SUPPLEMENTARY INFORMATION

Hippocampal hypoactivity, insensitivity to pro-psychotic drugs and potentiated latent inhibition in glutaminase-deficient mice: therapeutic implications for schizophrenia

Gaisler-Salomon *et al.*

DETAILED METHODS **2**

Enzymatic activity **2**

^{1}H Magnetic resonance spectroscopy **3**

Immunocytochemistry **4**

Behavioral Testing **5**

***In vivo* imaging** **15**

Slice physiology **16**

Microdialysis **17**

SUPPLEMENTAL FIGURES **20**

Figure S1. Magnetic resonance spectroscopy **20**

Figure S2. Probe placement in microdialysis study **22**

REFERENCES **23**

DETAILED METHODS

Enzymatic activity

Tissue preparation and measurement of GLS1 activity were performed according to established procedures (Conjard *et al*, 2002; Curthoys and Lowry, 1973). Tissue from frontal cortex (FC), hippocampus (HIPP) and thalamus (THAL), as well as tissue from whole brains (from a separate group of animals) were rapidly removed, fast frozen, and kept at -80 °C pending analysis. Glutaminase activity was measured by the production of glutamate, which was converted to a fluorescence readout using glutamate dehydrogenase, in a two-step assay. Homogenates were prepared at 0° C in 20 mM phosphate buffer (pH 7.4), containing 0.5 mM EDTA, 5 mM 2-mercaptoethanol, 25% (v/v) glycerol and 0.02% (w/v) BSA, and the assay conducted based on the following reactions:

1. Glutamine + $H_2O \rightarrow$ glutamate + NH_4^+ (catalyzed by glutaminase)
2. Glutamate + $NAD^+ \rightarrow$ 2-oxoglutarate + NH_4^+ + NADH + H^+ (fluorometric readout)

In Reaction 1, hydrolysis of glutamine was performed at 38 °C for 1 h. A 50 µl volume of reagent [75 mM Tris/HCl (pH 8.6), 30 mM glutamine, 150 mM phosphate (pH 8.0), 0.2 mM EDTA, 0.02% (w/v) BSA] was mixed with 10 µl (15-20 µg tissue) of homogenate. After 1 h, the assay was stopped with 10 µl of 0.8 M HCl. A 30 µl aliquot was transferred into a 96-well microtiter plate, and the accumulated glutamate was measured using Reaction 2. For this, 350 µl of a second reagent [90 mM Tris/HCl (pH 8.4), 1 mM NAD+, 300 µM ADP, 0.03% (w/v) H_2O_2 and 6 units/ml beef liver glutamate dehydrogenase] was added, and after 40 min, fluorescence was measured. Activity was expressed as glutamate produced per mg protein per hour. Calibration of Step 2 was done using glutamate standards (0-20 nmoles).

^{1}H Magnetic resonance spectroscopy

Sample collection for high resolution, magic angle spinning ^{1}H magnetic resonance spectroscopy (HR-MAS ^{1}H MRS), data acquisition and spectrum analysis followed established protocols (O'Leary-Moore *et al*, 2007; Perrine *et al*, 2008). HR-MAS ^{1}H MRS generates chemical spectra with enhanced resolution, allowing for more precise quantification of neurochemicals, particularly GABA, glutamate and glutamine (see Fig S1A, Supplementary Information). Briefly, mice were sacrificed by cerebral dislocation and the brain quickly extracted, placed in a chilled matrix and sliced into 2-mm thick coronal slices. Circular punches (1 or 2 mm in diameter) were made from FC, anterior (HIPP and THAL slices, following established anatomical landmarks (Paxinos and Franklin, 2001). Tissue punches consistently weighed ~2 mg. They were frozen immediately on dry ice and stored at −80 °C until HR-MAS ^{1}H MRS analysis.

Tissue punches were analyzed in groups by an operator blind to genotype. Tissue was rapidly weighed and the intact tissue sample placed into a Bruker zirconium rotor (2.9-mm dia., 10 μL capacity) containing 5 μL of phosphate buffered (pH 7.4) D_2O and 3-[(trimethylsilyl)]-1-propane sulfonic acid (TSP) to allow for locking on the center frequency and as a chemical shift reference (0.0 ppm), respectively. The rotor was inserted immediately into a MAS probe contained in a vertical wide-bore 11.7-Tesla 500 MHz Bruker magnet controlled with an Avance 500 spectrometer. The MAS probe held the sample at 4 °C and spun the rotor at 4.2 kHz while maintaining the rotor at the magic angle relative to the static magnetic field (B_0). A Carr–Purcell–Meiboom–Gill (CPMG) rotor-synchronized pulse sequence, TR = 3500 ms, bandwidth 8 kHz, 16k complex points, 32 averages (Cheng *et al*, 1997), was used to acquire the spectra with a total acquisition time of 223 sec.

The resulting spectra were analyzed with LCModel (Provencher, 1993), a program that utilizes a linear combination of a custom set of 27 neurochemical model spectra, the *basis set*, to fit known MR-visible neurochemicals and calculate absolute concentration values. Cramer-Rao bounds estimated the precision of the LCModel fit for each neurochemical, and were typically < 10% indicating an excellent fit of the data; measurements with a Cramer Rao bound >25% were excluded. Absolute concentrations of MR visible metabolites were corrected for wet tissue weight and expressed as nmol/mg tissue.

