Supplementary Information

Glutamate decarboxylase 67 deficiency in a subset of GABAergic neurons induces schizophrenia-related phenotypes

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Materials and Methods

Animals

PV-Cre; *GAD67*$^{flox/flox}$ and *PV-Cre*; *GAD67*$^{flox/+}$ mice were generated by crossing ~~*PV*~~*Pvalb*-Cre BAC transgenic ~~(*PV-Cre*~~[Tg(Pvalb-cre)] (hereafter, *PV-Cre*) mice (Tanahira *et al*, 2009) and *GAD67-floxed* (*Gad1*^{Tm1}) mice (Obata *et al*, 2008). We genotyped the mice with the *PV-Cre*, *GAD67*$^{+}$, *GAD67*^{flox}, or *GAD67*^{null} allele through PCR analysis. The primer sequences and the approximate length of the amplified DNA fragments are as follows: *PV-Cre* allele, 5'-GTCTCTGGTGTAGCTGATGATCCGAA-3' and 5'-CCCTGTTTCACTATCCAGGTTACGGA-3'; *GAD67* allele, P1 (5'-AAGGTGCACAAAACTGCCTGAGATTCA-3'), P2 (5'-TCTCCCCAAGACTTGTCACCTGCTG-3'), and P3 (5'-CCCCTTAGCTGTGAGCCTCAC-3'); *PV-Cre* allele, 321 bp; *GAD67 wild type* allele, 213 bp; *GAD67 floxed* allele, 316 bp; and *GAD67 null* allele, 127 bp. The crosses between *PV-Cre*; *GAD67*$^{flox/+}$ mice and *GAD67*$^{flox/flox}$ mice produced *PV-Cre*; *GAD67*$^{flox/null}$, *GAD67*$^{flox/null}$, *GAD67*$^{flox/+}$, *GAD67*$^{flox/flox}$, *PV-Cre*; *GAD67*$^{flox/flox}$, and

PV-Cre; *GAD67*$^{flox/+}$ mice. PV is expressed in the ovary and testis (Kägi *et al*, 1987; Pohl *et al*, 1995). Thus, PV-Cre-mediated recombination occurred in female germ cells in some cases (Kobayashi and Hensch, 2013). We excluded the null-mutant *PV-Cre*; *GAD67*$^{flox/null}$ and *GAD67*$^{flox/null}$ mice from further analysis. In behavioral tests and acute slice electrophysiology, male *PV-Cre*; *GAD67*$^{flox/+}$ mutant mice and littermate male controls (*GAD67*$^{flox/+}$) were used. *VGAT-floxed STOP-tdTomato* transgenic mice were used for identifying what type of GABAergic neuron subtypes express Cre in *PV-Cre* mice. The generation of *VGAT-floxed STOP-tdTomato* transgenic mice will be described in detail elsewhere. In brief, because the expression of tdTomato is driven by the VGAT promoter, the reporter expression is restricted to inhibitory neurons when the floxed STOP is removed by Cre.

Western blot analysis

Whole brains and cerebral cortices were collected from mice at postnatal day 49 (P49)-P62 and P90-105, respectively, and homogenized in ice-cold homogenization buffer [320 mM sucrose, 50 mM Tris-HCl (pH 7.2), 5 mM EDTA, and 1 mM PMSF].

The homogenates were centrifuged at 3,000 rpm for 10 min at 4°C, and the S1 fractions were obtained. Equal amounts of protein from the S1 fractions of whole brains or cerebral cortices were separated through 8% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Then, the proteins were transferred to a nitrocellulose membrane using a semidry transfer method, and immunoblots were probed with a rabbit anti-GAD65/67 antibody (Hanamura *et al*, 2010) (1 μg/ml) or a monoclonal anti-β-actin antibody (Abcam, #ab6276, 1:10000). The protein levels were visualized using the enhanced chemiluminescence (ECL) Western Blotting Analysis System (GE Healthcare Life Sciences, Buckinghamshire, UK) and imaged with light-capture (ATTO, Tokyo, Japan). Protein levels were quantified by densitometry of the immunochemical signal using ImageJ software (http://rsbweb.nih.gov/ij/) and normalized to the signal intensity obtained for β-actin.

Immunohistochemistry

The mice were deeply anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and transcardially perfused with phosphate-buffered saline (PBS; pH 7.4) followed by either

4% paraformaldehyde (PFA) in 0.1 M phosphate buffer (PB; pH 7.4) for GAD67 and PV staining or 4% PFA with 0.05% glutaraldehyde in 0.1 M PB for GABA staining. The brains were removed, post-fixed in the same fixative for 2 h at 4°C, and immersed in 15% and 30% sucrose in PBS overnight for cryoprotection. Free-floating sections (50 μm) were cut using a cryostat (CM3050; Leica Microsystems) and incubated in PBS with 0.3% Triton X-100 and 1% BSA for 1 h at room temperature (RT). Then, the sections were incubated for 24 h at RT in a mixture of primary antibodies diluted in PBS with 0.3% Triton X-100, 1% BSA, and 0.25% λ-carrageenan. The primary antibodies used were as follows: rabbit anti-parvalbumin antibody (Swant, #PV28, 1:5000); mouse anti-parvalbumin (Swant, #235, 1:5000); monoclonal mouse anti-GAD67 antibody (Millipore, #MAB5406, 1:1000); rabbit anti-GABA (Sigma, #A2052, 1:1000); rabbit anti-calretinin (Swant, # 7699/4, 1:2000); and rabbit anti-somatostatin-14 (Peninsula Laboratories, T-4103, 1:2000). The sections were washed in PBS and incubated for 2 h in a mixture of Alexa Fluor 488-, Alexa Fluor 594-, and Alexa 633-conjugated species-specific secondary antibodies (Molecular Probes) diluted 1:200 in PBS with 0.3% Triton X-100 and 1% BSA. After further

washes in PBS, the sections were mounted on glass slides with Fluoromount/Plus mounting medium (Diagnostic BioSystems). To visualize perineuronal nets, brain sections were incubated with the biotinylated lectin *Wisteria floribunda* agglutinin (WFA) (Vector Labs, 1:2000) at 4°C overnight. After washing in PBS, the sections were incubated with Alexa Fluor 594 conjugated to streptavidin for 20 min at room temperature. For quantitative analysis, images were captured with a confocal laser scanning microscope (FV1000; Olympus) for GAD67, parvalbumin, calretinin, and somatostatin or with a conventional epifluorescence microscope for GABA and PV double staining. The images were analyzed using ImageJ software (http://rsbweb.nih.gov/ij/). For each brain slice, all PV-, GAD67-, or GABA-positive cells were visually identified, and the intensities of the immunoreactive signals from the cell somata of each slice were measured. For GABA and PV double immunohistochemistry, the signal intensities were measured and then transformed into Z-scores using the mean and standard deviation of the control mice to equalize the variability of the immunohistochemical staining among the different samples due to differences in anti-GABA antibody penetration. Quantification of the fluorescent

intensities was carried out without any adjustment of brightness/contrast. Sections chosen for analysis were anatomically-matched between comparing groups. Stereological cell counting approach was employed from rectangular regions of interests (ROIs) of fixed size positioned over the prefrontal cortex (1,000 μm high × 500 μm wide). Fluorescent cells within each ROI were counted using the Cell Counter plugin for ImageJ. The representative images in the figures are shown with adjustment of brightness/contrast for visibility.

