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ORIGINAL INVESTIGATION

Antidepressant response to chronic citalopram treatment in eight inbred mouse strains

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Abstract
Rationale The antidepressant response exhibits a characteristic delay. BALB/cJ mice respond to chronic, but not subchronic, treatment with selective serotonin reuptake inhibitors (SSRIs), providing a model of antidepressant onset. Identification of other mouse strains exhibiting this phenotype will provide additional tools for studying mechanisms of the antidepressant response.
Objectives We aimed to identify inbred mouse strains that respond to chronic, but not subchronic, SSRI treatment in the forced swim test (FST). We also assessed whether response correlated with genotype at the functional C1473G polymorphism in tryptophan hydroxylase-2 (Tph2).
Methods BALB/cJ, three closely related strains (BALB/cByJ, SEA/GnJ, A/J), and four distantly related strains (C57BL/6J, C57BL/10J, CAST/EiJ, SM/J) received the SSRI citalopram (0–30 mg/kg/day in drinking water) for ~4 weeks and were assessed for locomotion and FST behavior. Citalopram-responsive strains were assessed identically following ~1 week of treatment. C1473G genotypes were determined.
Results BALB/cJ and related strains carried the 1473G allele and responded to chronic citalopram treatment in the FST. BALB/cJ, BALB/cByJ, and SEA/GnJ mice showed either no response or an attenuated response to subchronic treatment. Distantly related strains carried the 1473C allele and showed no response to citalopram. No relationship was found between the antidepressant response and baseline immobility or locomotion.
Conclusions BALB/cJ and related strains exhibit an antidepressant response to chronic SSRI treatment that emerges over time and is likely a heritable trait. This antidepressant response is associated with carrying the 1473G allele in Tph2. In conclusion, BALB/cJ and related strains provide valuable models for studying the therapeutic mechanisms of SSRIs.

Keywords Tryptophan hydroxylase 2 · Selective serotonin reuptake inhibitor · SSRI · Forced swim test · Inbred strain · Citalopram · Depression · BALB/cJ · Locomotion · Mice

Introduction

Selective serotonin reuptake inhibitors (SSRIs) provide first-line pharmacological treatment for depression (Koenig and Thase 2009). However, the mechanism of therapeutic action of SSRIs and other antidepressants still remains largely unknown. Although SSRIs increase extracellular serotonin levels within minutes to hours of administration, their therapeutic effects require several weeks of treatment to emerge (Blier 2003). The delayed therapeutic effects of SSRIs are thought to result from neuroplastic changes that develop downstream from 5-HTT blockade (Pittenger and Duman 2008). Animal behavioral models in which chronic, but not short-term, antidepressant treatment alters behavior are critically needed for studying the mechanisms underlying the antidepressant response (Cryan et al. 2002; Dulawa and Hen 2005).

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The forced swim test (FST) is a rodent drug screening model in which acute antidepressant treatment reduces immobility and/or increases active behaviors with high reliability and specificity (Borsini and Meli 1988; Porsolt et al. 1977). However, the behavioral response to acute injection is unlikely to reflect changes in depression-related behavior, since the therapeutic effects of antidepressant effects require weeks to emerge. We previously reported that the inbred BALB/cJ mouse strain responds to chronic, but not subchronic, treatment with antidepressants including SSRIs in the FST (Dulawa et al. 2004; Holick et al. 2008). Specifically, weeks of treatment with the SSRI fluoxetine in the drinking water was required to reduce immobility, while steady-state serum fluoxetine levels are achieved in less than 5 days (Dulawa et al. 2004; Santarelli et al. 2003). Thus, while the FST is traditionally used to screen antidepressants following acute drug injection, the FST can also be used as a model for studying the therapeutic effects of chronic antidepressant treatment in BALB/cJ mice. The neural mechanisms underlying the response to acute versus chronic antidepressant treatment in the FST are distinct; for example, the 5-HT1A receptor is required for the response to acute, but not chronic, treatment with the SSRI fluoxetine (Holick et al. 2008).

In the present experiments, we aimed to identify additional inbred strains that respond to chronic (~4 weeks), but not short-term (~1 week), treatment with SSRIs in the FST. We used two strategies to identify additional SSRI responsive strains: (1) we hypothesized that strains closely related to BALB/cJ might also exhibit sensitivity to chronic SSRI treatment and (2) we also selected strains distantly related to BALB/cJ and each other to identify more genetically diverse SSRI responsive strains. Thus, were selected three strains most closely related to BALB/cJ (BALB/cByJ, A/J, and SEA/GnJ) and four distantly related strains (C57BL/6J, C57BL/10J, CAST/EiJ, and SM/J) based on single nucleotide polymorphism databases (Petkov et al. 2004; Fig. 1).

Tryptophan hydroxylase-2 (Tph2) is the rate-limiting enzyme for the biosynthesis of brain serotonin (Zhang et al. 2004). Human Tph2 gene variants have also been associated with the SSRI response (Tsai et al. 2009; Tzvetkov et al. 2008), and the murine C1473G polymorphism in Tph2 has been associated with response to acute SSRI treatment in the FST (Cervo et al. 2005; Guzzetti et al. 2008). The 1473C allele is highly conserved across species, while the 1473G form is found in several inbred mouse strains including BALB/cJ and confers a 50% reduction in Tph2 enzymatic activity (Sakowski et al. 2006) and marked reductions in in vivo brain serotonin synthesis and tissue content (Cervo et al. 2005; Zhang et al. 2004). In the present studies, we determined C1473G genotype to evaluate whether this allele correlates with response to chronic (~4 weeks) SSRI treatment. We hypothesized that like BALB/cJ, other strains carrying the lower-functioning 1473G allele would respond to chronic SSRI treatment in the FST.