Immunocytochemistry

Mice were anesthetized with ketamine (100 mg/kg, Fort Dodge Laboratories, Fort Dodge, IA) and perfused with saline containing 0.5% ketamine (to minimize agonal glutamate release), and then with a fixative solution containing 4% formaldehyde, 0.3% glutaraldehyde and 0.5% ketamine in phosphate-buffered saline (PBS). Brains were removed and postfixed overnight at 4 °C in the same fixative solution, cryoprotected in a graded series of sucrose solutions, and frozen sections cut at 20 μm. Free-floating sections were washed in PBS, aldehydes quenched with glycine (100 mM), and non-specific binding blocked with 5% normal goat serum and 0.05% Triton X-100, for 30 minutes. Sections were incubated with Glu2 primary antiserum (1:2000; Immunostar, Hudson, WI), a monoclonal antiserum directed against the carbodiimide conjugate of glutamate (Madl *et al*, 1986) overnight, at 4° C, visualized with a rhodamine secondary (1:500; Chemicon/Millipore, Temecula, CA), and counterstained with the nuclear marker DAPI (Invitrogen) for 1-3 min. DAPI-stained nuclei were mainly neuronal, as astrocytic nuclei are larger and thus less prominent, and microglial nuclei much smaller. Fluorescent images were acquired (iVision-Mac, BioVision Technologies, Exton, PA) on a Zeiss Axiovert microscope with a 40x /1.3 n.a. oil immersion lens, a high-efficiency rhodamine filter (Omega

Optical, Brattleboro, VT) and a chilled CCD camera (Photometrics Sensys, Roper Scientific, Tucson, AZ). A series of five adjacent fields were imaged, capturing the first fluorescence exposure from each field to avoid bleaching artifacts. Background was subtracted using an adjacent no-primary section. Mosaics of the images were assembled and histograms of pixel intensity generated. Images were scored blind to genotype. Mean intensities were calculated from the histograms.

Behavioral Testing

Locomotor activity in the open field

Open field locomotor activity testing was conducted according to established methodology, in 43.2 x 43.2 x 30.5 cm Plexiglas activity chambers (Med Associates, St. Albans, VT), equipped with arrays of infrared photocell beams parallel with the chamber walls and floor, using Activity Monitor software (Med Associates). Baseline locomotor activity was monitored for 30 min. In a separate experiment, locomotor activity in response to amphetamine was measured in a new cohort of mice. On Days 1 and 2, mice were habituated to the open field. On Day 3, mice were put in the activity chamber for 30 min and then administered amphetamine 2.0 mg/kg i.p. (Sigma-Aldrich, St. Louis, MO) or saline, at a volume of 10 mL/kg inside the activity boxes, and activity was monitored for 90 min post-injection (amphetamine was dissolved in 0.9% saline). The total distance traveled during successive 10-min bins was recorded and analyzed.

Rotarod

Motor learning was determined using an accelerating rotarod apparatus (San Diego Instruments, San Diego, CA), as previously described (Sharma *et al*, 2005). Mice were tested using two trials per day (4 h rest between trials) on 3 consecutive days. Each trial consisted of three tests per animal, with at least a 15 min rest between tests. During each test, the rod accelerated from 4 to

40 rpm for 6 min and then continued at 40 rpm for the remaining 9 min. Trials lasted till the mouse fell off the rod or for the maximum of 15 min. Latency to fall was recorded and analyzed.

Light/Dark emergence test

The Light/Dark emergence test was conducted in the same open field chambers used for monitoring locomotor activity, as previously described (Belzung *et al*, 1987). Briefly, the open field box was divided into two equal compartments by a dark plastic insert, opaque to visible light but transparent to infrared light, which took up half of the chamber area. An opening located in the center of the dark wall at floor level allowed the mouse to move between the light and dark compartments. The light compartment was brightly illuminated with an 8-W fluorescent bulb (400 lux). Between each trial, the apparatus was cleaned with 70% ethanol. At the beginning of the test, mice were placed in the dark compartment and allowed to explore both chambers freely for 5 min. During the test, the time spent in each of the compartments was recorded, as were the number of transitions between compartments and the total ambulatory distance in each compartment.

Prepulse inhibition of startle

Mice were tested in four SR-Lab Systems (San Diego Instruments), as previously described (Paylor and Crawley, 1997). Background noise level in each chamber was 68 dB. Mice were habituated to the test chambers for 5 min, and then presented with acoustic trials that consisted of startle (pulse-alone) trials (120 dB), prepulse+pulse trials (72, 76 or 78 dB followed 100 ms later by a 120 dB pulse) and prepulse-alone trials (72, 76 or 78 dB). All pulses were 20 ms long. Sessions began and ended with 10 presentations of the pulse-alone trials; in between, each prepulse+pulse or prepulse alone trial type was presented 10 (prepulse+pulse) or 4 (prepulse alone) times in a pseudo-random order. A total number of 76 trials were run, with an inter-trial

interval averaging 40 sec (range 20–40 sec). Maximum startle amplitude, averaged across trials for each prepulse intensity, was used as the dependent variable. Percent prepulse inhibition (PPI) of the startle response was calculated as follows:

$$\left[1 - \frac{\text{startle response on prepulse+pulse trials}}{\text{startle response on pulse-alone trials}}\right] \times 100$$

Delayed Non-Match/Match-to-Sample

The test was performed as previously described (Dias and Aggleton, 2000; Kellendonk *et al*, 2006), with slight modifications. Briefly, testing was conducted in a Y maze with equal length arms. All arms of the maze were covered with black tape to minimize spatial cues. Mice were group housed and food deprived to 85% of their *ad libitum* body weight by limiting food intake to 1 h per day. After completing the task on each testing day, mice were given free access to food in individual cages for 60 min. Five days after the food deprivation protocol was initiated, mice were habituated to the maze for three days (3 daily trials); they were placed in the maze for 2 min and allowed to collect food rewards (Cheerios®) distributed in the maze. Testing began on day 4, and consisted of two consecutive tasks: a delayed non-match to sample (DNMTS) task and a delayed match to sample (DMTS) task. In the DNMTS phase, mice were tested in 4 trials per day, each trial consisting of 2 runs, a forced run and a choice run. Before each trial, both arms were baited. In the forced run, the randomly chosen right or left arm was open, while the entrance to the other arm was closed. The mouse was placed in a gated start box at one end of the maze. The trial started with the lifting of the gate; after the mouse collected the food from the open arm it was moved to a transfer cage. After a delay of 4 sec, the closed arm was opened, and the mouse placed in the same start box as before. The choice run was terminated as soon as the mouse reached the end of one arm. A correct choice was scored when the mouse visited the