Slice preparation and electrophysiology

Hippocampal slices were prepared from 12- to 16-week-old male *PV-Cre*; *GAD67*$^{flox/+}$ mice and littermate controls (*GAD67*$^{flox/+}$), using standard procedures (Miwa *et al*, 2008). Slices were perfused with a medium that was saturated with 95% O_2 and 5% CO_2 and contained (in mM) 119 NaCl, 2.5 KCl, 2.5 $CaCl_2$, 1.3 $MgSO_4$, 1.0 NaH_2PO_4, 26.2 $NaHCO_3$, and 11 glucose. The medium (at 25 ± 1°C) for recordings containing 100 μM picrotoxin, a $GABA_A$ receptor antagonist, was used for all of the experiments except for the recording of $GABA_A$ receptor-mediated IPSCs ($GABA_A$-IPSCs). Whole-cell

patch-clamp recordings were made from pyramidal cells in the CA1 region with a MultiClamp 700B patch-clamp amplifier (Molecular Devices, Union City, CA, USA). The pipette solution contained (in mM): 122.5 cesium gluconate, 17.5 CsCl, 10 HEPES, 0.2 EGTA, 8 NaCl, 2 Mg-ATP, and 0.3 Na_3-GTP (pH 7.2; 290–310 mOsm). In some experiments, biocytin (5 mg/ml) was added to the internal solution immediately before recording. The recording electrodes had resistances of 3–7 MΩ. The series resistance was 10–30 MΩ and was monitored online throughout the experiment. The experiments were rejected if the series resistance changed by more than 20%. The signal was filtered at 5 kHz and digitized at 20 kHz with pClamp9.2 software (Molecular Devices). For evoking synaptic responses, a bipolar stimulating electrode placed in the stratum radiatum and was stimulated at 0.1 Hz. The cells were voltage-clamped at −90 mV to record excitatory postsynaptic currents (EPSCs), unless otherwise indicated. When NMDA receptor-mediated EPSCs were recorded, 10 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) was present to block AMPA receptor-mediated EPSCs. $GABA_A$-IPSCs were evoked at 0 mV in the presence of CNQX (10 μM) and D-(–)-2-amino-5-phosphonovaleric acid (D-APV) (50 μM). The

rise times of NMDA EPSCs were measured as the time from 10 to 90% of the peak amplitude. The decay times of NMDA EPSCs were fitted to the sum of two exponential functions: $y = A_f\exp(-t/\tau_f) + A_s\exp(-t/\tau_s)$, where A is the current amplitude, t is time, τ is the decay time constant, and the subscripts 'f' and 's' denote fast and slow components, respectively. Experiments were carried out in a genotype-blinded manner.

Behavioral analyses

All behavioral tests were performed with age-matched, male mutant mice and littermate control mice that were 12 weeks old at the start of testing. Heterozygous *GAD67*$^{flox/+}$ mice were used as control animals. The mice were housed in a room with a 12-h light/dark cycle (lights on at 7:00 a.m.) with access to food and water ad libitum. Behavioral testing was performed between 8:00 a.m. and 6:00 p.m., except for the open field test (7:00 a.m. to 7:00 p.m.) because of the limited laboratory capacity. The genotypes were blinded to the investigators during behavioral testing. The first group performed the behavioral tests in the following sequence: general health and neurological screening; elevated plus maze; light/dark transition; acoustic startle

response and prepulse inhibition test; rotarod test; Y-maze test; Crawley's sociability and preference for social novelty test; and contextual and cued fear conditioning test. The second group performed the behavioral tests in the following sequence: Porsolt forced swim test; open field test; and sensitivity to MK-801. The order of testing was chosen from least invasive to most invasive, to decrease the chance that behavioral responses were altered by prior test history. Each behavioral test was separated from each other at least by 1 day.

For general health and neurological screenings, the body weight, condition of the coat, righting reflex, whisker twitch, and ear twitch were evaluated. Muscular strength was measured using the wire hang test. In the wire hang test, the mouse was placed on a metal wire mesh, and then the mesh was inverted. Latency to fall from the mesh was recorded with a 60-s cutoff time.

For the light/dark transition test, the apparatus used consisted of a cage (42 cm × 21 cm × 25 cm) divided into two chambers of the same size divided by a partition containing a sliding door (O'Hara & Co., Ltd., Tokyo, Japan). One of the chambers was brightly illuminated (390 lux), whereas the other was dark (3 lux). The mice were

placed into the dark side and allowed to move freely between both chambers with the door open for 10 min. The total number of transitions between the chambers, time spent in each side, first latency to enter the light side, and distance traveled were recorded. The data were collected and analyzed using ImageJ LD1 software (O'Hara & Co., Ltd., Tokyo, Japan).

For the elevated plus maze test, the apparatus consisted of two open arms and two closed arms (O'Hara & Co., Ltd., Tokyo, Japan). Each arm was the same size (25 cm × 5.5 cm), and the closed arms had 15-cm-high transparent walls. The floor of the arms and the central square (5.5 cm × 5.5 cm) were made of white plastic. The apparatus was placed 50 cm above the floor. The light condition was 70 lux at the central square of the maze. The mice were allowed to move freely in the maze for 10 min. The time spent in the open arms, distance traveled, and the number of entries into the open arms were recorded. The data were collected and analyzed using ImageJ EP1 software (O'Hara & Co., Ltd., Tokyo, Japan).

For the acoustic startle response and prepulse inhibition (PPI) test, an acoustic startle reflex measurement system (O'Hara & Co., Ltd., Tokyo, Japan) was used. The

startle response was assessed with various stimuli intensities. Five times of 70 to 120 dB (70, 75, 80, 85, 90, 95, 100, 110, 120 dB) white noise stimuli (40 ms) were presented in quasi-random order and random inter-trial intervals (10-20 s). In the PPI session, mice experienced five trial types: no stimulus; startle stimulus (120 dB, 40 ms) only; prepulse 70 dB (20 ms, lead time 100 ms) and pulse 120 dB; prepulse 75 dB (20 ms, lead time 100 ms) and pulse 120 dB; and prepulse 80 dB (20 ms, lead time 100 ms) and pulse 120 dB. Each trial was repeated ten times in quasi-random order and random inter-trial interval (10-20 s). Prepulse inhibition was defined as the percent decline of the startle response: 100 - [(startle amplitude after prepulse and pulse)/ (startle amplitude after pulse only)] × 100.

