Materials and Methods

Reverse-transcriptase PCR and quantitative real-time PCR

For measurement of monoamine oxidase A and B (MAO-A and MAO-B) expression and activity (n=7 per genotype), the frontal cortex and hippocampus were rapidly dissected and frozen on liquid nitrogen. Total RNA was prepared from the samples with the RNeasy Lipid Tissue Mini Kit (Qiagen, Inc.) and converted to single-stranded cDNA by the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Inc.). Quantitative real-time PCR experiments were performed with the cDNA with a Roche Light Cycler and 1x SYBR Green universal PCR master mix (Invitrogen, Inc.) as described previously [3]. The fluorescence signal was quantified by the LightCycler Data Analysis software (Roche, Inc.) with the second derivative maximum method. The threshold cycle for each sample was chosen within the linear range. Monoamine oxidase A and B (MAO-A and MAO-B) were normalized to levels of glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The primer sequences were the following:

MAO-B Forward, 5'-GAAGTTGAGCGGCTGATACAC-3';

MAO-B Reverse, 5'-GCATCACTGGGAATCTCTTGG-3';

MAO-A Forward, 5'-GCCCAGTATCACAGGCCAC-3';

MAO-A Reverse, 5'-CGGGCTTCCAGAACCAAGA-3';

GAPDH Forward, 5'-CATGTTCCAGTATGACTCCACTC-3';

GAPDH Reverse, 5'-GGCCTCACCCCATTTGATGT-3'.

Expression levels were calculated from the threshold cycles as $2^{-(MAO-GAPDH)}$. Data were normalized to the mean of C57BL/6 C/C mice.

MAO activity assay

MAO activity in dissected frontal cortex and hippocampus tissue was detected with the Amplex red monoamine oxidase assay kit (cat no. A12214. Invitrogen, Carlsbad, California). Amplex red reacts with H2O2, a byproduct of MAO activity, to produce the stable fluorophore resorufin. The reaction is catalyzed by horse radish peroxidase [4]. Here, the assay was optimized to measure MAO activity with 5-HT as the enzymatic substrate. Three comparisons were performed: 1. BALB/c G/G *versus* C57Bl/6 C/C, 2. C57Bl/6 C/C *versus* C57Bl/6 G/G, and 3. BALB/c G/G *versus* BALB/c C/C. Frozen tissues were weighed and homogenized by sonication in ice cold 50 x (w/v) lysis buffer (50mM NaH2PO4, 5 mM KCl, 120 mM NaCl, 0.5% (v/v) Triton X-100, pH 7.4). The homogenate was centrifuged (15000g, 10 min.) and the supernatant recovered and further diluted 40 x (v/v) in reaction buffer (50 mM NaH2PO4, pH 7.4) to a final sample dilution of 0.5 μg tissue/μl. Each comparison was performed within a single 96 well plate. Triplicates of 100 μl sample (or reaction buffer for blank samples) were incubated for 30 min. at 37 ºC with 0.5 μM of the MAO inhibitors pargyline and clorgyline (non-specific activity) or in the absence of MAO inhibitors (total activity) in 96 well plates. Next, 50 μl of 1000μM (final 250 μM) 5-HT was added to all sample wells. A 50μl of a mixture of Amplex red (800 μM, final 200 μM) and horse radish peroxidase solution (2U/ml, final 0.5U/ml) was then added to all sample wells to start the reaction, and immediately thereafter fluorescence at 0 min. was determined. Fluorescence development (λ_{ex}=544, λ_{em}=590) of resorufin was measured every 20 min. for 120 min. with a plate reader (NOVOstar, BMG Labtech, Offenburg, Germany). Nmol resorufin was interpolated from

a series of resorufin standard samples (10, 30, 100, 300 and 1000 nmol per 200ul) included on each plate. MAO activity was expressed as nmol of resorufin formed per 50ug tissue. Specific (MAO derived) activity was calculated as “total” resorufin produced – “non-specific” resorufin produced.

Tail suspension assay

Mice (n=13-18 per group) were assessed for immobility in a tail suspension apparatus (Med-Associates, St. Albans, VT) as described previously [1, 2]. Mice were randomly assigned to either the vehicle (saline) group or the escitalopram (5mg/kg) group and injected i.p. 30 min. before testing. Escitalopram oxalate was a generous gift from H. Lundbeck A/S (Copenhagen, DK) and was injected intraperitoneally (i.p.) in a volume of 10ml/kg. The 5mg/kg dose was chosen for the tail suspension assay on the basis of pilot experiments. During testing, mice were suspended for 6 min. by a tape placed at the tip of the tail. Total immobility time was measured as the time spent below a preset threshold established in pilot experiments. The Med-Associate settings used to define immobility in this experiment were the following: threshold=1, gain=1. Mice that climbed their tails during the experiment were excluded from analysis. Data were analyzed for both the MAO and tail suspension assays by a two-way Analysis of Variance with genotype and drug as independent variables. For the tail suspension assay, data were also analyzed by a three-way Analysis of Variance with strain, genotype, and drug as independent variables.

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[4] M. Zhou, Z. Diwu, N. Panchuk-Voloshina, R.P. Haugland, A stable nonfluorescent derivative of resorufin for the fluorometric determination of trace hydrogen peroxide: applications in detecting the activity of phagocyte NADPH oxidase and other oxidases, Anal Biochem 253 (1997) 162-168.

Figure S1.