baited arm in the choice run, i.e. the arm *opposite* to that visited on the forced run. The inter-trial delay was at least 20 min to avoid proactive interference from the last trial. Start arms were alternated in a pseudorandom order on successive training days. The number of correct trials was recorded. Criterion was reached when over three days 11 out of 12 choices were correct. The number of days required to reach criterion was assessed for each mouse. A non-reinforced probe trial was run on the day after a mouse had reached criterion. Three animals did not reach criterion. All animals that reached criterion responded correctly on the probe trial.

A subset of the mice that completed the DNMTS task and performed successfully on the probe trial continued on to a MTS task. The MTS task differed from the DNMTS task in that mice in the choice run were reinforced for visiting the *same* arm as in the forced run. Acquisition in the MTS portion of the experiment was subdivided into two main phases (Dias *et al*, 2000). The first *perseveration* phase corresponded to when mice were performing below chance in any given session (< 2 out of 4 correct trials), i.e. when the mice initially tried to solve the matching task by nonmatching to sample. The second *learning* phase commenced when performance was significantly at or above chance (≥ 2 out of 4 trials), with the criterion defined as three consecutive sessions performed at or above chance level. The number of days required to reach criterion, i.e. to switch from the perseverative to the learning phase, was assessed for each mouse.

Fixed Interval conditioning and interval timing

Fixed Interval (FI) conditioning and interval timing was measured as previously described (Drew et al., 2005), in eight identical experimental chambers (Med Associates) equipped with liquid dippers. Each chamber was located in a light- and sound-attenuating cabinet equipped with an exhaust fan, which generated 72 dB background noise. Inside a dipper alcove, an infrared

photocell detector (4 mm into the opening) was used to record head entries. A reward of one drop of evaporated milk could be provided by raising the dipper. A retractable lever was mounted on the same wall as the feeder. A house light (Med Associates) located at the top of the chamber was illuminated throughout the sessions. An audio speaker was positioned 8.5 cm from the floor on the wall opposite the feeder trough. The speaker delivered a brief tone (90 db, 2500 Hz, 250 ms) to signal that the liquid dipper was raised. To motivate mice to earn rewards in the operant tasks, they were limited to 1 hr daily access to food in the home cage. Water was continuously available in the home cage.

Operant lever press training

Mice were first trained to consume the liquid reward from the dipper. They were placed inside the chambers with the dipper in the raised position, providing access to a drop of evaporated milk. The dipper was retracted 10 sec after the first head entry into the feeder trough. A variable inter-trial interval (ITI) ensued, followed by a new trial identical to the first. The session ended after 30 min or 20 dipper presentations. On the following day, mice received another session similar to the first, except that the dipper was raised for 8s and then lowered whether or not mice had made a head entry. Sessions continued till mice reached the criterion of at least 20 of 30 head entries during dipper presentations in one session. Sessions were scheduled once per day, 5 days per week.

Mice were then trained to press a lever to earn reward. At the beginning of the first session the lever was extended into the chamber, and every lever press was rewarded. In this and all subsequent sessions, the reward consisted of raising the dipper for 5 sec, allowing access to the drop of milk. To familiarize mice with the retraction and extension of the lever, after the twentieth reinforcement, the lever was retracted. After a variable delay, the lever was extended,

and the cycle repeated. If a mouse did not earn a minimum of 100 reinforcements in the session, it repeated the procedure the next day. These sessions lasted for 8 hours. In the next training session, the lever was extended and retracted after every two reinforcements and then re-extended after a variable ITI. The session ended when the mouse earned 40 reinforcements, or one hour elapsed. Mice continued training till they reached the criterion of earning 40 rewards in one session. Mice then moved to FI training.

Fixed Interval training

In Fixed Interval (FI) training, lever presses were reinforced after a minimum interval had elapsed from the bar extension until reward became available. Mice began on FI-4s schedule, meaning that the first lever press occurring more than 4s after lever extension was reinforced. The bar was retracted with each dipper presentation and followed by a variable ITI (mean = 30s, range = 110s). Each subsequent trial began with the reinsertion of the lever. When a mouse earned at least 40 rewards in one session, the FI duration was extended in the next session. The FI durations were 4, 8, 12 and 16 sec (16s). When a mouse reached the criterion of 40 rewards in one session on the FI-16s schedule it was moved to peak interval (PI) training.

Peak Interval training

Peak Interval (PI) training was used to test the accuracy of temporal information processing on the 16-s FI. Sessions consisted of two trial types: *Fixed Interval* FI-16s trials (as described above) and *Peak* trials. Peak trials were probe trials in which the lever was extended for 64 sec but lever presses were not reinforced. On these trials, the likelihood of responding increases as the expected time of reward (*i.e.* 16 sec) approaches, and then declines. FI and Peak trials were ordered randomly, and the ITI was varied (as above). The first session of PI training consisted of 12 Peak and 48 FI-24s trials. If the mice earned at least 40 rewards, every session thereafter

consisted of a maximum of 24 Peak and 36 FI-24s trials. The session duration was capped at 70 min; if mice did not complete all 60 Peak and FI trials in this period, the session ended. Mice were trained for a minimum of 11 sessions. Response rates (responses/sec) and latency to respond during FI training and PI trials were recorded and analyzed.

