Supplementary material

Generation of the mouse line that does not express S-COMT

Comt cloning. A mouse PAC library RPCI21 (UK HGMP Resource Centre, Cambridge, UK) was screened with a ~1.7 kb probe (generated by PCR amplification of PAC DNA using primers Comt_int_F1 atgaccaaatgattataggcatggt, and Comt_ex2_R1 gctgggtgatgg-cagcgtagtc). The DNA from one positive clone was digested with Mph1103I and a ~7.8kb fragment isolated was sub-cloned into pLITMUS-29 (New England Biolabs Ipswich, MA, USA) Mph1103I site.

MET2 mutagenesis and construction of targeting vector. The ~1.3kb Bsp1407I(BsrGI)/Kpn2I(BspEI) fragment containing exon2 with MET2 was further sub-cloned into pLITMUS-38. The MET2 was mutated by PCR and an additional restriction site, BshTI, was generated for control after homology recombination. The Bsp1407I/Kpn2I was sub-cloned back into main Mph1103I fragment. The ~1.2kb KpnI fragment was sub-cloned into pBluscript KS (Stratagene, La Jolla, CA, USA), and two new restriction sites XhoI and SalI were created near the first KpnI site by PCR and sub-cloned back into the Mph1103I fragment. The DTA cassette was cloned via NotI/SalI into pGEM11. The modified (Mut_MET2/XhoI/SalI) ~7.8kb fragment was sub-cloned into pGEM11_DTA vector **(Figure 1A main manuscript, Table S1)**. One of the Mph1103I restriction sites (5'arm) was deleted by partial digestion, Klenow and self-ligation. The ~3.9kb SalI (newly generated) /Mph1103I fragment (3'arm) was sub-cloned into the vector containing Lox-TK-NEO-Lox cassette and opened by XhoI/Mph1103I, where SalI and XhoI sites were eliminated. The new ~8.8kb SalI/Mph1103 fragment (floxTKNEO_3'arm) was sub-cloned back into main vector opened by XhoI/Mph1103I, where SalI XhoI sites have been eliminated again.

Recombination in ES cells, generation of chimeras, breeding F1 and F2 generations. 20 µg of targeting vector was introduced in 3B4 ES cells by electroporation (GenePulserII, Bio-Rad, Hercules CA, USA, conditions: 24V 500 µF, 0.4 cuv). Neomycin resistant recombinants were selected and treated with Cre recombinase to remove the *neo* gene after which the ES cells were used for blastocyst injection. 11% of the clones were positive for homologous recombination. Chimerical males were mated with C57BL/6J females, and DNA from tail biopsies of F1 pups were typed by PCR (see main manuscript). F1 heterozygous mice were mated and F2 mice of all three genotypes were obtained.

Western immunoblotting

The samples were diluted 1:20 with a homogenization buffer (10 mM Na_2HPO_4, pH 7,4, containing 0,5 mM dithiothreitol) and subsequently homogenized using a sonicator (Rinco Ultrasonics sonicator, Arbon, Switzerland). The homogenates were centrifuged at 890 X *g* at +4 °C for 10 min and the supernatants were collected. The 20 µg samples were then diluted with Laemmli buffer, loaded and electrophoresed in a 12 % SDS-polyacrylamide gel. Subsequently, samples were transferred onto Protran® nitrocellulose transfer membrane (Schleicher & Schuell Bioscience GmbH, Dassel, Germany). After blocking non-specific binding with 5 % non-fat dry milk in Tris-buffered saline (TBS), the membranes were incubated with mouse anti-COMT monoclonal antibody (1:10 000; BD Bioscience Pharmingen, San Diego, CA, USA). Following this, the membranes were incubated with a goat anti-mouse horseradish peroxidasse conjugate (1:2000; R&D Systems, Minneapolis, MN, USA). Bands of the blots were visualized using chemiluminescent substrate (Thermo scientific, Rockford, IL, USA) on a GeneGnome chemiluminescent detector (SynGene, Synoptics LTD, UK). A representative western immunoblotting was done on one randomly selected male and one randomly selected female from each genotype.

Light microscopic immunohistochemistry

Briefly, sections were dewaxed in xylene and rehydrated with graded alcohols. The antigen retrieval was processed in a microwave oven in citrate buffer (pH 6.0) and endogenous peroxidase activity was inactivated with 5 % hydrogen peroxide. Nonspecific binding was blocked with M.O.M. Basic kit (Vector Laboratories, Burlingame, CA, USA). The slides were incubated for 60 min with a mouse anti-COMT monoclonal antibody (dilution 1:20; Product #611970, BD Biosciences, San Jose, CA, USA), followed by washing with phosphate-buffered saline (PBS). The slides were then incubated with the rabbit anti-mouse HRP-conjugated secondary antibody (dilution 1:100; Product #31437, Pierce Biotechnology, Rockford, IL, USA) for 30 min, after which the slides were washed with PBS. The brown colour was developed with 0.05% 3,3'-diaminobenzidine (DAB) and 0.03 % hydrogen peroxide in PBS. Finally the slides were counterstained with Mayer's haematoxylin, washed, dehydrated and mounted with Depex (BDH, Poole, UK).