Monoamine oxidase expression and activity. A-D) Expression of monoamine oxidase A and B (MAO-A and MAO-B, respectively) was measured in the frontal cortex and hippocampus by quantitative real-time PCR. Data were normalized to glyceraldehyde 3-phosphate dehydrogenase expression and then normalized to the mean of C57B/6J C/C mice. E-F) MAO activity was measured in the frontal cortex by the Amplex red MAO assay kit (Invitrogen, Carlsbad). Graphs depict specific MAO activity, total activity (Tot.), and non-specific activity (NS). A) In the frontal cortex, MAO-A expression was slightly lower in BALB/c mice than in C57Bl/6 mice but was unaffected by *mTph2* genotype. B) In the frontal cortex, MAO-B expression was 50% lower in BALB/c mice than in C57Bl/6 mice but was also unaffected by *mTph2* genotype. C) There was no effect of strain or *mTph2* genotype on MAO-A expression in the frontal cortex. D) MAO-B expression in the hippocampus was unaffected by *mTph2* genotype but was significantly lower in BALB/c mice than in C57Bl/6 mice. E) The rate of 5-HT metabolism by MAO in the frontal cortex did not differ between strains. F) Among the BALB/c mice, there was no effect of *mTph2* genotype on MAO activity. G) There was no genotype effect on MAO activity among the C57Bl/6 mice. Data are presented as mean ± SEM. N=7. **, p<.01; ***, p<.001 C57Bl/6 vs. BALB/c.

Figure S2.

Effect of escitalopram on the tail suspension test. Mice were injected i.p. with vehicle or escitalopram (5mg/kg) 30 min. before testing. During testing, mice were suspended by their tails for 6 min., and total immobility time was measured. Escitalopram significantly

decreased immobility time in both strains, but had a greater effect on the BALB/c mice than the C57Bl/6 mice. There was no effect of genotype. A) BALB/c congenics (N=13-18). B) C57Bl/6 congenics (N=10-17). Data are presented as mean ± SEM. Escit., escitalopram. *, $p<.05$; ***, $p<.001$ vehicle vs. escitalopram.

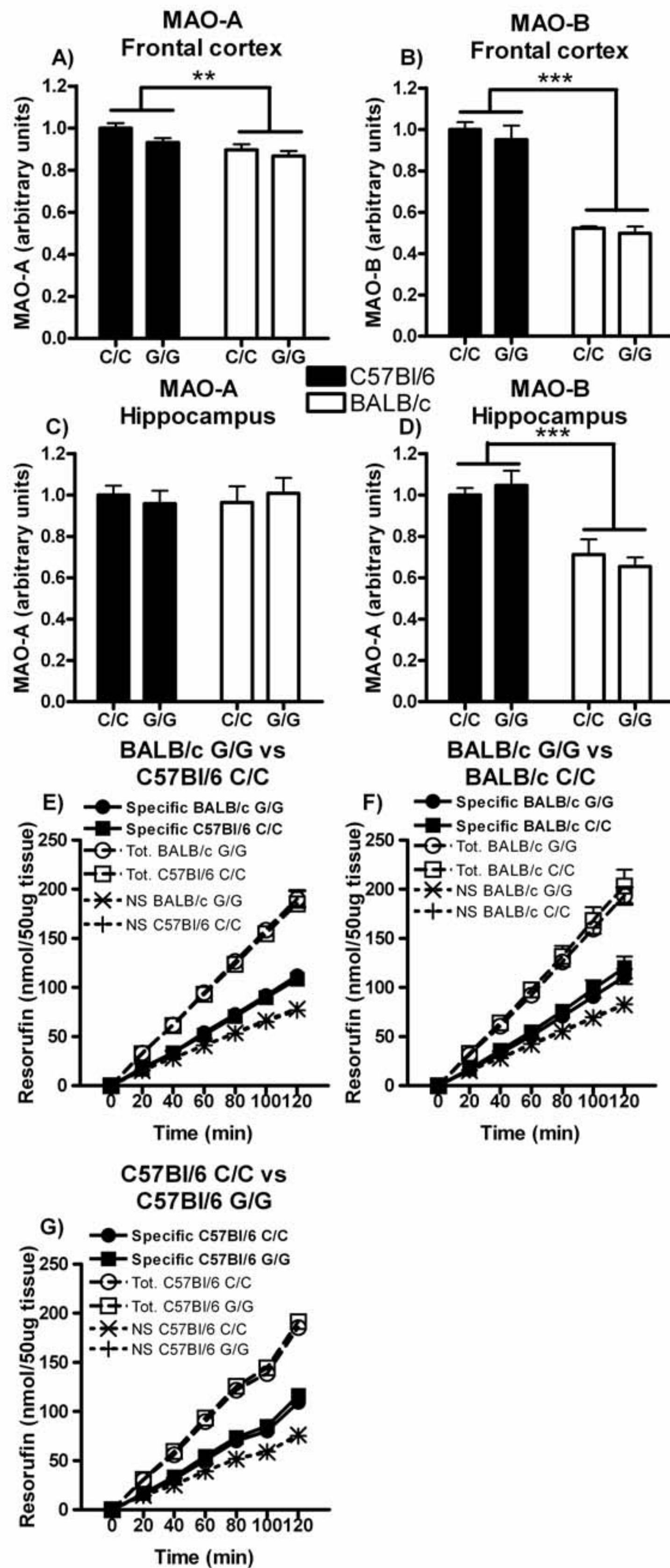

Figure S1.

Figure S2.