Fear conditioning to tone and context

Fear conditioning was performed according to standard procedure, as previously described (Saxe *et al*, 2007). Conditioning was conducted in 20×16×20.5 cm sound- and light-attenuated chambers (Med Associates). A house light mounted directly above the chamber provided ambient illumination. Behavior was recorded with a digital video camera mounted above the conditioning chamber, and analyzed with FreezeFrame software (Actimetrics, Evanston, IL), which assesses freezing by measuring changes in pixel intensity between successive video frames.

The fear conditioning procedure was conducted over 3 days. On day 1, mice received 3 pairings between a tone conditioned stimulus (CS; 20 sec, 80 dB, 2 KHz) and a co-terminating shock (unconditioned stimulus, US; 1 sec, 0.4 mA). The ITI was 120 sec, with additional 120 sec intervals before the first and after the last pairings. Chambers were cleaned with 70% isopropanol between each set of mice and scented with a paper towel dabbed in limonene solution placed beneath the chamber floor. Freezing was scored during the 20s of each tone presentation. On day 2, the procedure and context were changed in several ways to test conditioned fear to the tone CS in the absence of contextual cues associated with shock; the floor and walls of the chamber were covered with white and dark green plastic inserts; the chamber was scented with mint; between runs, chambers were cleaned with a non-alcohol disinfectant. Each mouse was placed in the chamber for 4 min. The tone was presented twice for 20 sec at 120

and 200 sec after the start of the session. No shocks were administered. Freezing was scored for the 20 sec before the first tone presentation (pre-tone freezing) and during the 20 sec of the first tone presentation (tone-period freezing). On day 3, mice were tested for conditioned fear to the training context. The testing procedure and context were identical to those used on day 1, except the CS was not presented. Mice were placed in the chamber for 3 min, and the entire session was scored for freezing behavior. Data not recorded due to apparatus failure (failure to record the session) were dropped from the statistical analysis.

Morris Water Maze

Testing was conducted according to standard procedure, as previously described (Saxe *et al*, 2007), in a 170 cm in diameter pool. During training a white circular platform (14.6 cm in diameter) was submerged 0.5 cm below the surface of the water in the center of one quadrant of the pool. Water in the pool was kept at a temperature of 21 °C and was colored with white nontoxic tempera paint. Movement was recorded using a video tracking system (HVS Image, Hampton, UK), and custom software was used to calculate total distance traveled and percent time in each quadrant. The procedure consisted of four stages: (i) habituation (ii) visible platform training (iii) hidden platform training, and (iv) memory testing. To habituate the animals to the procedure, mice were individually placed in a bucket in which the circular platform was submerged less than 0.5 cm below water level. Mice remained in the water until they reached the platform and remained on it for a 20 sec. Habituation consisted of one trial per day for 2 days (Days 1-2). Visible platform training (Day 3) consisted of two trials, with an ITI of 20 min. Mice were placed in the water at one of four locations around the perimeter of the pool. They were allowed 2 min to find the submerged platform marked by a visible cue above the water. The location of the platform was fixed. Hidden platform training (Days 4-11) was done exactly as

above except there was no visible marker to guide the mice requiring them to use extra-maze cues located in the room. Time (sec) to reach the hidden platform was recorded and analyzed on each trial. Two probe trials were conducted 1 h and 7 days after the last hidden platform training session. During probe trials, the platform was removed from the pool, and the mouse was placed in the center of the pool and taken out of the water 60 sec later. Percent time in each quadrant was recorded and analyzed.

Novel Object Recognition Test

Testing was conducted in 43.2×43.2×30.5 cm Plexiglas activity chambers (Med Associates) equipped with video cameras positioned 15 cm above the chambers, following established methodology (Ennaceur and Delacour, 1988). To facilitate scoring, 9×9 cm clear plastic grids were placed on the chamber floor. The objects were children's wooden building blocks of comparable height (approximately 3×3×3 cm) but of different shapes (e.g., square, circle or triangle) and brightness levels (e.g., bright yellow, dark blue), placed 12 cm away from two diagonal corners and fixed to the chamber floor with Velcro to prevent them from being moved. The experiment was performed in a dimly lit room, to reduce anxiety levels. Four mice were run in parallel in four separate experimental chambers. For two days prior to behavioral testing, mice were given daily 15 min exploration periods in the NOR box to ensure habituation to the empty apparatus and test room environment. Body weights were recorded and activity levels monitored during the habituation period. On Day 3, the Object Recognition test was conducted in two phases, a sample and a retention test phase. During the sample phase, two objects (A1 and A2) were placed in the experimental chamber. At the beginning of the sample phase, each mouse was placed in the center of the box and allowed to explore the two objects freely for a period of 10 min. Mice were then returned to their home cage for a 2 hr ITI. During this time, the box and

objects were cleaned with 10% ethanol, and one of the objects was replaced with a novel object. The location of the novel object in the retention trial (left or right) was counterbalanced between groups, and objects used in the sample phase for some animals were used as novel objects in the test phase for others. Following the 2 hr ITI, mice were returned to the box for the retention test phase, during which they were allowed to explore the familiar and novel object in the test box for 10 min. Experiments were video recorded from which object exploration times, for both sample and retention phases, were extracted manually using Stopwatch 0.8.12.2 software (CDSecretary, Shanghai, China) by an experimenter blinded to the experimental conditions. Object exploration was defined as the mice sniffing, licking or touching the objects with forepaws while sniffing but not by leaning against, turning around, standing or sitting on the objects (Grayson *et al*, 2007).