The contextual and cued fear conditioning test was conducted in a small chamber surrounded by a sound-attenuated chamber (O'Hara & Co., Ltd., Tokyo, Japan). This test consisted of a conditioning trial (Day 1), a contextual test trial (Day 2), and a cued test trial (Day 3). The mice were placed in a clear plastic conditioning chamber (10 cm × 10 cm × 10 cm) equipped with a stainless steel grid floor, which was wired to a shock generator for 170 s and then presented with a tone of 65 dB/10 kHz for 10 s

through a speaker on the ceiling of the sound-attenuating chamber. At the end of the tone presentation, the mice were given a foot shock (0.4 mA, 2 s). Freezing responses were monitored for 1 min after the foot shock, and the mice were then returned to their home cages. A contextual test was performed in the same conditioning chamber for 6 min in the absence of the tone at 24 h after the conditioning trial. Furthermore, a cued test was performed in a novel chamber with contexts different from those of the conditioning chamber to minimize freezing caused by contextual fear conditioning; the test chamber was different from the conditioning chamber in brightness (30 lux), color (white), and floor structure (with wood chip bedding and no grid). The cued test was conducted 24 h after the contextual test was finished and consisted of a 3 min exploration period (no CS) to evaluate nonspecific contextual fear followed by a 3 min CS period (no foot shock) to evaluate the acquired cued fear. The percent time exhibiting a freezing response (immobility excluding respiration and heartbeat) was measured as an index of fear memory. The data were collected and analyzed using TimeFZ1 (O'Hara & Co., Ltd., Tokyo, Japan).

For the rotarod test, a mouse was placed on a drum (3 cm diameter; O'Hara &

Co., Ltd., Tokyo, Japan) rotating at 4 rpm. Then, the rotation of the rotarod was accelerated from 4 to 40 rpm over a 300-s period at a constant rate. The amount of time each mouse was able to maintain its balance on the rod was measured. The mice were trained for two consecutive days and received four trials per day at 2-h intervals between trials.

For the Y-maze test, the Y maze apparatus consisted of three arms (40 cm × 9 cm × 25 cm) made of transparent Plexiglas joined in the middle to form a "Y" shape. The apparatus was placed in a diffusely illuminated room (70 lux at the center triangle of the maze). Each mouse was placed at the end of one arm and allowed to move freely through the maze during an 8 min session. Alternation behavior was defined as consecutive entries into each of the three different arms without repetition (for instance, ABC, CBA…). The percentage of spontaneous alternation was calculated as follows: the number of alternation (entries into three different arms consecutively) divided by the total possible alternations (i.e., the number of arm entries - 2) × 100.

For Crawley's sociability and preference for social novelty test, the social test apparatus consists of a rectangular, three-chambered box illuminated by LEDs (O'Hara

& Co., Ltd., Tokyo, Japan). This test consisted of a period of habituation, a sociability test, and a social novelty test. A subject mouse was first placed in the middle chamber and habituated to the entire test box for 10 min. After habituation, the subject mouse was removed from the apparatus and an unfamiliar male mouse (stranger 1) that had no prior contact with the subject mouse was placed in one of the side chambers. The location of stranger 1 in the left or right side chamber was systemically alternated between trials. The stranger 1 mouse was enclosed in a small cylindrical wire cage (diameter of 9 cm, 11 cm height, horizontal wires spaced 8 mm apart). An identical empty cage was placed in the opposite chamber. The subject mouse was then placed in the middle chamber and was allowed to move freely for 10 min (sociability test). At the end of the sociability test session, the subject mouse was removed from the apparatus, and an additional novel unfamiliar mouse (stranger 2) was enclosed in the wire cage that had previously been empty. The subject mouse was placed in the middle chamber and allowed to explore the apparatus for additional 10 min to assess social memory (social novelty test). The stranger mice used in this experiment were male C57BL/6J mice (15-16 weeks old). The mouse behavior was recorded and time spent within a

7-cm distance of each cage was measured by ImageJ CSI software (O'Hara & Co., Ltd., Tokyo, Japan).

For the open field test and sensitivity to MK-801, each mouse was placed in one corner of the apparatus (50 × 50 × 40 cm; O'Hara & Co., Ltd., Tokyo, Japan) and was allowed to move freely for 30 min and then MK-801 (0.2 mg/kg; Sigma) was administered intraperitoneally, and the mouse was returned to the same arena for 90 min. This MK-801 dose is sufficient to activate locomotor activity (Karlsson *et al*, 2008). The behavior of each mouse was monitored by a CCD camera equipped on the ceiling of the rack for the open field. Distance traveled (cm), vertical activity (rearing measured by counting the number of photobeam interruptions), and time spent in the center area of the field (36% of the field) were measured, and the data were collected every 5 min. The data were collected and analyzed using ImageJ OFC software (O'Hara & Co., Ltd., Tokyo, Japan).

For the Porsolt forced swim test, the mice were placed in a Plexiglas cylinder (22 cm height × 12 cm diameter), and the immobility and the distance traveled were recorded over a 10-min test period (Day 1). Retention tests were administered 24 h after

training (Day 2). The data acquisition and analysis were performed automatically using TimeFZ1 software (O'Hara & Co**.**, Ltd., Tokyo, Japan)**.**

