Supporting Information

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SI Text

Mouse Genotyping. Mouse genotyping was performed (The Jackson Laboratories, Bar Harbor, Maine) using the genomic DNA extracted using the Extract-N-AMP tissue PCR kit (Sigma) according to the manufacturer's instructions. The Gly-39 allele was identified by PCR amplification with the primers: 5′ CCA AGA ACC AAG AGC TAG TCA GGG TCC TTG GCA GAT GGG C; 5′ TTA GGC TCA CGT CAG CTA CC. For Glu39Gly, PCR conditions were as follows: 1 cycle (5 min at 95°C) and 40 cycles (45 s 95°C, 45 s at 65°C, 1 min at 72°C) using 0.8 μM dNTPs and Taq Polimerase (Promega, Madison, Wisconsin), followed by digestion with HaeIII for 1 h at 37°C and submitted to electrophoresis. For genotyping of Arg152Lys we used sense primer AACTGTGAGCTCCACGCCC and antisense primer CCTTTGAAAATCGGGCAGATC at 94°C for 5 min followed by 35 cycles of 94°C 30 sec, 52°C 30 sec, and 72°C 30 sec and finishing with a 5-min extension cycle at 72°C to produce a 213 bp amplicon subjected to restriction digestion with MnlI.

Marble Burying. Mice were housed with same sex littermates (2–5 per cage). Before beginning the marble burying assay, the mice were placed in individual cages (16 × 32 × 13 cm) containing 5 cm of beta chip sawdust bedding for 15 min to acclimate to the new surroundings. After 15 min the mice were briefly removed from the cage, the bedding was smoothed and slightly compacted, and 25 1.5-cm blue marbles were placed in the cage in 5 rows. The mice were returned to the cage and allowed 30 min to investigate the marbles. At the end of the study, the mice were returned to their home cages and the number of marbles buried at least two-thirds of the way in the bedding was recorded.

Nestlet Shredding. Mice were housed with same sex littermates (2–5 per cage). Before beginning the nestlet shredding experiment, the mice were placed in individual cages (16 × 32 × 13 cm) for 15 min to acclimate to the new surroundings. After a 15-min acclimation, a preweighed cotton nestlet (~5 × 5 × 0.3 cm, ~2 g) was placed in the middle of the cage. After 60 min, the mouse was removed and the portion of the nestlet not shredded for nest building was reweighed and the weight was subtracted from the initial weight of the nestlet.

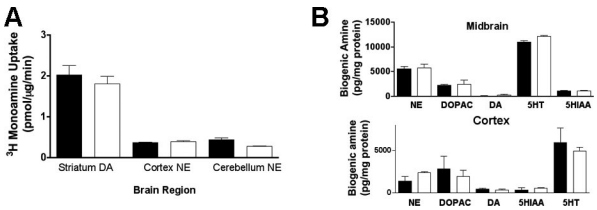

Fig. S1. Monoamine uptake (*A*) and neurochemistry (*B*) of inbred mouse lines bearing the ER (CBA/J, ■) or GK (C57BL/6J, □) mSERT haplotype. No significant differences were found in dopamine (DA), norepinephrine (NE) uptake. No differences were found in monoamine tissue levels between the two strains. Student's *t*-test for DA: $P = 0.456$. One-way ANOVA analysis of variance for NE: cortical NE: $P < 0.05$, cerebellar NE: $P < 0.05$.

