).

In the CNS, ethanol acts on GABA receptors and GABAergic neurons to further disinhibit downstream glutamatergic pyramidal neurons (Dao et al., 2021; Duman et al., 2019). Thus, we focus on the roles of CamK2A^{+} pyramidal neurons and Vgat^{+} inhibitory GABAergic neurons in ethanol-induce LORR mediated by GPR158 in mice (Puentes-Mestril et al., 2021; Schuske et al., 2007; Priya et al., 2019). Our study has been demonstrated that conditional *Gpr158* knockouts in Camk2A^{+} and Vgat^{+} neurons caused longer LORR durations by 69% and 92%, respectively, while an absence of GPR158 in GABAergic neurons resulted in an increased latency to LORR. Of interest, the latency to LORR was significantly increased in the mice with a selective knockout of *Gpr158* in GABAergic, but not pyramidal neurons, suggesting

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that a specific role of GABAergic neurons in LORR. As reported, GPR158 is an orphan receptor densely expressed in the PFC, striatum and hippocampus, while pyramidal neurons and inhibitory interneurons are the main neuronal types in these brain areas (Broccoli et al., 2018; Chen et al., 2021; Ostrovskaya et al., 2018; Puentes-Mestril et al., 2021; Schuske et al., 2007). Here, we conditionally knocked out the gene *Gpr158* under the control of the promoters *CamK2A* and *Vgat*, which are frequently used to mark pyramidal and GABAergic inhibitory neurons, respectively (Natividad et al., 2018; Ye et al., 2021; Huang et al., 2022). The protein CamK2 has 4 isoforms α, β, δ, and γ, among which the α isoform CamK2A is the main subunit within the CNS and densely distributed in excitatory glutamatergic neurons of the forebrain (Hanson & Schulman, 1992; Puentes-Mestril et al., 2021). Vgat is responsible for transportation and exocytotic release of GABA, located in the presynaptic terminal of GABAergic interneurons (Natividad et al., 2018; Ye et al., 2021; Huang et al., 2022). Taken together, GPR158 in pyramidal and inhibitory neurons both participate in mediating sensitivity to ethanol.

In the CNS, GPR158 can bind heparan sulfate proteoglycan (HSPG) glypican 4 (GPC4) to synergically organize the structure and mediate the function of the CA3 and CA1 mossy fiber synapses (Spanagel et al., 2014; Broccoli et al., 2018; Çetereisi et al., 2019). As a membrane receptor, GPR158 may modulate synaptic transmission by directly binding G protein signaling 7 (RGS7) to the plasma membrane and further affecting production of cyclic adenosine 3',5'-monophosphate (cAMP) in the

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cytoplasm (Harris et al., 1995; Itakura et al., 2019). GPR158-RGS7 complex can modulate GABA receptors through P/Q/N type voltage-gated $Ca2^{+}$ ion channels and G-protein inwardly rectifying K^{+} (GIRK) to exert an inhibitory effect on neuronal activity (Ostrovskaya et al., 2018; Orlandi et al., 2015). GABAA receptors are involved in ethanol-induced responses, while ethanol acts as a positive allosteric modulator of GABAA receptors which are widely expressed in the mesocorticolimbic system (Rudolph & Antkowiak, 2004; Kumar et al., 2012; Karlsson et al., 2012; Wei et al., 2020). Excessive ethanol ingestion may lead to a pervasive inhibition on cortical activity via docking to GABAA receptors to induce a sedation and sleeping state via regulating protein kinase A (PKA) activity (Zhuang et al., 2019; Vore et al., 2021; Koob & Colrain, 2020). Thus, GPR158 may modulate ethanol-induced LORR by regulation of the activity of GPR158-RGS7 complex and GABAA receptor-mediated fast inhibition.

Honestly, our study still has certain limitations. Firstly, here we used global and cell-type specific knockouts of *Gpr158*, but not time-specific knockouts, suggesting the potential impact of the developmental compensation cannot be excluded as *Gpr158* is theoretically deleted at the early stage of life (Eisener-Dorman et al., 2009). Secondly, pharmacological proofs may be needed to better support this conclusion, due to the lack of available ligands to modulate GPR158 activity as an orphan receptor nowadays (Sutton et al., 2018; Fu et al., 2022). However, our results

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concluded from the 3 transgenic mouse lines could still provide significant references on the role of GPR158 in the sedative-hypnotic sensitivity to ethanol.

