SUPPLEMENTAL DATA

Selective impairment in attentional set-shifting in *COMT Val-tg* mice

Due to the new paradigm here employed, we further analyzed with a separate 1-way ANOVA the performance of the control mice (not expressing the *COMT Val-tg*). The analysis of trials to criterion revealed an effect of the discrimination ($F_{8,56}$=4.76; P<0.0005). In particular, the control mice performed the EDS stage poorly compared to all the stages other than the CDRe (P<0.05; Supplemental Fig. S2). Moreover, the control mice required more trials to learn the CDRe compared to the SD and CD stages (P<0.05). Similarly, the analysis of the time to reach criterion revealed an effect of the discrimination ($F_{8,56}$=5.90; P<0.0001). Control mice required much more time to solve the EDS stage compared to all the other discriminations encountered (P<0.05; Supplemental Fig. S2).

COMT genotype and amphetamine treatment did not impact on sniffing behavior

To determine if abnormal sniffing behavior dependent on *COMT* manipulation or amphetamine treatment may affect recognition memory performance and keep the mice close to the objects for non-cognitive reasons, we quantified sniffing behavior during the retention trial phase carried out 1 hour after the acquisition phase and amphetamine treatment. For a better understanding of the impact of sniffing behavior in the novel object recognition test we subdivided the sniffing as done in the open field arena or directed towards the objects. Analysis of the time spent sniffing in the open field arena showed no genotype effect ($F_{1,35}$= 0.31, P=0.58), no amphetamine treatment effect ($F_{1,35}$= 1.09, P=0.30), and no genotype x amphetamine treatment effect ($F_{1,35}$= 0.02, P=0.90; Supplemental Fig. S4). In the same way, the analysis of sniffing behavior directed towards the objects revealed no genotype effect ($F_{1,35}$= 0.01, P=0.92), no amphetamine treatment effect ($F_{1,35}$= 0.98, P=0.33), and no genotype

x amphetamine treatment effect ($F_{1,35}$= 0.67, P=0.42; Supplemental Fig. S4). Thus, we can exclude that abnormal sniffing behavior is responsible for the differences saw in the time spent exploring the objects during the retention trial of the novel object recognition task.

SUPPLEMENTAL METHODS

Immunohistochemistry. Separate animals including *COMT Val-tg* mice and their littermate controls were set up for immunohistochemical examination of the human COMT. Animals were anesthetized with an overdose of sodium pentobarbital (80 mg/kg). The thoracic cavity was opened, the inferior vena cava was clamped, and a needle Teflon catheter was inserted into the apex of the heart and routed to the entrance of the aorta. Fifty units of heparin were injected into the catheter, and the right atrium was punctured to allow drainage. The animal was then perfused transcardially with 20 ml of 0.15 M NaCl followed by 20 ml of phosphate-buffered 4% paraformaldehyde followed by another 20 ml of 0.15 M NaCl. The brains were post-fixed overnight in phosphate-buffered 4% paraformaldehyde and then stored in 25% sucrose until sectioned. Brains were cut into sequential 25 μm coronal sections using a freezing-stage microtome (Jung Histoslide 2000, Deerfield, IL). The sections were stored at -20°C in tissue culture dishes containing cryoprotectant until they were processed.

The tissue was rinsed with PBS and treated with a solution of 1% sodium borohydride for 20 min. Endogenous peroxidase activity was stopped by incubation with 2% H_2O_2 for 20 min. Nonspecific antigenic sites were blocked by a 20 min step in diluted normal serum. Tissues were incubated with the human COMT primary antibody (1:1,000), overnight at 4°C. Then the sections were first incubated at RT with a biotinylated secondary antibody (Vector, Burlingame, CA) for 30 min and second with the peroxidase-conjugated biotin–avidin complex (Vectastain ABC kit) for 40 min. Finally, the peroxidase was revealed by immersion in DAB (Vector). Then the tissue slices were double stained by 0.75 % cresyl violet acetate (Raymond A Lamb, England), Tissue slices were visualized using a Axipphot microscope connected to a digital camera (Q Imaging, Canada) and a computer running the Bioquant software package (R&M Biometrics, Nashville, TN). The tissue slices were visualized using 10× and 40× objective lenses, and the brain regions of interest were observed. The numbers of

total human COMT Val positive cells in PFC and striatum and the numbers of total cells were counted separately on each section. Using the Bioquant software package, each individual immunoreactive cell was marked during the counting process, eliminating the possibility of double counting identified cells.