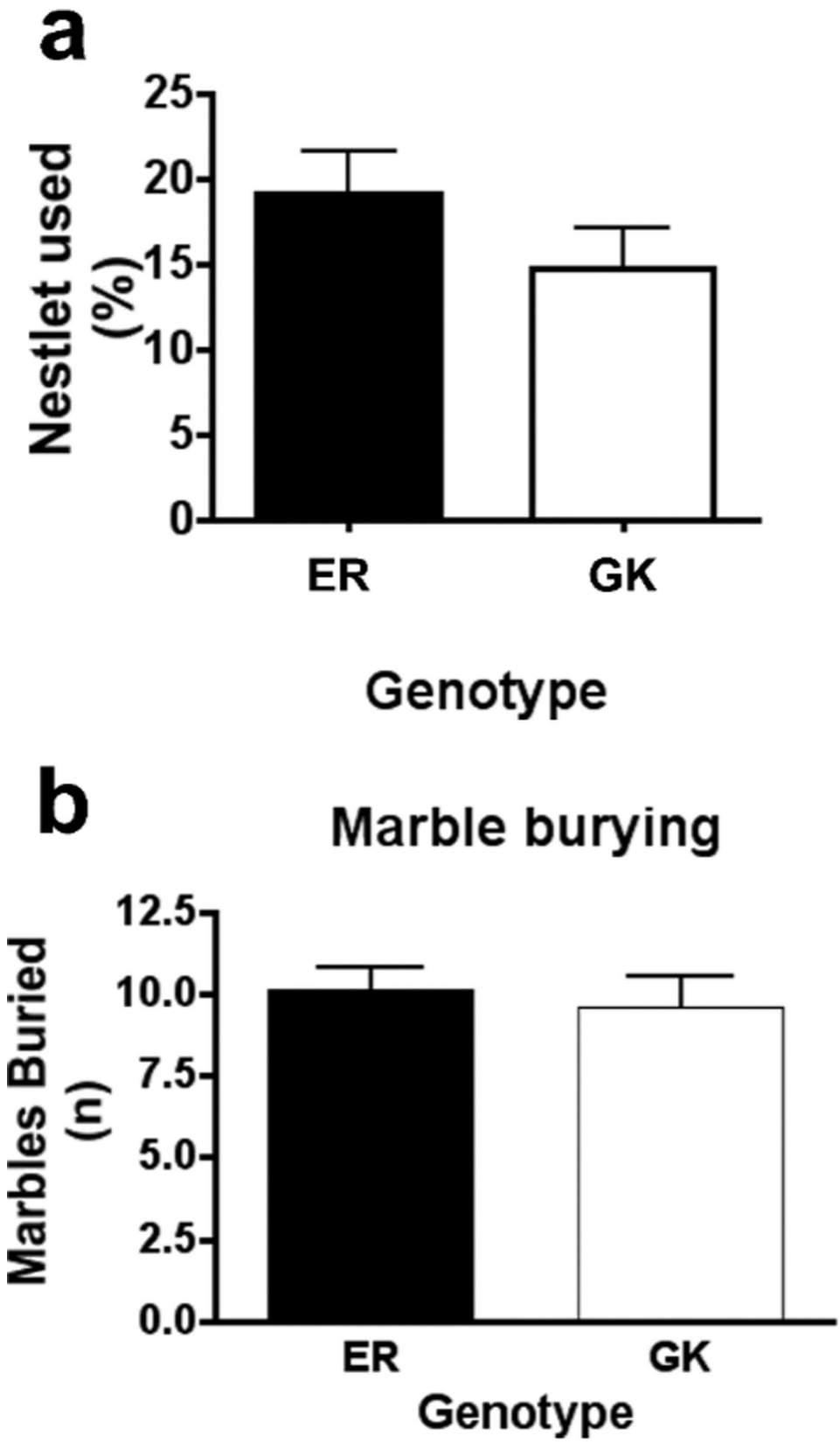

Fig. S2. Nestlet shredding (females) and marble burying (males) behavior in BXD mouse lines. No significant differences were found between ER and GK lines. Nestlet shredding Student's *t*-test: $P = 0.116$, $n = 5$ lines (ER) and 6 lines (GK). Marble burying Student's *t*-test: $P = 0.8915$, $n = 8$ lines (ER), 7 lines (GK).