To conclude, GPR158 participates in mediating the behavioral sensitivity to the sedative-hypnotic effect of ethanol. The results give the public and research community a better understanding of the vulnerability to ethanol-induced hypnosis and dependency.

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Conflict of Interest

The authors declare no conflict of interest.

Acknowledgement

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Figure Legends

Figure 1 Experimental design. LORR, loss of righting reflex; OFT, open field test.; BEC, blood ethanol concentration.

Figure 2 Animal model validation. Western blot results validated that the expression of GPR158 was significantly decreased in *Gpr158*$^{-/-}$ mice (A). Representative images for genotyping *Gpr158*$^{-/-}$ (band size 312 bp), *CamK2A-Cre* (band size 500 bp), *Vgat-Cre* (band size 200 bp) and *Gpr158*$^{fl/fl}$ (band size 577 bp) mice were present from B-E. Only the homozygous transgenic mice were used in this study.

Figure 3 Global *Gpr158* knockout increased LORR duration, but not latency to LORR or locomotor activity. In LORR test, the latency to LORR did not change compared to WT littermates (A), while *Gpr158*$^{-/-}$ mice had a longer LORR duration (B) (n = 8/genotype). In the OFT, distance traveled (C), moving speed (D) or time spent in the center (E) did not differ between *Gpr158*$^{-/-}$ mice and WT littermates. NS, not significant; * $p < 0.05$ compared to the control group. Data represented as the mean ± SEM.

Figure 4 Global *Gpr158* knockout did not affect ethanol metabolism. Blood ethanol concentrations (BECs) were measured at 30, 60, 120, 240 and 480 min after an ethanol injection at a dose of 3.5 g/kg (n = 5/genotype). Data represented as the mean ± SEM.

Figure 5 Global *Gpr158* knockout did not affect sensitivity to the effect of ethanol on motor activity. (A) *Gpr158*$^{-/-}$ mice did not show different distance traveled

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(A), moving speed (B) or time spent in the center (C) after the ethanol injection compared to WT littermates (WT: n=6; KO: n=6) in the OFTs. Ethanol was injected at a low dose at 0.5 g/kg and a high dose at 2 g/kg spanned with a 3-d break for the OFT. NS, not significant; * $p < 0.05$ compared to the control group. Data represented as the mean ± SEM.

Figure 6 Conditional *Gpr158* knockout in Camk2A$^+$ neurons increased LORR duration, but not latency to LORR or locomotor activity. In the test, the latency to LORR did not change between *Camk2A-Cre;Gpr158$^{fl/fl}$* mice and the control group (A), while *Camk2A-Cre;Gpr158$^{fl/fl}$* mice had a longer LORR duration (B) (n = 9/genotype). In the OFT, distance traveled (C), moving speed (D) or time spent in the center (E) was not different between *Camk2A-Cre;Gpr158$^{fl/fl}$* mice and the control group. NS, not significant; *$p < 0.05$ compared to the control group. Data represented as the mean ± SEM.

Figure 7 Conditional *Gpr158* knockout in Vgat$^+$ neurons increased LORR duration and reduced latency to LORR, but not locomotor activity. In the test, *Vgat-Cre;Gpr158$^{fl/fl}$* mice had a shorter latency to LORR (A) and a longer LORR duration (B) compared to the control group (control: n = 10; Vgat-KO: n = 8) after an ethanol injection at a dose of 3.5 g/kg. In the OFT, distance traveled, moving speed or time spent in the center was not different between *Vgat-Cre;Gpr158$^{fl/fl}$* mice and the control group. NS, not significant; *$p < 0.05$ compared to the control group. Data represented as the mean ± SEM.