Latent inhibition

Testing was conducted in the sound-attenuated fear conditioning chambers (described above), following established methodology (Yee *et al*, 2006). The procedure consisted of 4 phases: pre-exposure, conditioning, context test, and tone test. Within each genotype, mice were randomly assigned to either the non-pre-exposed (NPE) or pre-exposed (PE) condition. In pre-exposure, PE mice received 40 presentations of a 30 sec tone CS (80 dB, 2 KHz) at a variable interstimulus interval of 40 ± 30 sec; NPE mice were placed in the chamber for the same time. Conditioning commenced immediately after pre-exposure, without removing the animals from the chambers. Conditioning comprised three discrete trials of CS-US pairings. Each trial began with the 30 sec tone CS (identical to the one used during pre-exposure) followed immediately by the delivery of a 1 sec, 0.7 mA foot shock US. Trials were run at 180 sec intervals, with a final 180 sec after the last trial. Clozapine (Sigma-Aldrich, dissolved in 1.5% DMSO in saline, pH adjusted to 7.4 with

0.05% acetic acid) at a dose of 1.5 mg/kg and a volume of 5 mL/kg, or saline, was injected i.p. 15 min before the start of the pre-exposure phase.

The context test took place 24 h later; mice were returned to the same chambers and observed for a period of 480 sec in the absence of any discrete stimulus. During pre-exposure and conditioning, and in the context test, each chamber was scented with a paper towel dabbed with limonene solution placed beneath the chamber floor. The tone test was conducted another 24 h later, when the animals were returned to the chambers. After a 180 sec acclimation period, the tone CS was turned on for 480 sec.

The tone test was conducted in a different context, with white and dark green plastic inserts to cover the floor and the wall of the chambers and mint-scented paper towels. Freezing was measured on all three days of the experiment. On the tone test day, percent pre-tone freezing was defined as the average freezing during the 180 sec preceding tone onset. The freezing ratio for each animal represents freezing levels before and after tone presentation, and was calculated using the formula B/(A+B), where A is the percent freezing during the 30 sec immediately preceding tone onset and B the percent freezing during the tone.

In vivo imaging

A modified mouse imaging protocol (Moreno *et al*, 2006; Pereira *et al*, 2007) was used to generate baseline and post-ketamine relative cerebral blood volume (rCBV) maps. Briefly, seven sets of axial T2-weighted images were acquired sequentially. Each set consisted of 10 images, acquired over 16 min. The contrast agent Gadodiamide was injected (13 mmol/kg i.p.) after a pre-contrast set. After 3 post-contrast sets, ketamine (30 mg/kg, diluted in saline) was injected at a volume of 10 mg/mL through the catheter. Three further post-ketamine sets were acquired. rCBV was mapped as changes in the transverse relaxation rate (R^2) induced by the contrast agent.

rCBV maps were measured from steady-state T2-weighted images as CBV $R^2 = \ln(S_{pre}/S_{post})/TE$, where TE is the effective echo time, S_{pre} is the signal before the contrast administration, and S_{post} is the signal after the contrast agent reaches steady-state. The derived maps were normalized to the maximum 4-pixel signal value of the posterior cerebral vein. Images in set 4 were used to construct baseline/pre-ketamine rCBV maps. Images in sets 5, 6 and 7 were used to construct post-ketamine rCBV maps.

Visualized anatomical landmarks were used together with standard atlases to define regions of interest: the FC consisted of the infralimbic (IL) and prelimbic (PrL) cortex as well as the medial, ventral and lateral orbitofrontal cortex (OFC) and was bordered by the olfactory bulb (anterior), the lateral septum (LS; medial posterior), the caudate-putamen (CPu) and sensory area 1 (S1; lateral posterior). The THAL consisted mainly of anterior and ventral thalamic nuclei, and was bordered by the lateral and third ventricles (anterior) and the internal capsule (lateral). The HIPP consisted of five subregions: the dentate gyrus (DG), the CA3 subfield, the CA1 subfield, the subiculum (SUB), and the entorhinal cortex (EC), identified according to anatomical landmarks as previously described (Moreno *et al*, 2006; Pereira *et al*, 2007). Normalized CBV measurements from each subregion were used for group data analysis.

Slice physiology

Whole cell recordings were obtained from transverse hippocampal or coronal anterior cingulate cortex slices, 400 µm thick. The recording chamber was continuously perfused (1 ml/min) with standard ACSF (in mM): 125 NaCl, 2.5 KCl, 25 $NaHCO_3$, 1.25 NaH_2PO_4, 2 $CaCl_2$, 1 $MgCl_2$ and 25 glucose, pH 7.4, saturated with 95% O_2-5% CO_2, including the $GABA_A$ antagonist gabazine (SR95531; 10 µM), at room temperature (22-24 ºC). Pyramidal neurons were identified by their location, size and shape under Nomarksi optics. Patch pipettes were fabricated from standard

wall borosilicate capillaries and had resistances of 3-5 MΩ. The pipette solution contained (in mM): 140 Cs^+-gluconate, 10 HEPES, 0.1 $CaCl_2$, 2 $MgCl_2$, 1 EGTA, 2 ATP-Na_2 and 0.1 GTP-Na_2, pH 7.3, and included QX-314 (5 mM) to block unclamped and antidromic Na^+ currents. Spontaneous EPSCs (sEPSCs) were recorded at -75 mV from CA1 pyramidal neurons (HIPP) or large pyramidal neurons in layer V/VI (anterior cingulate) under voltage clamp with an Axopatch 200B amplifier (Molecular Devices, Sunnyvale, CA). Once recordings had stabilized (< 5 min), sEPSCs were collected for 1 min. Data were filtered at 5 kHz and sampled at 200 μsec. The amplitude and frequency of sEPSCs was measured using Axograph 3.6 (Molecular Devices) with a template function, and met the criteria of amplitude > 5 pA and rise time < 2 msec. A second cell was recorded in healthy slices, but no more than two cells per slice.

Measurements of synaptic strength were done at 29 °C as previously described (Yano *et al*, 2006). Hippocampal slices were placed in an interface chamber, and allowed to recover for 90 min before recording. CA1 field EPSPs (fEPSPs) were evoked and recorded with both stimulating and recording electrodes in the CA1 stratum radiatum. Stimulus voltages (*V*) was plotted against the slope of the fEPSP to determine the input-output relationship. All electrophysiology experiments were done blind to genotype. Slices with unstable responses during baseline stimulation were discarded.