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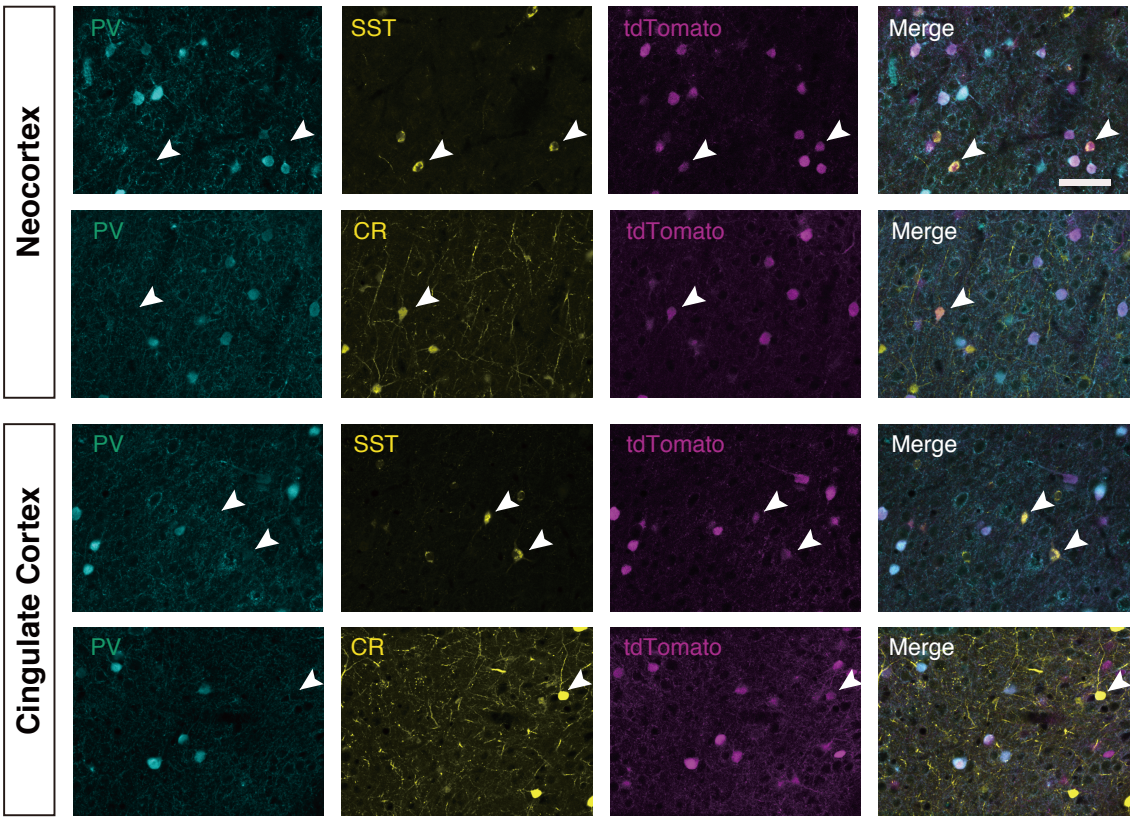
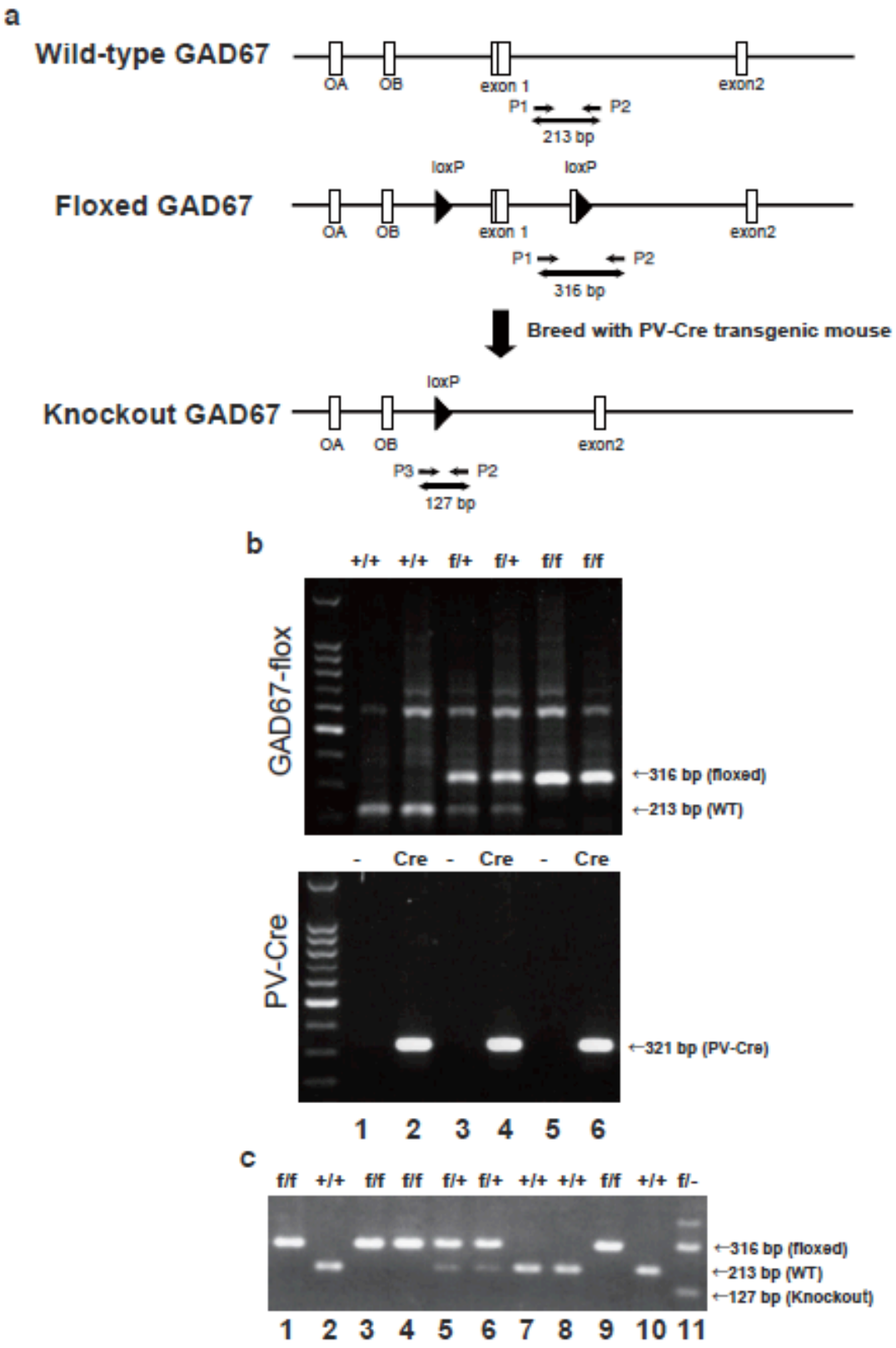


Figure S1 Expression patterns of functional Cre in GABAergic neurons in layer II/III of the cerebral cortex from 3-month-old *PV-Cre*; *VGAT-floxed STOP-tdTomato* mice. To visualize GABAergic neuron-specific Cre activity of *PV-Cre* mice, we crossed them with GABAergic neuron-specific tdTomato reporter mice, *VGAT-floxed STOP-tdTomato* transgenic mice. Because the expression of tdTomato is driven under the control of the promotor for vesicular GABA transporter (VGAT), which is specifically expressed in GABAergic neurons, the reporter is restricted to GABAergic neurons when the *floxed STOP* cassette is removed by Cre. The generation and characterization of *VGAT-floxed STOP-tdTomato* transgenic mice will be described in detail elsewhere. Cre-dependent recombination and thereby tdTomato expression (magenta) occurred in PV-positive cell lineage of GABAergic neurons. PV (cyan) and somatostatin (SST) or calretinin (CR) (yellow) positive cells are shown in the neocortex and cingulate cortex. Note that tdTomato expression was observed primarily in PV-positive neurons. A few tdTomato-positive cells were not positive for PV, but at least in part, were positive for SST or calretinin (CR) (arrow heads). Scale bar, 50 μm.


a
Wild-type GAD67
OA
OB
exon 1
exon2
P1
P2
213 bp
loxP
loxP
Floxed GAD67
OA
OB
exon 1
exon2
P1
P2
316 bp
Breed with PV-Cre transgenic mouse
loxP
Knockout GAD67
OA
OB
exon2
P3
P2
127 bp
b
+/+ +/+ f/+ f/+ f/f f/f
GAD67-flox
←316 bp (floxed)
←213 bp (WT)
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PV-Cre
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1 2 3 4 5 6
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f/f +/+ f/f f/f f/+ f/+ +/+ +/+ f/f +/+ f/-
←316 bp (floxed)
←213 bp (WT)
←127 bp (Knockout)
1 2 3 4 5 6 7 8 9 10 11

Figure S2 PV promotor-mediated disruption of the mouse GAD67 gene. (a) A schematic representation of GAD67 genomic DNA (Wild-type GAD67), targeted genome (Floxed GAD67), and Cre-mediated deleted genome (Knockout GAD67). Exon 1 of GAD67 was deleted using the Cre-loxP system. The arrows indicate PCR primers (P1, P2, and P3). (b) Representative results of PCR genotyping for GAD67-flox (upper; +, wild-type allele; f, floxed allele), and for PV-Cre (bottom; -, no PCR product corresponding to the Cre transgene; Cre, the presence of PCR product corresponding to the Cre transgene). (c) Using the primers that detect the knockout allele (P2 and P3), we observed mutant mice with the GAD67-null allele (i.e., *GAD67*$^{flox/null}$ and *PV-Cre*; *GAD67*$^{flox/null}$) at a frequency of less than 5%. An example of *GAD67* $^{flox/null}$ (f/-) is shown in lane 11.