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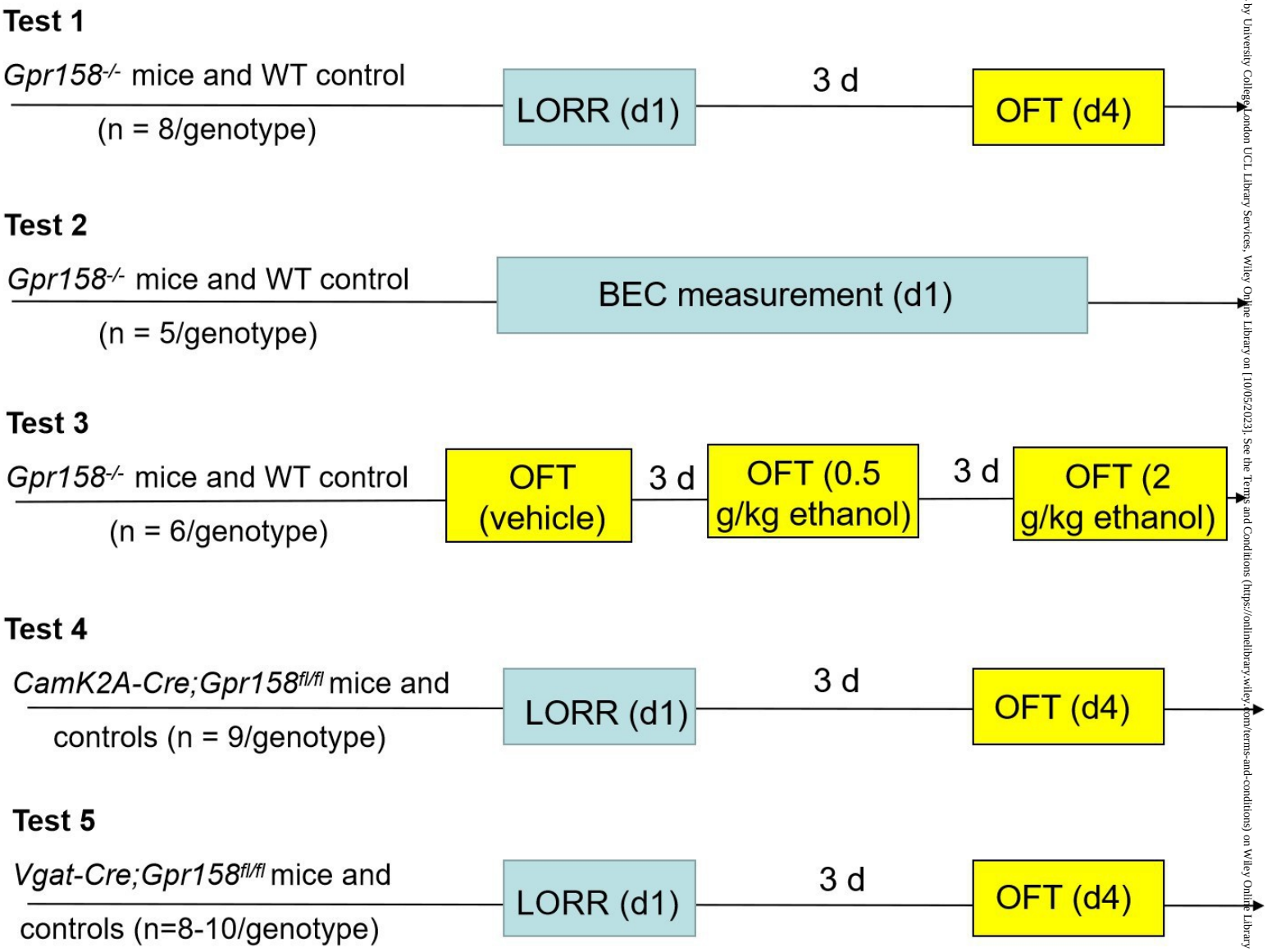