Microdialysis

WT-GLS1 het pairs were matched for date of birth, holding conditions, and microdialysis and HPLC conditions. Specifically, littermates (7/12 pairs) or cage-mates of each genotype, were handled and tested in parallel, undergoing surgery and all testing simultaneously. Mice were anesthetized with isoflurane (1-2% in oxygen) and stereotaxically implanted with microdialysis guide cannulae (MBR 2-5, Bioanalytical Systems, West Lafayette, IN) in the striatum/nucleus

accumbens with target coordinates (relative to Bregma) +1 mm anterior, + 1.3 mm lateral and 2.7 mm below the skull surface. Following surgery, mice were habituated to the manipulation of the stylet in the cannula for 2-3 days. For the microdialysis session, mice were placed in testing chambers (30.5 cm high x 30.5 cm diameter buckets, in sound-attenuating chambers and low ambient lighting) 30-45 min prior to insertion of the microdialysis probe. The microdialysis probes (MBR 2-5; membrane diameter=0.22 mm, length=2.0 mm) were slowly inserted to extend 2 mm ventral to the tip of the guide cannula, and then perfused at a flow rate of 2.0 µl/min with an artificial cerebrospinal fluid (aCSF) containing (in mM): 126.5 NaCl, 27.5 $NaHCO_3$, 2.4 KCl, 0.5 Na_2SO_4, 0.5 KH_2PO_4, 1.1 $CaCl_2$, 0.8 $MgCl_2$, 4.9 D-glucose. Dialysate collection began 2.5 h after probe insertion, into tubes containing 10 μl of an antioxidant cocktail of glutathione and acetic acid. Four consecutive 15-min baseline samples were collected. Amphetamine 2.0 mg/kg was then administered and four more 15-min samples were collected.

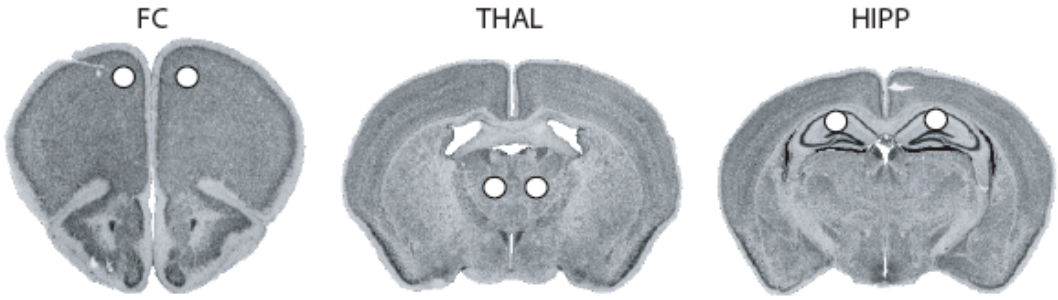
Quantification of dopamine (DA) in the dialysis samples was performed by HPLC with electrochemical detection. Briefly, DA and its metabolites were separated with a reverse phase C18 column (ChromSep SS 100 x 3.0MM, Inertsil 3 ODS-3; Varian, Palo Alto, CA) and a mobile phase composed of (in mM): 75 NaH_2PO_4, 25 citric acid, 25 µM EDTA, 100 µl/L tetraethylamine, 2.2 mM 1-Octanesulfonic acid sodium salt; and, 10% Acetonitrile, 2% Methanol. DA was oxidized with a coulometric electrode (Model 5014, ESA, Chelmsford, MA) set to a potential of 350 mV. The concentration of DA and its metabolites were quantified using an external standard curve from standards prepared in the same aCSF/preservative mixture as the brain dialysates. At the end of the experiments, animals were euthanized and the dialysis probe was carefully removed from the guide cannula. The head mount with guide cannula was removed with the surrounding cranium, following by removal of the brain. The brain was post-

fixed to 10% formalin, cryoprotected, and sectioned at 40 μm. Sections surrounding the dialysis probe tract were Nissl-stained and probe placement in the medial or central striatum was verified.