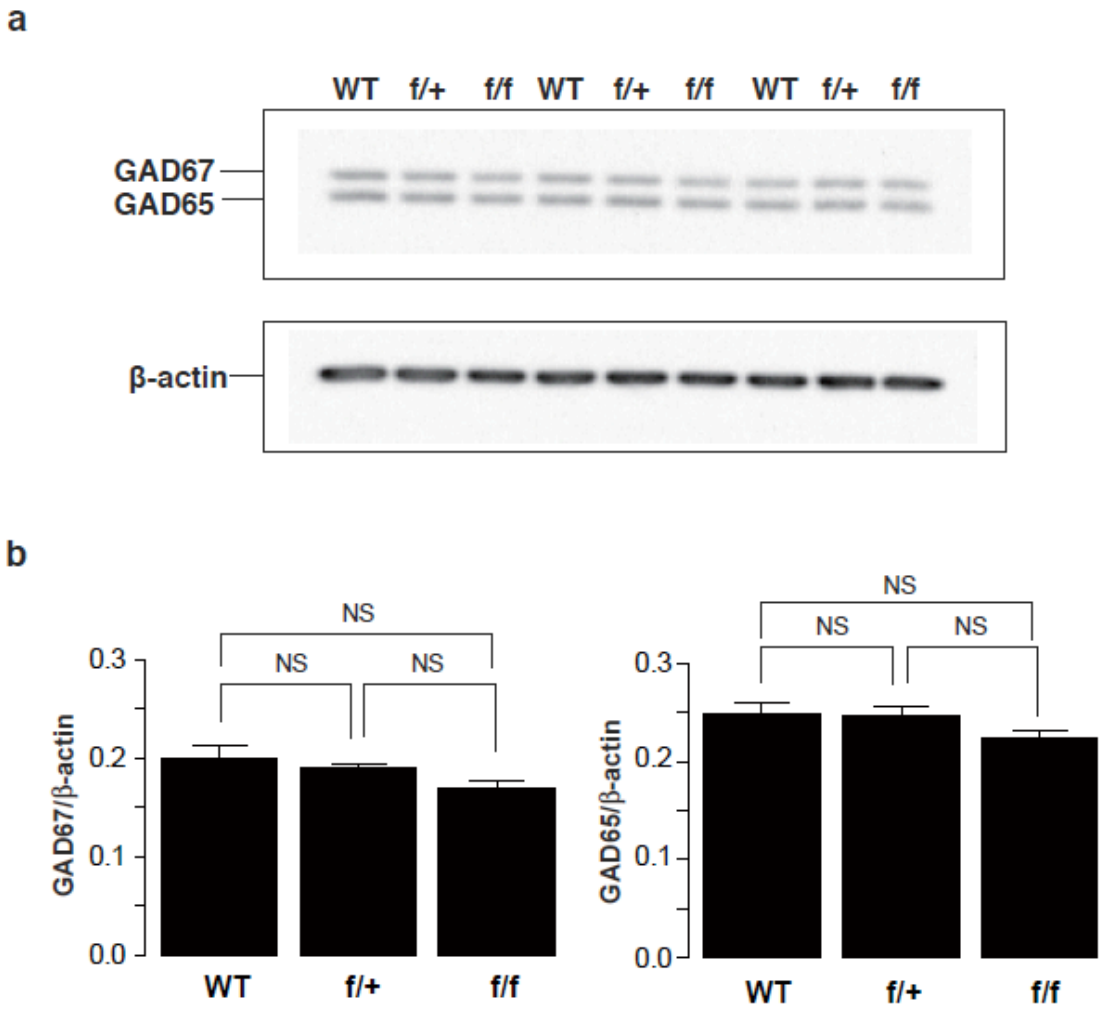

Figure S3 No significant genotype effects on the expression level of GAD65 and GAD67 in whole brain homogenates from wild-type, *GAD67*$^{flox/+}$, and *GAD67*$^{flox/flox}$ mice. (a) Western blot analysis of the whole brain of wild-type (WT), heterozygous GAD67$^{flox/+}$ (f/+), and homozygous GAD67$^{flox/flox}$ (f/f) mice. (b) Densitometric analysis of the expression levels of GAD67 (left) and GAD65 (right). To evaluate the expression levels of GAD65 and GAD67, the intensities of the bands in b were divided by their corresponding control (β-actin). The relative expression levels of GAD67 (left; WT, 0.199 ± 0.015; f/+, 0.190 ± 0.0035; f/f, 0.170 ± 0.0068) and GAD65 (right; WT, 0.249 ± 0.011; f/+, 0.246 ± 0.011; f/f, 0.224 ± 0.0061). n = 3 for each genotype. The data are presented as the mean ± s.e.m. P values were calculated using one-way ANOVA.

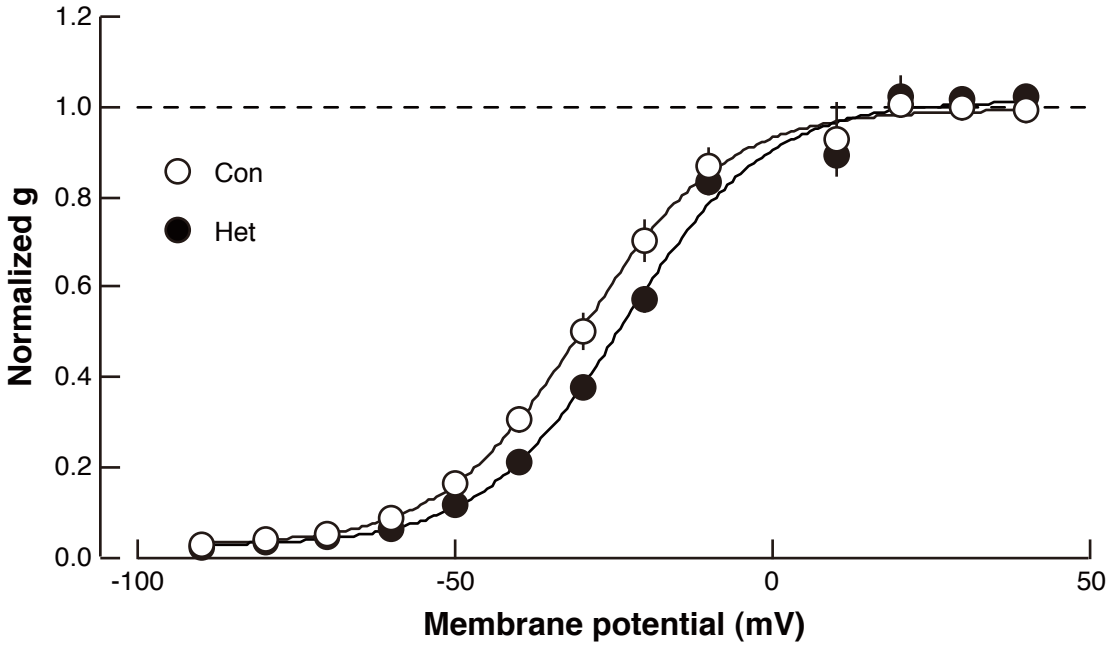

Figure 4 The conductance-voltage (*g*-*V*) relationship of NMDA synaptic currents. The conductance-voltage plots of NMDA EPSCs of *GAD67*$^{flox/+}$ (Con, open circles, n = 9) and *PV-Cre*; *GAD67*$^{flox/+}$ (Het, filled circles, n = 8) mice. The conductance was normalized to the maximum value of conductance in each cell. The data were fitted with the Boltzmann function. The membrane potential at which the NMDA receptor channel exhibited 50% of the maximal conductance (V_{50}) in conductance-voltage plots was -29.7 ± 1.5 mV and -23.1 ± 1.1 mV of *GAD67*$^{flox/+}$ and *PV-Cre*; *GAD67*$^{flox/+}$ mice, respectively. There was a significant difference (P = 0.0042) in the V_{50} between the two genotypes.