Immunoblotting. Specific brain regions were obtained from the *COMT Val-tg* mice and their littermate controls and from *COMT*+/+, +/- and -/- by gross dissection. The brain tissues were then homogenized and diluted to 1.5 μg/ μl of total protein content in lysis buffer. After be denatured same amount protein of each sample was loaded onto precast 10% Bis-TRIS polyacrylamide gels (Invitrogen, Carlsbad, CA), and proteins were separated by electrophoresis. Each gel contained a molecular weight marker ladder (SeeBlue Plus 2; Invitrogen Carlsbad, CA) and control pooled samples used for normalization in triplicate. Gels were transferred onto nitrocellulose membranes for 2 hrs at room temperature at 85V in Neupage transfer buffer with 20% methanol. Membranes were blocked for 1 hour in 10% goat serum in Tris buffered saline (TBS) with 0.1% Tween-20 (TBS-T), and then incubated with the polyclonal rabbit anti-COMT antibody (1:7000 dilution, Chemicon, Temecula, CA), rabbit anti-Glutamic acid decarboxylase 67 (GAD67) antibody (1:3,000 dilution), rabbit anti-TH antibody (1:100,000 dilution), goat polyclonal anti CaMKKβ (1:1000 dilution), rabbit polyclonal anti CaMKI (1:200 dilution), mouse monoclonal anti CaMKII (1:5000, dilution), goat polyclonal anti CaMKIV (1:200 dilution), and rabbit polyclonal anti CaMKKα (1:1000 dilution), antibodies obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Blots were rinsed in TBS-T, incubated in the peroxidase-conjugated goat anti-rabbit, goat anti-mouse antibody (1:10,000 dilution, Chemicon, Temecula, CA) and donkey anti goat antibodies (1:1000, Santa Cruz, CA) separately for 1 hour in 5% normal goat serum in TBS-T and rinsed in TBS-T. Blots were developed in ECL-plus (GE Healthcare, Piscataway, NJ) and exposed

to Kodak Bio-Max film. Films were digitized using a scanner, and the resulting images were analyzed using NIH Image gel plotting macros. The values for all samples were expressed as a percentage of the mean of controls on the same gel.

COMT activity assay. The COMT enzyme activity assay is based on the organic solvent extraction method that separates the radioactive product, the methylated catechol, and the free radioactive co-enzyme, ^{3}H-S-adenosyl-methionine (SAM; (Zurcher and Da Prada, 1982). One hundred µg of mouse brain tissue protein at a concentration of 5µg/µl was transferred to a fresh microcentrifuge tube from each sample and equilibrated to temperature shortly before the enzyme assay. Five hundred microliters of the substrate mixture containing 10 mM Tris, pH 7.4, 1 mM MgCl2, 1.5 µCi of ^{3}H-adenosyl-S-methionine (SAM), 10 µM of catechol and 1 µM of DTT was added to each tube. The tubes were then incubated at 37 °C for 20 min. The reactions were immediately terminated by adding 500 µl of 1M HCl. The radioisotope-labeled catechol products from the reactions were extracted and determined by mixing the reaction mixture with 10 ml scintillation fluid (Flow I, Molecular Diagnosis) and measuring the radioactivity of the mixture in a scintillation counter. COMT enzyme activity is calculated as DPM per mg total protein and reported as the percentage of the mean of controls. To establish a baseline control for non-specific reactions that do not depend on COMT, 5 µl of the specific COMT inhibitor, tolcapone (10 mg/ml), was added to a tube containing 100 µg of the human DLPFC sample. The high concentration of potent inhibitor block the specific reaction catalyzed by COMT, and the radioactivity from this reaction served as a baseline. COMT enzyme activity in the frontal cortex of the COMT Val-tg mice and their controls are measured as radioactivity of the ^{3}H-labeled methylated product in DPM per mg of total protein and presented as the percentage of the mean of controls.