Figure1 paradigm.jpg

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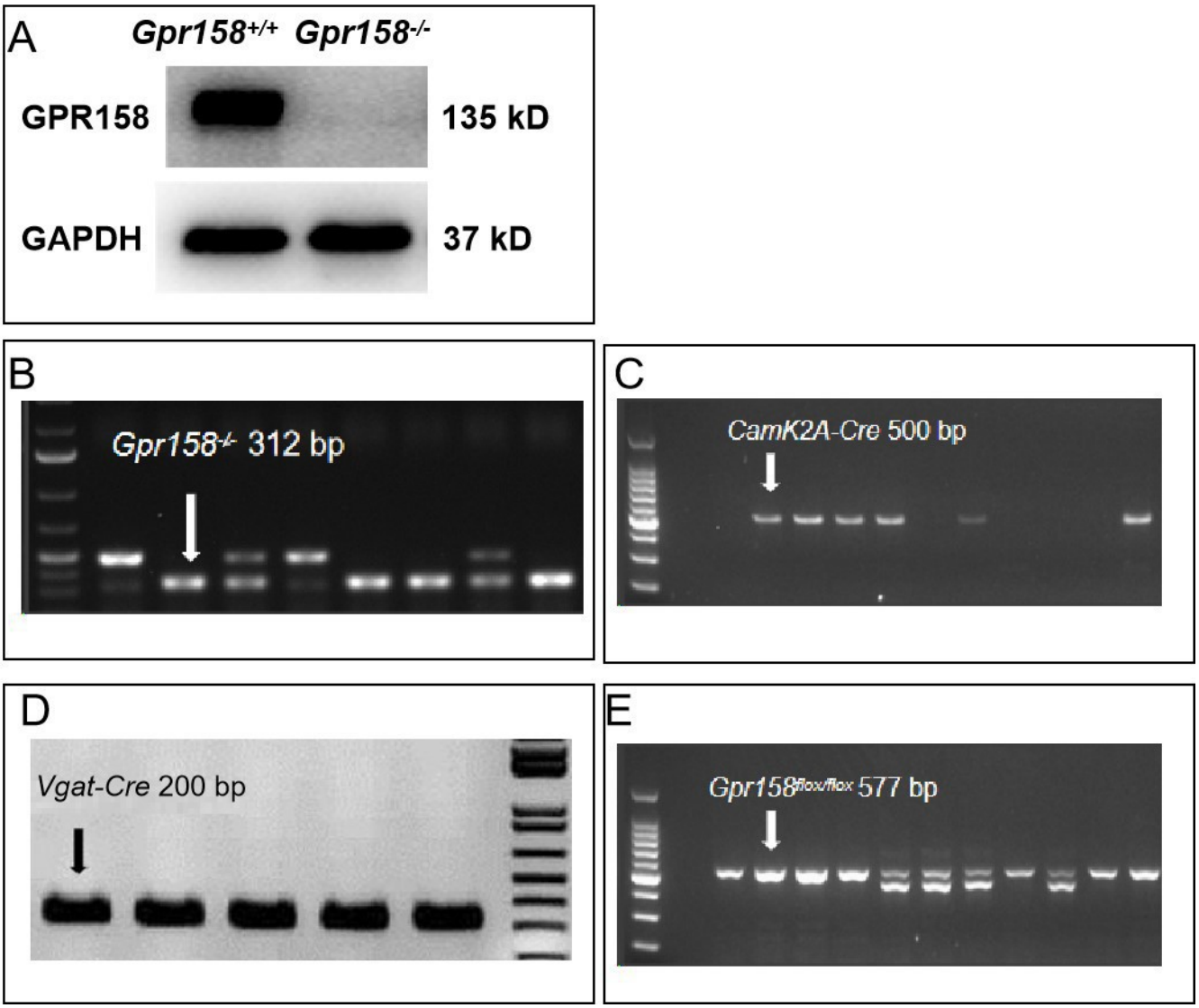