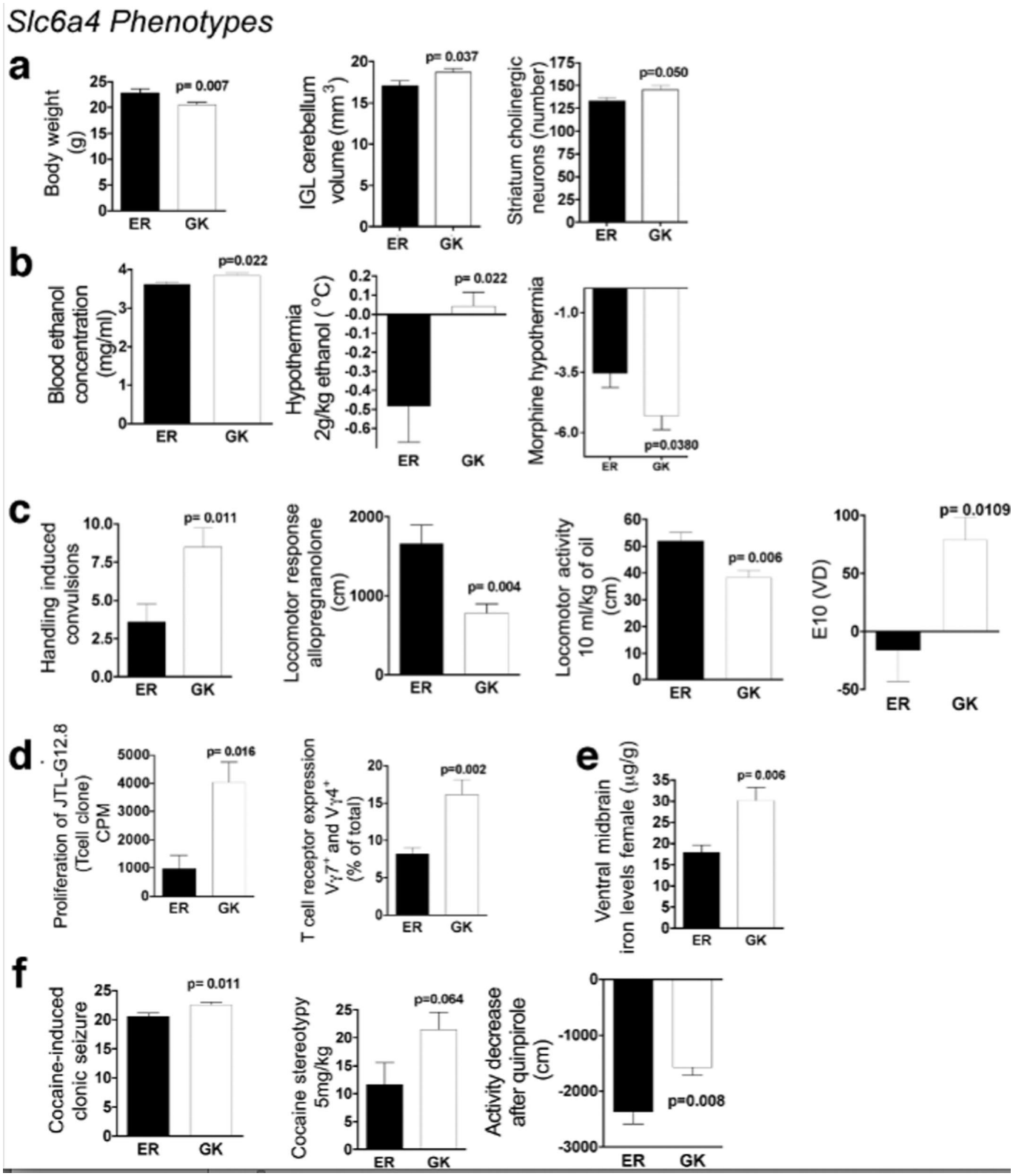

Fig. S3. Phenotypes influenced by mSERT haplotype in BXD lines, where (■) represent ER lines and (□) GK lines (Pubmed ID shown in parenthesis). (*a*) Anatomical phenotypes include body weight (979472), cerebellar volume (11438585), and number of striatal cholinergic neurons (8968368). (*b*) Ethanol-associated traits: blood ethanol concentration (10656187), hypothermia induced by 2g/kg ethanol (8627539), and morphine hypothermia (1632590). (*c*) Anxiety-related phenotypes: handling-induced convulsions (8512534), total distance (cm) traveled in response to 10 mg/kg allopregnanolone (11895174), total distance traveled after 10 mL/kg oil (11106859), and ''velocity difference'' between the light and dark compartments from the 0–10′ interval after injection with 1.8 g/kg of ethanol (data not shown). (*d*) Immune system phenotypes: proliferation of T cells (6203970) and T cell receptor expression (9159147). (*e*) Iron midbrain levels in females. (*f*) DA-signaling phenotypes: cocaine dose necessary to induce clonic seizure (mg/kg) (10734168), cocaine stereotypy (5 mg/kg) (10591541), and quinpirole-induced decrease in activity (110540779).