RNA Extraction and Reverse Transcription. Brain tissue was pulverized and stored at –80°C. Total RNA was isolated from 20-30 mg of tissue from the frontal cortex, using RNA tissue kit (Fisher Scientific/5Prime, catalog # FP-2302410) according to the manufacturer's protocol. The yield of total RNA was determined by absorbance at 260 nm using Nanodrop. RNA quality was assessed using an Agilent Bioanalzer 2100 (Agilent Technologies, Palo Alto, CA, USA). Agilent traces did not show anomalous peaks indicating genomic DNA contamination in any sample and all RIN values were >9.0. First-strand cDNA was generated from 1-2 μg total RNA using GeneAmp RNA PCR kit with random hexamers and Oligo$(dT)_{16}$ in conjunction with MuLV reverse transcriptase (Applied Biosystems, Foster City, CA, USA).

Quantitative Real-Time PCR. Expression levels of mRNAs were measured by real-time quantitative (RT-PCR) using ABI Prism 7900 sequence detection system, 384-well format (Applied Biosystems, Foster City, CA, USA). Each 10 μl reaction contained cDNA template obtained from 60 ng of RNA, 900 nM of primer, 250 nM of probe, and 1X Taqman Universal PCR Mastermix (Applied Biosystems) with Hot Goldstar DNA Polymerase, dNTPs with dUTP, uracil-N-glycosylase. We used ABI Assay-on-Demand for α-CaMKII (Mm00437967_m1), β-CaMKII (Mm00432296_m1), γ-CaMKII (Mm00618054_m1), δ-CaMKII (Mm00499266_m1), CaMKKβ (Mm00520236_m1), CaMKIV (Mm00437978_m1), Adcy1 (Mm011878929_m1), CNR1 (Mm00432621_s1), PKA (Mm00660092_m1), FEZ1 (Mm00805945_m1), and LIS1 (Mm00443070_m1). For normalization, we used two housekeeping genes, actin (Mm00607939_s1) and β-glucuronidase (GUSB; Mm00446953_m1, ABI, Assays-on-Demand).

PCR cycle parameters were 50°C for 2 min, 95°C for 10 min, 40 cycles of 95°C for 15s, and 59°C or 60°C for 1 min. PCR data were acquired from the Sequence Detector

Software (SDS version 2.0, Applied Biosystems) and quantified by a standard curve method using serial dilutions of pooled cDNA derived from RNA obtained from all subjects. The R2 value of the curve was 0.996, the slope was -3.9 and controls that lacked template cDNA emitted no detectable signal. All samples were measured in a single plate, and their cycles at threshold (Ct) values were in the linear range of the standard curve. All measurements were performed in triplicate.

Hot plate and Tail flick tests. The analgesia tests examined the subject's pain threshold and sensitivity. The hot plate and tail flick tests assess centrally mediated and spinal pain reflexes, respectively (Crawley, 2007). In the hot plate test, each mouse was placed on a 55ºC hot plate (Columbus Instruments, Columbus, OH, USA) and a timer was manually activated from the moment that the mouse's paws reached the plate. The time to the first hindpaw licking, jumping, or vocalizing was recorded, which served as the latency time for the mouse to react. If the mouse failed to react after 30 sec, the trial was terminated and the mouse was removed. In the tail-flick test, the mouse's tail was placed over a light beam in the apparatus, and the time interval from when the light beam was on to the tail flick was measured. None of the mice tested reached the cut off of 10 sec of no response.

SUPPLEMENTAL REFERENCES

Crawley JN (2007) What's Wrong With My Mouse? Behavioral Phenotyping of Transgenic and Knockout Mice, Second Edition Edition. Hoboken, NJ John Wiley & Sons, Inc.

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