Figure2 Model validation.jpg

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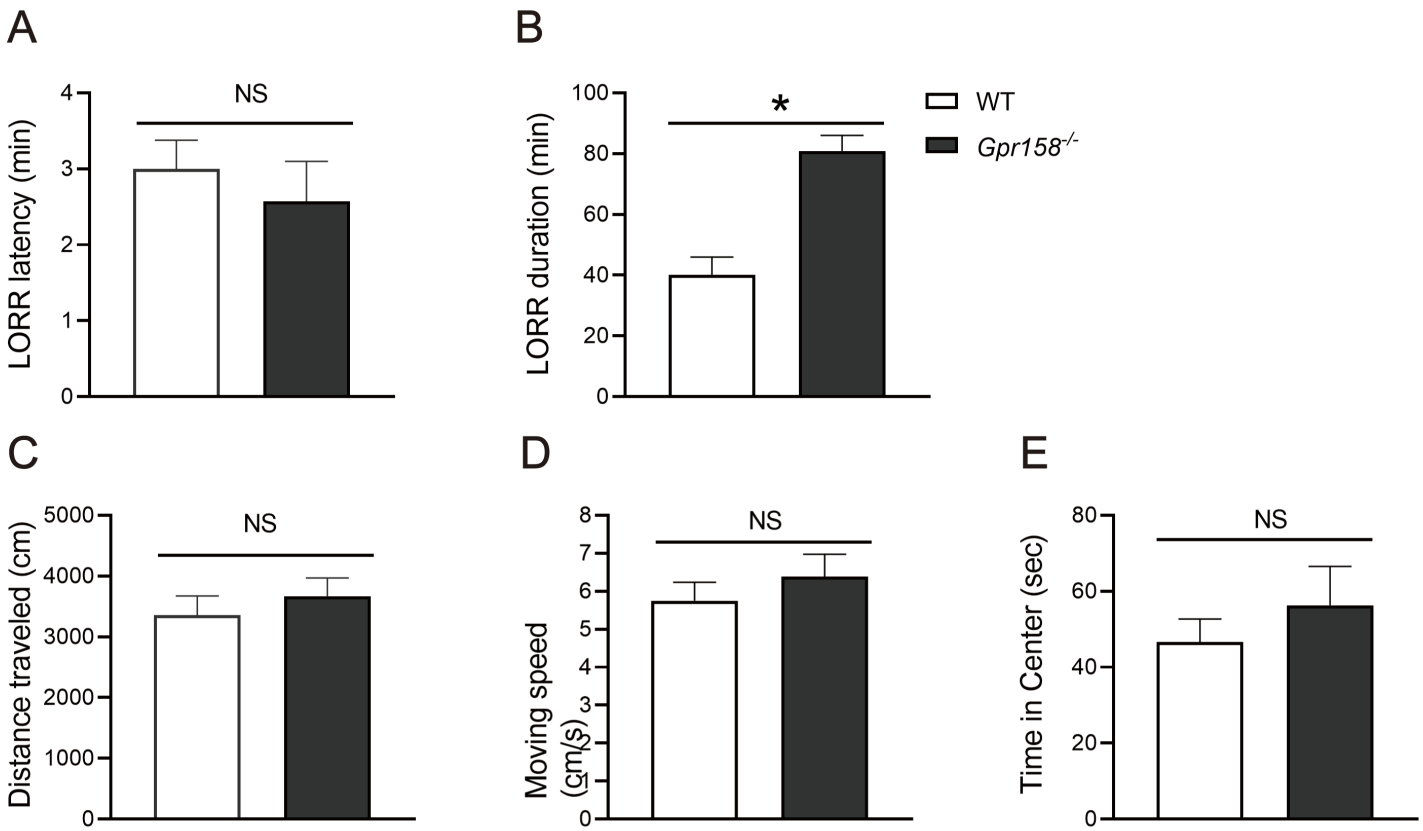

Figure3 KO.tif

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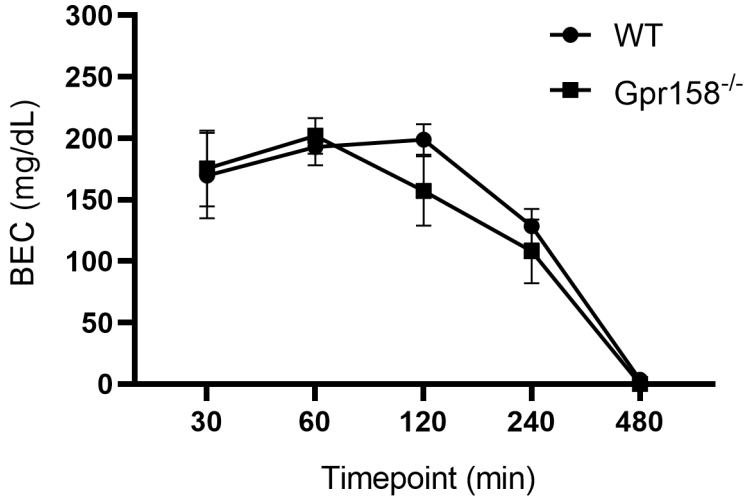