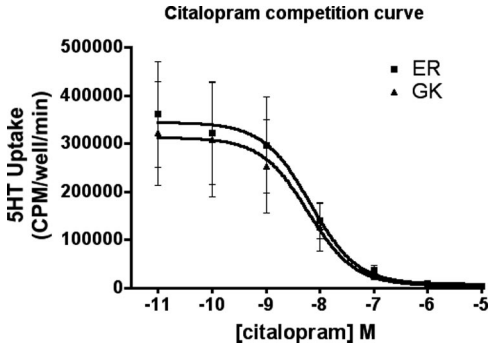

Fig. S4. Analysis of mSERT haplotype influence on citalopram sensitivity. Cells were transfected with either the ER or GK mSERT cDNAs and evaluated for [3H]5-HT transport as described in *Experimental Procedures* in the presence of increasing concentrations of unlabeled citalopram. Citalopram competition curves show no differences between ER and GK citalopram binding affinities EC_{50} (ER = 6.5175e-009 ± 0.3791M; GK = 5.9044e-009 ± 0.4331M). Student's *t*-test: $P = 0.942$, $n = 5$.

Table S1. Genotypes of common inbred mouse lines for *Tph2* and *Slc6a4* polymorphisms

Mouse line	Tph2 (aa pos 447)	Slc6a4 (aa pos 39/152)
129Xi/J	Pro	Glu/Arg
A/J	Arg	Glu/Arg*
AKR/J	Pro	Glu/Arg*
BALB/cJ	Arg	Glu/Arg
BUB/J	Pro	Glu/Arg*
BTBR T + tf	Pro	Glu/Arg*
C3H/J	Pro	Glu/Arg*
C57BLKS/J	Pro	Glu/Arg
C57BL/6JJ	Pro	Gly/Lys
C57BL/10J	Pro	Gly/Lys
C57BR/J	Pro	Gly/Lys
C57L/J	Pro	Gly/Lys
C58/J	Pro	Glu/Arg
CAST/Ei	Pro	Gly/Lys
CBA/J	Pro	Glu/Arg
CE/J	Pro	Glu/Arg*
CZECHII/EiJ	Pro	Glu/Arg*
DBA/J	Arg	Glu/Arg
FVB/NJ	Pro	Glu/Arg*
SPRET/J	Pro	Glu/Arg*
JF1/MS	Pro	Glu/Arg*
KK/HIJ	Pro	Glu/Arg*
LP/J	Pro	Glu/Arg*
MA/MyJ	Pro	Glu/Arg*
MOLF/EiJ	Pro	Glu/Arg
MSM/Ms	Pro	Glu/Arg*
NON/LtJ	Pro	Glu/Arg*
NZB/BINJ	Pro	Glu/Arg*
NZW/LacJ	Pro	Glu/Arg*
PERA/EiJ	Pro	Glu/Arg*
PL/J	Pro	Glu/Arg*
RIIIS/J	Pro	Glu/Arg*
SEA/GnJ	Arg	Glu/Arg*
SJL/J	Pro	Glu/Arg*

*Data from the Mouse Phenome database, not confirmed by authors.