Tissue samples were photographed with a digital camera connected to an Olympus BX40 microscope and DP50 imaging device (Olympus Corporation, Tokyo, Japan). Only minor corrections to brightness and contrast were made with Adobe Photoshop CS2 (version 9.0, Adobe Systems Incorporated, San Jose, CA, USA).

COMT activity-assay

Briefly, the enzyme preparation was incubated at 37 ºC in 100 mM phosphate buffer (pH 7.4) containing 5 mM $MgCl_2$, 200 μM S-adenosyl-L-methionine (Sigma, St. Louis, MO, USA) and 500 μM 3,4-dihydroxybenzoic acid (Sigma, St. Louis, MO, USA). The reaction products, vanillic and isovanillic acid, were analyzed with high-performance liquid chromatographic (HPLC) system with electrochemical detection. The system consisted of a sample autoinjector (Jasco AS-2057, Tokyo, Japan), a pump (Merck Hitachi LaChrom L-7100, Tokyo, Japan), an RP-18 column (3 mm, 4.6 x 150 mm; Waters Spherisorb, Milford, MA, USA), a coulometric detector (ESA Coulochem

model 5100A detector and a model 5014B cell; ESA Inc., Chelmsford, MA, USA; detector potential 0.50 mV) and an integrator (Shimadzu C-R5A; Shimadzu Corporation, Kyoto, Japan). Mobile phase was 0.1 M Na_2HPO_4 (pH 3.3), 0.15 mM EDTA and 25 % methanol with flow-rate of 0.8 $mlmin^{-1}$.

Assay of monoamines in plasma and tissue samples

The column (Spherisorb ODS2 3 mm, 4.6 x 100 mm; Waters, Milford, MA, USA) was kept at a temperature of 38 °C with a column heater (Croco-Cil, Bordeaux, France). The mobile phase consisted of 0.1 M NaH_2PO4 buffer, 7 % MeOH, 200 mgl^{-1} octane sulphonic acid and 450 mgl^{-1} EDTA. The pH of the mobile phase was set to 3.0 using H_3PO_4. The flow rate of 1 $mlmin^{-1}$ was set by the pump (ESA Model 582 Solvent Delivery Module; ESA Chelmsford, MA, USA) equipped with a pulse damper (SSI LP-21, Scientific systems, State Collage, PA, USA). With a CMA/200 autoinjector (CMA, Stockholm, Sweden) 50 µl of the filtrate was injected into the system. Monoamines were detected using ESA CoulArray Electrode Array electrochemical detector and the chromatograms were processed and integrated using CoulArray for windows –software (ESA, Chelmsford, MA, USA). The values were calculated as $ngml^{-1}$ of plasma or $ngml^{-1}$ tissue.

Vascular space contribution

Since the animals were not perfused, the contribution of blood L-dopa and 3-OMD levels to the concentration in tissue samples was estimated. Parenchymal concentrations were calculated at each time point according to the equation $C_i = C_m - V_pC_p$ where C_i was the actual tissue concentration, C_m was the concentration measured in the tissue sample, V_p was the relative amount of vascular space and C_p was the concentration in plasma. Vascular space contribution in different tissues varies depending on the tissue and method used to prepare them. In

the liver, the relative space of sinusoidal lumina has been reported to be 10.6 % (Blouin et al. 1977), whereas in the rat brain the vascular space contribution has been set at 2 % (Morgan et al. 1992). We used an estimate of 5 % in all calculations. For L-dopa, vascular space contribution of the total concentration at a given time point were 1.35 ± 0.16 (mean % ± S.E.M.) in the duodenum and 5.47 ± 0.39 in the liver**.** For 3-OMD, it was 1.23 ± 0.22 in the duodenum and 1.85 ± 0.33 in the liver. In the brain, vascular space contribution of the total concentrations of L-dopa were 6.60 ± 0.85 in the PFC and 8.00 ± 1.23 in the striatum**.** For 3-OMD, it was 2.21 ± 0.39 in the PFC and 2.33 ± 0.44 in the striatum.

We did not recalculate the tissue levels of L-dopa and 3-OMD since the contribution of the blood to total tissue concentrations was always less than 10% and evidently very similar in all animals. Therefore comparisons of the three groups were not invalidated.

References

Blouin A, Bolender RP, Weibel ER (1977). Distribution of organelles and membranes between hepatocytes and nonhepatocytes in the rat liver parenchyma. A stereological study. J Cell Biol 72:441-455.

Morgan ME, Chi SC, Murakami K, Mitsuya H, Anderson BD (1992). Central nervous system targeting of 2',3'-dideoxyinosine via adenosine deaminase-activated 6-halo-dideoxypurine prodrugs. Antimicrob Agents Chemother 36:2156-2165.

Table S1. Primers used for COMT_Met2 Target vector construction and testing.