Figure4 Metabolism .tif

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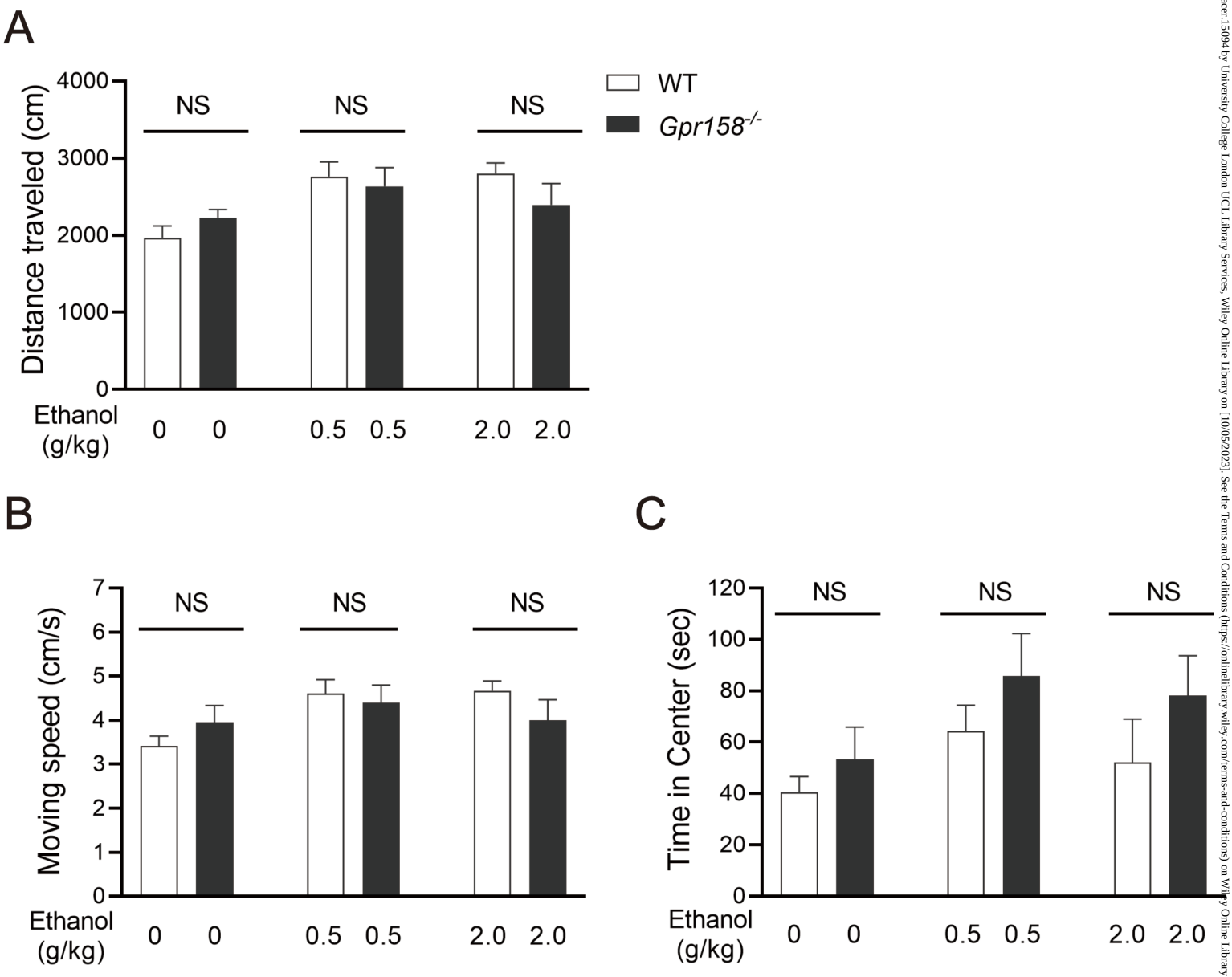