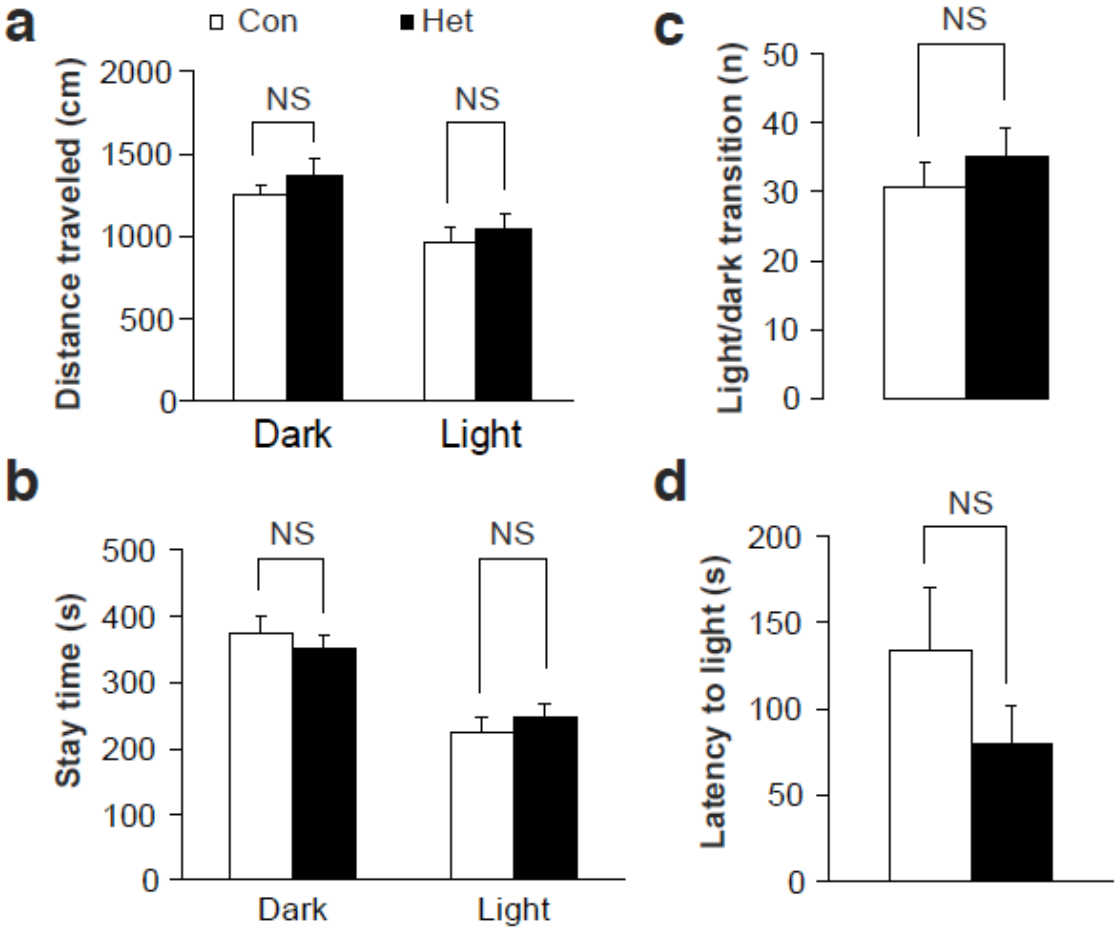

Figure 5 Normal behaviors of *PV-Cre*; *GAD67*$^{flox/+}$ mice in the light/dark transition test. (a) Distance traveled in the light/dark compartments (Dark, Con, 1281.2 ± 68.8 cm, Het, 1369.4 ± 96.8 cm, t = -0.8556, P = 0.3986; Light, Con, 983.7 ± 93.4, Het, 1050.7 ± 85.9, t = -0.6014, P = 0.5518). (b) Time spent in the light/dark compartments (Dark, Con 373.5 ± 21.7, Het, 352.9 ± 20.1, t = 0.7923, P = 0.434; Light, Con 226.5 ± 21.7, Het, 247.1 ± 20.1, t = -0.7913, P = 0.4346). (c) The number of transitions between the light and dark sides (Con, 31.8 ± 3.8; Het, 35.2 ± 4.0; t = -0.6998, P = 0.4891). (d) Latency to enter the light box (Con, 126.3 ± 35.1; Het, 80.0 ± 22.1; t = -1.293, P = 0.2062). No significant difference was observed between genotypes in this test. Control mice (Con, n = 18); *PV-Cre*; *GAD67*$^{flox/+}$ mice (Het, n = 16). The data are presented as the mean ± s.e.m. P values were calculated by Welch's two sample t-test.