Primer	DNA sequence
Control primer 1 (Neo R1 pos)	ctgtgctcgacgttgtcactg
Control primer 2 (3'arm_Comt_R1_pos)	tgcatctgcctagaacctcaag
Control primer 3 (5'arm_Comt_F1_pos)	gaccttgaactcagagttacacc
Control primer 5 (Mut_Met_Comt_R1_contr)	ccttactcctctccggacca
Control primer 6 (Mut_Met_Comt_F1_contr)	tggagtgcttaccttgtacacat

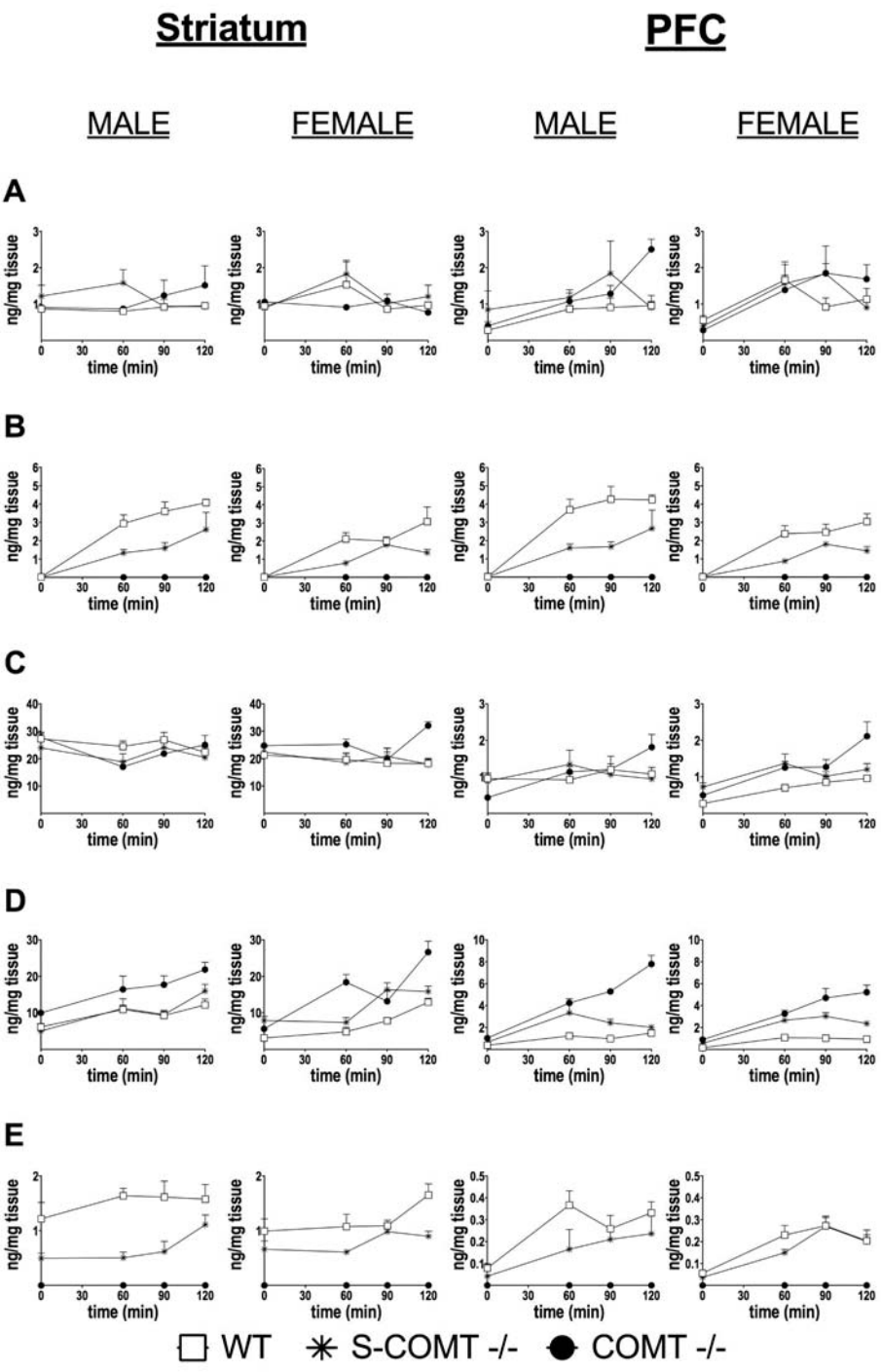

Figure S1. The effect of COMT genotype (S-COMT deficiency or full COMT-ko) on the time course of striatal and PFC levels of L-dopa **(A)**, 3-OMD **(B)**, dopamine **(C)**, DOPAC **(D)** and HVA **(E)** after oral administration of L-dopa and carbidopa (10 $mgkg^{-1}$ and 30 $mgkg^{-1}$, respectively). Data represents group means. n (male/female) = 13/13 (WT), 6/7 (S-COMT -/-), 6/7 (COMT -/-)