Table S2. Traits impacted by *Slc6a4* and *Tph2*

ID	Pubmed	Phenotype	Sample	*P Tph2*	*P Slc6a4*
10647	11529276	Body weight [g]	35	0.9256055	0.0023588
10246	14744041	Ventral midbrain iron levels, female [ug/g]	15	0.1599624	0.0032347
10465	9159147	T cell receptor expression, V-gamma-7 positive and V-gamma-4]	25	0.415533	0.0030925
10454	11895174	Locomotor response to 10 mg/kg allopregnanolone	24	0.6072188	0.0032256
10237	6203970	Proliferation of JTL-G12.8 (T cell clone) without 50 μg/mL GAT	25	0.6465703	0.0042395
10245	14744041	Ventral midbrain iron levels, male [ug/g]	15	0.119462	0.0041624
10051	11054779	Activity decrease after administration of 0.03 mg/kg quinpirole	24	0.1576165	0.0034425
10241	14744041	Caudate-putamen iron levels, male [ug/g]	15	0.2266541	0.0055225
10084	8627539	Hypothermia tolerance (positive scores) or sensitization (negative scores) 2 g/kg	23	0.1133265	0.0085527
10570	11106859	Locomotor activity after 10 mL/kg of oil in first 15 min of 30-min activity test	22	0.7054719	0.0083203
10276	10591541	Dopamine transporter caudate-putamen, males	18	0.784576	0.0080978
10027	8512534	Nitrous oxide withdrawal handling induced convulsions, difference from baseline	19	0.3124916	0.0119643
10203	10734168	Cocaine in mg/kg (infused via tail vein) to induce clonic seizure [mg/kg]	24	0.8513991	0.0141609
10272	10591541	Drd2 expression in ventral midbrain, male and female mean	18	0.074708	0.0163878
10136	10581484	Ethanol acceptance, female raw mean consumption	23	0.0660183	0.0142815
10270	10591541	Drd2 expression in ventral midbrain, males	18	0.1013146	0.0141119
10040	10656187	Blood ethanol concentration (BEC) from the retro-orbital sinus	24	0.2886647	0.0252485
10581	7695038	Ethanol acceptance total consumption over 24 hr, female [g/kg]	21	0.2352512	0.0370645
10064	9655868	Area under the 25-hr curve for withdrawal following 72 hr exposure to air	25	0.0058179	0.286601
10047	11054779	Tolerance to quinpirole induced hypothermia on day 2 as compared to day 1	24	0.0057739	0.5700668
10350	12183685	Ethanol induced ataxia, time for regain of balance on dowel test (1) [min]	30	0.0132397	0.0511413
10048	11054779	Tolerance to quinpirole induced hypothermia on day 3 as compared to day 1	24	0.0103794	0.5423341
10599	14657177	Total lateral geniculate nucleus endothelial cell number $\times$ 1000	32	0.078951	0.3134177
10669	10102277	Retinal area [mm2]	26	0.0583644	0.1560597
10347	12183685	Ethanol induced ataxia, initial sensitivity blood ethanol concentrations (BECo)	30	0.0240444	0.0637918

**P* values are derived from the additive linear mixed model used to identify novel phenotypes.

Table S3. Traits impacted by *Slc6a4* and *Tph2* epistasis

ID	Pubmed	Phenotype	*N*	*P* value
10649	9412494	Retinal ganglion cell number (× 1000)	26	0.0005229
10670	10102277	Retinal ganglion cell number (× 1000)	26	0.0005229
10600	14657177	Retinal ganglion number (× 1000)	34	0.0008895
10650	9412494	Retinal ganglion cell number (residual cells × 1000 corrected for brain and body	26	0.0019426
10780	15922957	Coagulation Factor IX (cF.IX) levels following injection of 3e11 vg/mouse AAV2-c	18	0.0033972
10752	12925895	Total cholesterol levels following 16-week high-fat diet	18	0.0042743
10668	10102277	Lens weight [mg]	26	0.0046043
10579	8748968	Plasma corticosterone 6 hr post EtOH female [ug/dl]	25	0.0051004
10795	16407118	Cell proliferation in the adult dentate gyrus (Ki67; residuals)	29	0.0058015
10001	11438585	Cerebellum weight [mg]	34	0.0097921
10753	12925895	LDL cholesterol levels following 16-week high-fat diet	18	0.0099457
10827		Total iron-binding capacity of blood	29	0.0215391
10587	7625571	Ethanol acceptance-consumption of 10% ethanol in 24 h after 24 h of water	21	0.0220154
10671	10102277	Eye weight, regression corrected for age, sex, body and brain weight (mg)	26	0.0229984
10725	16910173	Frontal cortex zinc levels, females	15	0.0239297

**P* values are derived from the additive linear mixed model used to identify novel phenotypes.