Figure5.tif

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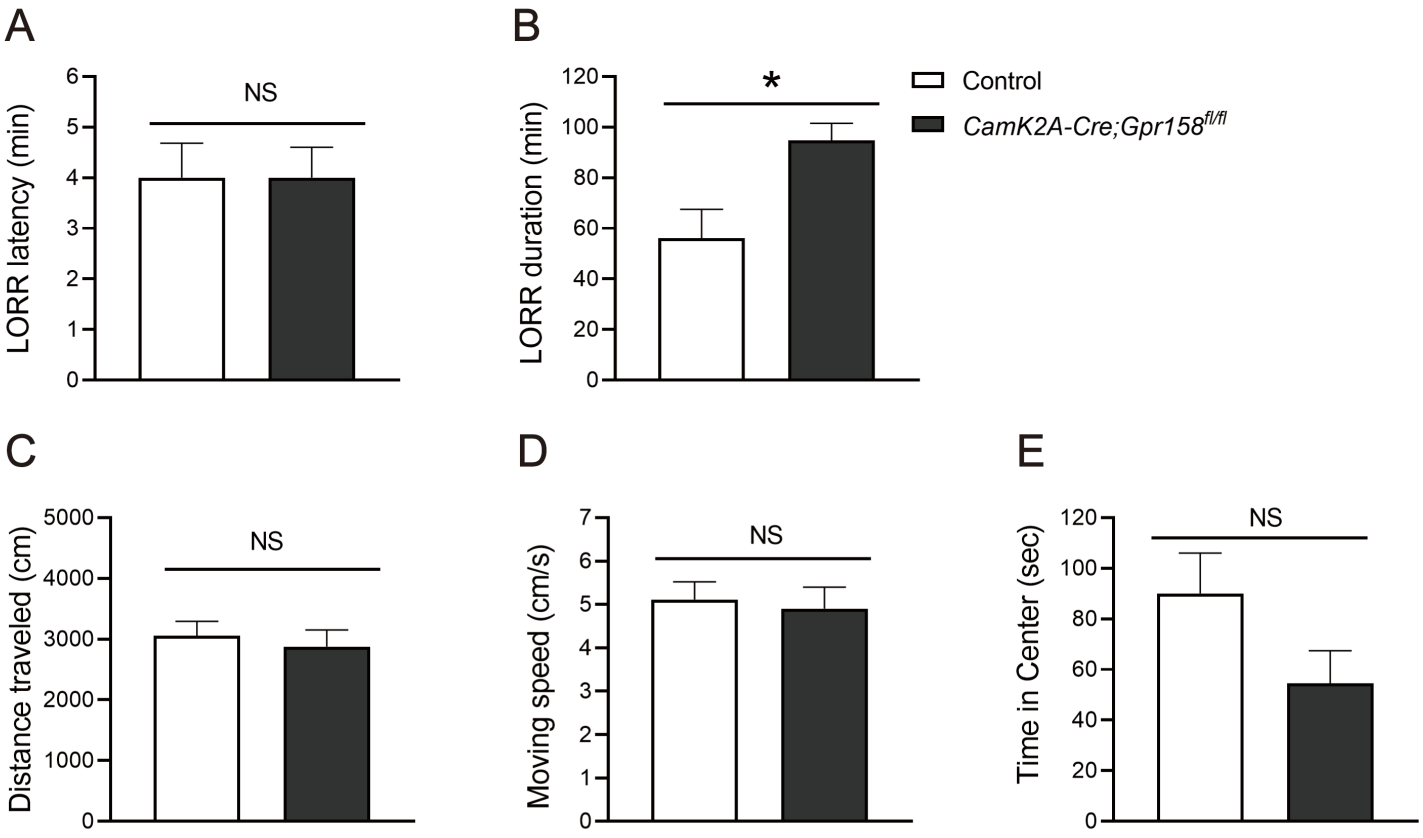