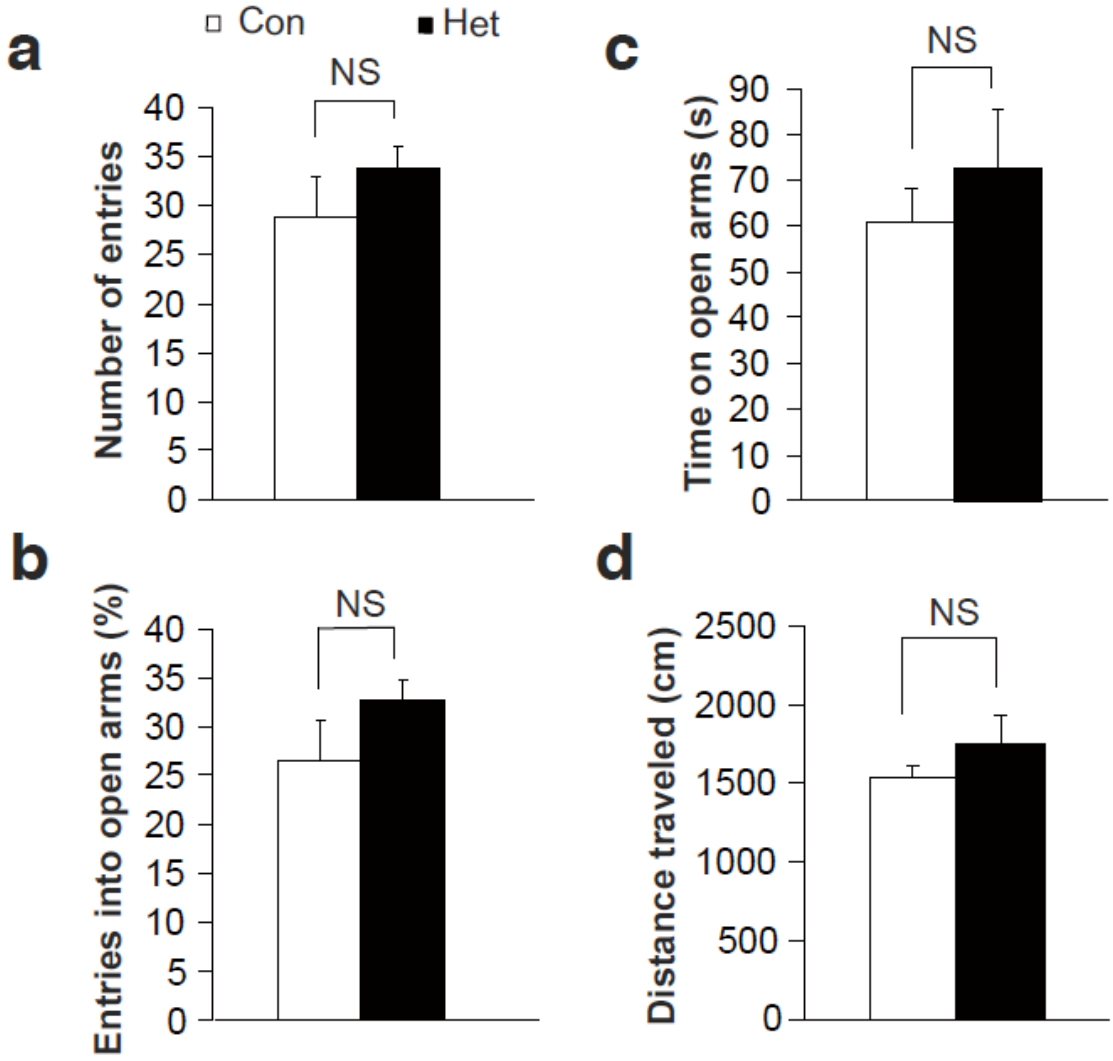

Figure 6 Normal anxiety-like behaviors of *PV-Cre*; *GAD67*$^{flox/+}$ mice in the elevated plus maze. (a) The number of arm entries (Con, 28.9 ± 2.1; Het, 33.9 ± 4.0; t = -1.0886, P = 0.2878). (b) The percentage of entries into open arms (Con, 26.6 ± 2.2%; Het, 32.6 ± 4.1%; t = -1.3067, P = 0.2041). (c) The time spent in open arms (Con, 60.9 ± 7.2 s; Het, 72.7 ± 12.6 s; t = -0.8169, P = 0.4219). (d) The distance traveled (Con, 1531.8 ± 77.8 cm; Het, 1753.6 ± 176.0 cm; t = -1.1527, P = 0.2621). No significant differences were observed between genotypes in this test. Control mice (Con, n = 18); *PV-Cre*; *GAD67*$^{flox/+}$ mice (Het, n = 16). The data are presented as the mean ± s.e.m. P values were calculated using Welch's two sample t-test.

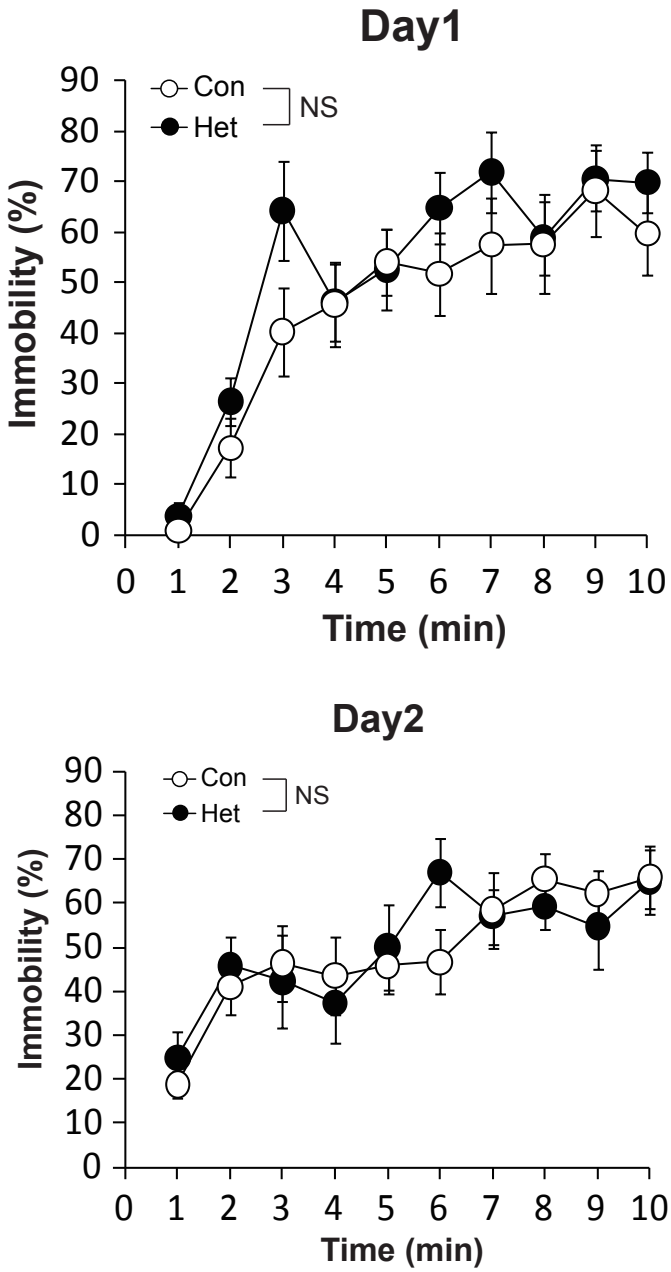

Figure 7 Normal performance in behavioral despair of *PV-Cre*; *GAD67*$^{flox/+}$ mice in the Porsolt forced swim test. There was no significant difference in the percentage of immobility time between the genotypes in each block for Day 1 (upper, $F(1,20) = 2.2465$, $P = 0.1495$) and Day 2 (bottom, $F(1,20) = 0.0248$, $P = 0.8764$). Control mice (Con, open circles, $n = 11$); *PV-Cre*; *GAD67*$^{flox/+}$ mice (Het, filled circles, $n = 11$). The data are presented as the mean ± s.e.m. *P* values were calculated using two-way repeated measures ANOVA.