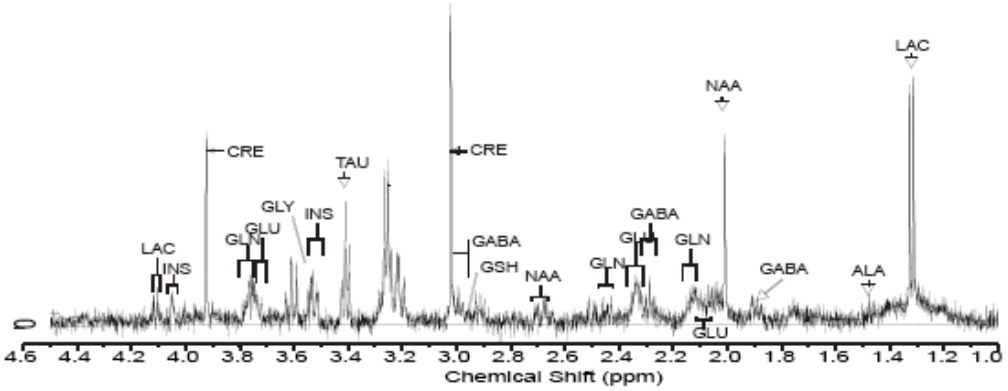
SUPPLEMENTAL FIGURES

Figure S1. Magnetic resonance spectroscopy

A



B

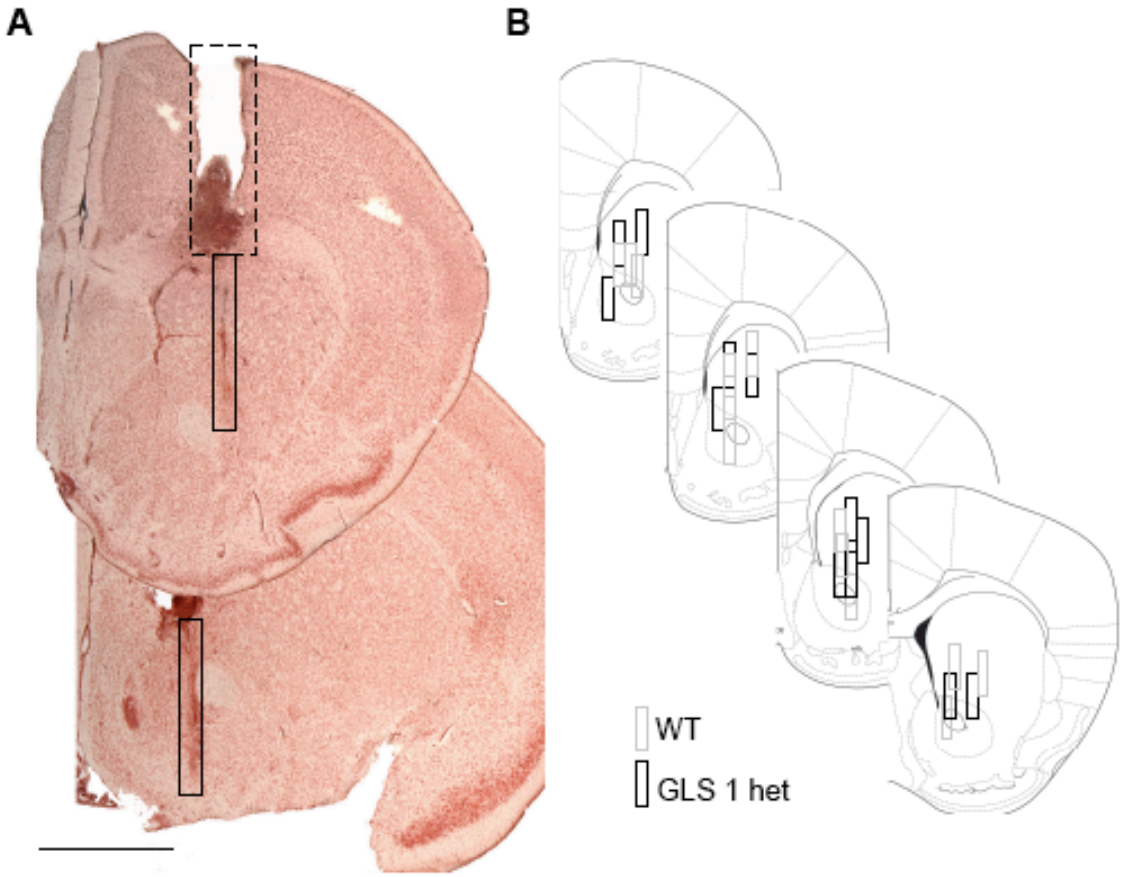


C

	FC		HIPPO		THAL	
	WT (n=15)	GLS1 HETS (n=10)	WT (n=8)	GLS1 HETS (n=7)	WT (n=8)	GLS1 HETS (n=7)
Alanine	0.75 (.03)	0.66 (.06)	0.51 (.04)	0.58 (.05)	0.58 (.03)	0.57 (.1)
GABA	4.55 (.16)	4.98 (.34)	2.77 (.16)	***2.14 (.17)***	5.7 (.19)	5.78 (.34)
Glutamine	2.57 (.13)	**3.44 (.29)**	2.73 (.21)	3.18 (.14)	2.99 (.24)	3.64 (.27)
Glycine	1.34 (.03)	1.33 (.06)	0.97 (.11)	0.84 (.06)	1.16 (.04)	1.25 (.05)
Glutamate	11.35 (.34)	***9.54 (.5)***	10.49 (.2)	***9.13 (.38)***	8.55 (.61)	8.05 (.54)
Glutathione(GSH)	1.13 (.04)	1.39 (.57)	1.09 (.05)	1.16 (.1)	1.08 (.12)	1.19 (.09)
Inositol	6.68 (.28)	6.3 (.34)	5.98 (.35)	***4.98 (.31)***	6.87 (.35)	7.35 (.34)
Lactate	11.14 (.25)	11.32 (.55)	10.38 (.64)	***9.36 (.64)***	8.66 (.49)	9.73 (.37)
N-acetylaspartate(NAA)	8.05 (.18)	7.92 (.35)	5.63 (.22)	5 (.37)	6.51 (.25)	6.97 (.37)
GABA/Creatinine	0.64 (.01)	0.71 (.04)	0.53 (.03)	***0.42 (.02)***	0.8 (.04)	0.76 (.02)
Glutamine/Creatinine	0.37 (.02)	**0.49 (.03)**	0.43 (.03)	**0.52 (.02)**	0.42 (.01)	0.47 (.04)
Glutamate/Creatinine	1.61 (.03)	***1.36 (.06)***	1.28 (.03)	1.17 (.05)	1.2 (.04)	***1.04 (.03)***
Glutamate/Glutamine	4.35 (.01)	***2.7 (.04)***	3.03 (.02)	***2.22 (.02)***	2.78 (.01)	***2.17 (.04)***

A. Location of tissue samples (obtained with 1 mm punches, Fine Science Tools) from the frontal cortex (FC), thalamus (THAL) and hippocampus (HIPP) were obtained following established coordinates (Paxinos *et al*, 2001). **B.** Sample spectra of the HIPP of a WT mouse obtained using HR-MAS 1H MRS at 11.7 T. HR-MAS visible neurochemicals include alanine (ALA), aspartate (ASP), betaine (BET), choline (CHO), creatine (Cr), GABA, glutamate (GLU), glutamine (GLN), glutathione (GSH), glycerophosphorylcholine (GPC), glycine (GLY), lactate (LAC), *myo*-inositol (mI), N-acetylaspartate (NAA), *N*-acetyl-aspartyl-glutamate (NAAG), phosphorylcholine (PCH), phosphorylethanolamine (PEA), and taurine (TAU). Signals for most chemicals have more than one resonance peak, a characteristic accounted for in the LCModel analysis.. (B) Mean (SEM) levels of selected HR-MAS visible neurochemicals. Bold, right-shifted: Significant ($p<0.05$) increase compared to WT. Bold, left-shifted: Significant ($p<0.05$) decrease compared to WT. Images of brain sections are from http://www.mbl.org.