Figure6 CamK2A-KO.tif

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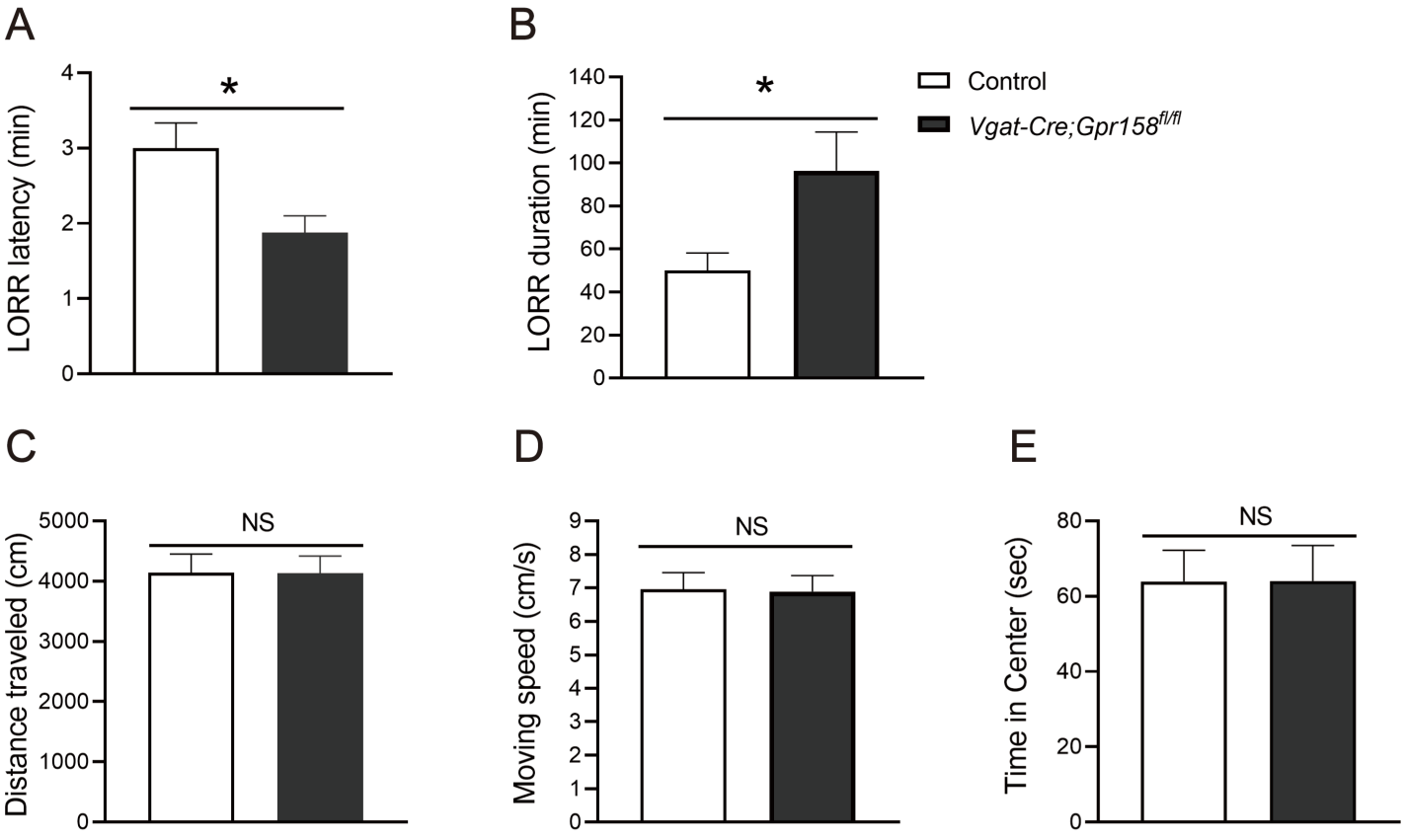

Figure7 Vgat-KO.tif

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