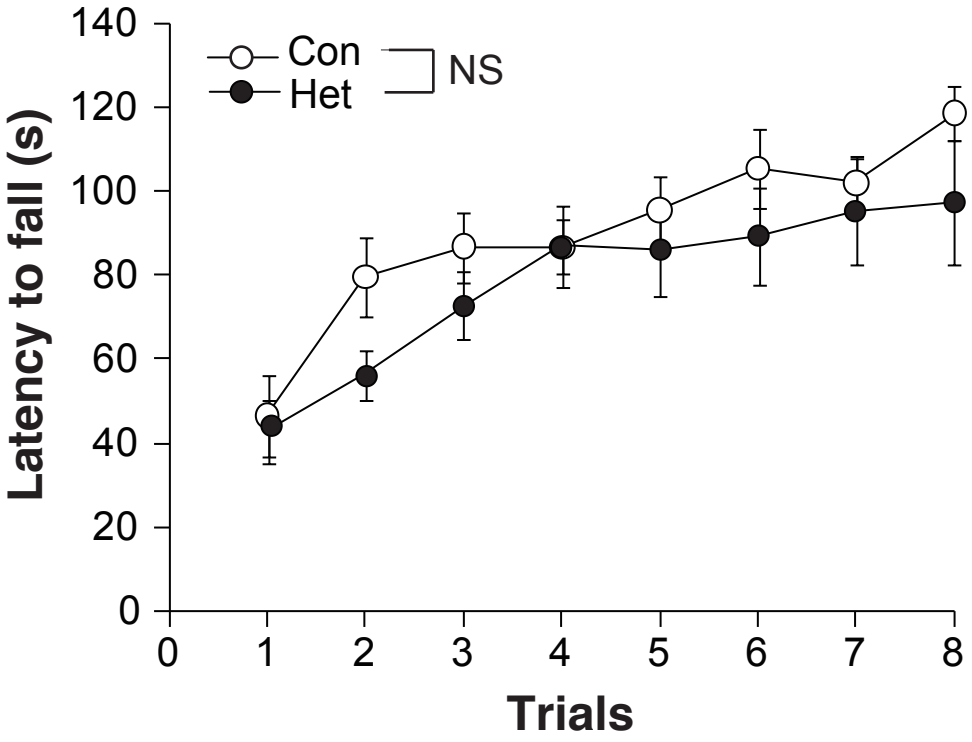

Figure 8 Normal motor coordination/motor learning of *PV-Cre*; *GAD67*$^{flox/+}$ mice. Rod rotation was accelerated from 4 to 40 rpm over a 300-s period. No significant differences in the latency to fall from an accelerating rotarod were observed between genotypes (F (1,31) = 1.4291, P = 0.2410). Control mice (Con, open circles, n = 17); *PV-Cre*; *GAD67*$^{flox/+}$ mice (Het, filled circles, n = 16). The data are presented as the mean ± s.e.m. P values were calculated using two-way repeated measures ANOVA.

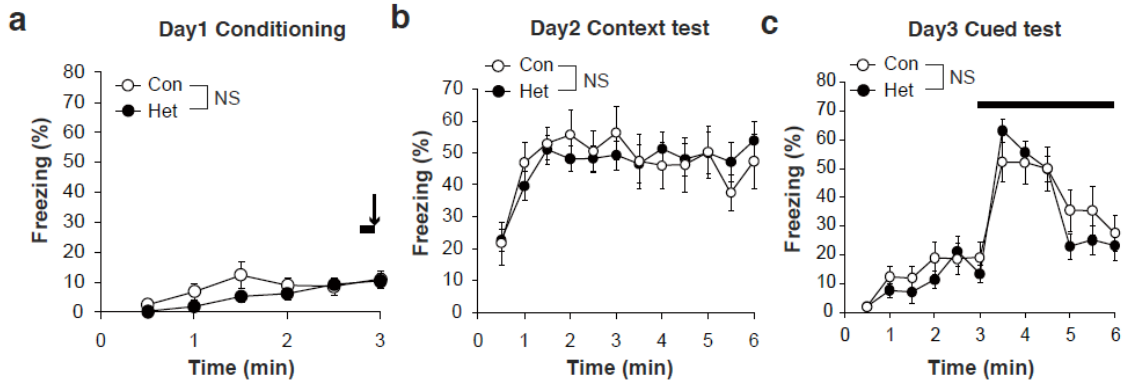

Figure S9 Normal contextual and cued fear conditioning in *PV-Cre*; *GAD67*$^{flox/+}$ mice. (a) Percentage of freezing time of controls (Con, open circles) and *PV-Cre*; *GAD67*$^{flox/+}$ mice (Het, filled circles) in fear conditioning on Day 1. At 170 s after the mice were placed in the conditioning chamber, a tone was presented for 10 s (bar), and at the end of the tone, the mice were administered a foot shock (arrow). (b) The percentage of freezing time in the contextual testing 24 h after conditioning (Day 2). (c) The percentage of freezing time in the cued test 48 h after conditioning (Day 3). The tone was presented 3 min after mice were place into a test chamber with altered context (bar). No significant genotype effects on freezing time were found in any of the tests (Day 1, F (1,20) = 2.3492, P = 0.1410; Day 2, F (1,20) = 0.0013, P = 0.9710; Day 3, F (1,20) = 0.3303, P = 0.5718). Control mice (Con, n = 11); *PV-Cre*; *GAD67*$^{flox/+}$ mice (Het, n = 11). The data are presented as the mean ± s.e.m. P values were calculated using two-way repeated measures ANOVA.

Table S1 Colocalization of chemical markers for GABAergic neurons with tdTomato[1]

a

	PV (%)	SST (%)	tdTomato only (%)
Motor cortex			
Layer II/III (*n* = 181)	68.5 ± 3.0	22.0 ± 3.6	9.5 ± 2.2
Layer V (*n* = 156)	69.9 ± 4.0	22.8 ± 3.8	7.3 ± 1.9
Somatosensory cortex			
Layer II/III (*n* = 141)	65.5 ± 3.8	24.5 ± 3.4	10.0 ± 2.1
Layer V (*n* = 146)	74.6 ± 5.5	14.8 ± 3.7	10.7 ± 3.0
Cingulate cortex			
Layer II/III (Anterior) (*n* = 165)	72.8 ± 4.5	17.7 ± 3.4	9.5 ± 2.4
Layer II/III (Posterior) (*n* = 122)	61.0 ± 5.2	27.9 ± 3.6	11.1 ± 4.1

b

	PV (%)	CR (%)	tdTomato only (%)
Motor cortex			
Layer II/III (*n* = 138)	64.5 ± 3.3	13.9 ± 4.1	21.7 ± 9.1
Layer V (*n* = 143)	75.6 ± 3.5	2.6 ± 1.2	21.8 ± 3.2
Somatosensory cortex			
Layer II/III (*n* = 166)	62.7 ± 1.7	12.4 ± 1.1	24.9 ± 1.8
Layer V (*n* = 199)	76.8 ± 2.8	0.38 ± 0.4	22.8 ± 2.7
Cingulate cortex			
Layer II/III (Anterior) (*n* = 184)	65.3 ± 4.6	12.6 ± 2.6	22.1 ± 3.8
Layer II/III (Posterior) (*n* = 149)	60.3 ± 4.8	13.3 ± 3.8	26.5 ± 5.1

[1]The table shows the proportion of parvalbumin (PV)-positive and somatostatin (SST)-positive cells or calretinin (CR)-positive cells among tdTomato-positive GABAergic neurons in *PV-Cre*; *VGAT-floxed STOP-tdTomato* mice. (a) The percentages of PV-positive cells, SST-positive cells, and cells negative for both PV and SST (tdTomato only) among tdTomato-positive GABAergic neurons by double immunolabeling with antibodies for PV and SST. (b) The percentages of PV-positive cells, CR-positive cells, and cells negative for both PV and SST (tdTomato only) among tdTomato-positive GABAergic neurons by double immunolabeling with antibodies for PV and CR. The data are presented as the mean ± s.e.m.