Figure S2. Probe placement in microdialysis study



Photomicrographic and schematic representation of placements of microdialysis guide cannulae and probes. **A.** Typical appearance in non-perfused, post-fixed brain of the placements of the microdialysis probe membrane (within dashed-border box) and the guide cannula (within solid-border box). The probe was removed prior to sacrificing the animal so the minimal tissue damage is likely representative of the area damaged *in vivo*. However the guide cannula was removed post-mortem from unfixed tissue (when the skull cap was taken off); thus, much of the apparent damage surrounding the guide cannula occurred post-mortem. Scale bar represents 1.0 mm, corrected for shrinkage. **B.** Distribution of probe tips for WT (gray rectangles) and GLS 1 hets (black rectangles). Schematics adapted from Paxinos and Franklin (2001).

REFERENCES

Belzung C, Misslin R, Vogel E, Dodd RH, Chapouthier G (1987). Anxiogenic effects of methyl-beta-carboline-3-carboxylate in a light/dark choice situation. *Pharmacol Biochem Behav* **28**(1): 29-33.

Cheng LL, Ma MJ, Becerra L, Ptak T, Tracey I, Lackner A*, et al* (1997). Quantitative neuropathology by high resolution magic angle spinning proton magnetic resonance spectroscopy. *Proc Natl Acad Sci U S A* **94**(12): 6408-6413.

Conjard A, Brun V, Martin M, Baverel G, Ferrier B (2002). Effect of starvation on glutamine ammoniagenesis and gluconeogenesis in isolated mouse kidney tubules. *Biochem J* **368**(Pt 1): 301-308.

Curthoys NP, Lowry OH (1973). The distribution of glutaminase isoenzymes in the various structures of the nephron in normal, acidotic, and alkalotic rat kidney. *J Biol Chem* **248**(1): 162-168.

Dias R, Aggleton JP (2000). Effects of selective excitotoxic prefrontal lesions on acquisition of nonmatching- and matching-to-place in the T-maze in the rat: differential involvement of the prelimbic-infralimbic and anterior cingulate cortices in providing behavioural flexibility. *Eur J Neurosci* **12**(12): 4457-4466.

Ennaceur A, Delacour J (1988). A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. *Behav Brain Res* **31**(1): 47-59.

Grayson B, Idris NF, Neill JC (2007). Atypical antipsychotics attenuate a sub-chronic PCP-induced cognitive deficit in the novel object recognition task in the rat. *Behav Brain Res* **184**(1): 31-38.

Kellendonk C, Simpson EH, Polan HJ, Malleret G, Vronskaya S, Winiger V*, et al* (2006). Transient and selective overexpression of dopamine D2 receptors in the striatum causes persistent abnormalities in prefrontal cortex functioning. *Neuron* **49**(4): 603-615.

Madl JE, Larson AA, Beitz AJ (1986). Monoclonal antibody specific for carbodiimide-fixed glutamate: immunocytochemical localization in the rat CNS. *J Histochem Cytochem* **34**(3): 317-326.

O'Leary-Moore SK, Galloway MP, McMechan AP, Hannigan JH, Bowen SE (2007). Region-dependent alterations in glutamate and GABA measured by high-resolution magnetic resonance spectroscopy following acute binge inhalation of toluene in juvenile rats. *Neurotoxicol Teratol* **29**(4): 466-475.

Paxinos G, Franklin KBJ (2001). *The Mouse Brain in Stereotaxic Coordinates*, 2nd edn. Academic Press: San Diego.

Paylor R, Crawley JN (1997). Inbred strain differences in prepulse inhibition of the mouse startle response. *Psychopharmacology (Berl)* **132**(2): 169-180.

Perrine SA, Michaels MS, Ghoddoussi F, Hyde EM, Tancer ME, Galloway MP (2008). Cardiac effects of MDMA on the metabolic profile determined with (1)H-magnetic resonance spectroscopy in the rat. *NMR Biomed* **22**(4): 419-425.

Provencher SW (1993). Estimation of metabolite concentrations from localized in vivo proton NMR spectra. *Magn Reson Med* **30**(6): 672-679.

Saxe MD, Malleret G, Vronskaya S, Mendez I, Garcia AD, Sofroniew MV*, et al* (2007). Paradoxical influence of hippocampal neurogenesis on working memory. *Proc Natl Acad Sci U S A* **104**(11): 4642-4646.

Sharma N, Baxter MG, Petravicz J, Bragg DC, Schienda A, Standaert DG*, et al* (2005). Impaired motor learning in mice expressing torsinA with the DYT1 dystonia mutation. *J Neurosci* **25**(22): 5351-5355.

Yano H, Ninan I, Zhang H, Milner TA, Arancio O, Chao MV (2006). BDNF-mediated neurotransmission relies upon a myosin VI motor complex. *Nat Neurosci* **9**(8): 1009-1018.

Yee BK, Balic E, Singer P, Schwerdel C, Grampp T, Gabernet L*, et al* (2006). Disruption of glycine transporter 1 restricted to forebrain neurons is associated with a procognitive and antipsychotic phenotypic profile. *J Neurosci* **26**(12): 3169-3181.