	A/J	BALB/cJ	BALB/cByJ	SEA/GnJ	SM/J	C57BL/10J	CAST/EiJ	C57BL/6J
A/J								
BALB/cJ	361							
BALB/cByJ	363	3						
SEA/GnJ	464	301	304					
SM/J	736	551	567	502				
C57BL/10J	833	823	841	737	683			
CAST/EIJ	710	704	713	669	657	746		
C57BL/6J	882	861	880	761	731	49	769	

Fig. 1 The number of single nucleotide polymorphisms between different mouse strains are shown. A total of 1,638 markers were evaluated for 102 mouse strains (Petkov et al. 2004). Of all strains examined, BALB/cJ were most closely related to BALB/cByJ, A/J, and SEA/EiJ strains. Only the eight strains used in the present studies are shown

Methods

Animals

BALB/cJ, BALB/cByJ, SEA/GnJ, A/J, C57BL/6J, C57BL/10J, CAST/EiJ, and SM/J (Jackson Laboratories, Bar Harbor, ME, USA) 8–12 weeks of age and weighing 12–25 g were used for all studies (see Supplemental results). Female mice were used for all experiments due to female preponderance in depressive disorders (Parker and Hadzi-Pavlovic 2004). Mice were housed in groups of four to five and maintained on a 12:12-h L/D schedule (lights on at 0600 hours). Food and water were provided ad libitum. Behavioral testing occurred during the light phase between 0600 and 1600 hours. Animal testing was conducted in accord with the NIH laboratory animal care guidelines and with Institutional Animal Care and Use Committee approval.

Drugs

The SSRI citalopram was purchased from ANAWA Biomedical Services and Products (Switzerland) and was provided to animals in the drinking water. We used the SSRI citalopram because it is highly selective for 5-HTT (Bezchlibnyk-Butler et al. 2000).

The concentration of citalopram provided was calculated based on average daily water consumption and body weight

for each strain. Mice received 0, 10, or 30 mg/kg/day citalopram for chronic studies and 0 or 20 mg/kg/day citalopram for subchronic studies. We chose an intermediate dose of 20 mg/kg/day for subchronic studies to conserve drug and mice, since the 10- and 30-mg/kg/day doses were found to produce similar behavioral effects. Drug-treated water was protected from light in opaque water bottles. Vehicle-treated animals received regular (tap) drinking water, and drug solutions were replaced every 3 to 4 days. Each mouse received only one drug dose and duration of treatment (subchronic or chronic).

Forced swimming test

We used the modified FST, which is a modified version of the traditional rat FST (Porsolt et al. 1979) and was designed to increase sensitivity for detecting SSRIs (Detke et al. 1995, 1997). We performed the modified FST as described previously (Dulawa et al. 2004; Holick et al. 2008). Briefly, mice were placed into white opaque plastic buckets (61 cm high, 48 cm diameter) filled 48 cm high with 23–25°C tap water for 6 min each on two consecutive days. Swim sessions were videotaped from a tripod-mounted camera positioned directly above swim buckets. Animals' behavior was analyzed by a trained observer blind to treatment conditions using a time sampling technique in which the predominant behavior (swimming, immobility, or climbing) is scored every 5 s for the last 4 min of the test (Cryan and Lucki 2000). Reductions in immobility and/or increases in swimming or climbing reflect antidepressant effects (Detke et al. 1997).

Open field test

Locomotor activity was quantified using 42 cm long× 42 cm wide×30 cm high plexiglass activity chambers (Accuscan, Columbus, OH, USA) equipped with 16 infrared beams (2.54 cm apart) along each wall to determine the paths taken by mice. Each activity chamber was enclosed within an environmental control chamber with overhead lighting (Accuscan, Columbus, OH, USA). Paths were stored as *x*–*y* coordinate sequences. Overall locomotor activity was quantified as the total distance traveled (centimeters).

Plasma citalopram measurement

At the end of all behavioral testing, mice were sacrificed by decapitation, and trunk blood was collected in heparinized tubes. Blood was spun at 2,000×*g* for 5 min, and plasma supernatant was removed stored at −80°C. Plasma citalopram was measured using a previously published liquid chromatographic method (Oyehaug et al. 1982). This procedure was modified slightly by using a more polar reversed-phase column (trimethylsilyl bonded silica) and altering the mobile phase to 72:28 phosphate buffer/acetonitrile with the pH adjusted to 3.2 with phosphoric acid and *n*-butylamine with a flow rate at 1.5 ml/min. A fluorescence detector set at 235 $nm_{excit.}$. and 300 $nm_{emiss.}$ produced clean chromatograms with no interference from other drugs or endogenous material. The method was validated from 300 ng/ml to the lower limit of quantitation (2.5 ng/ml) resulting in an intra-day variation of no more than 10% for all three compounds at seven concentrations of the calibration curve. Inter-day variation did not exceed 6.8% for the three quality controls over 16 consecutive days.

Genotyping

To determine the C1473G genotype of each inbred strain, we performed PCR on mouse genomic DNA. For BALB/cJ, BALB/cByJ, SEA/GnJ, A/J, C57BL/6J, C57BL/10J, and SM/J strains, primer sequences were forward—5′ TCTGTGGCTGCTGCTCGCTGAGCT 3′ and reverse—5′ AACTCACCGACTGGAATTGTGCGA 3′. Different primers were developed for the CAST/EiJ strain, which contained polymorphisms in this area: forward—5′ GTCGACTAGTAACTGAGAAGT3′ and reverse—5′ CAAATAGGATCATCTTTCA 3′. PCR conditions were as follows: one cycle (5 min at 94 C) and 35 cycles (30 s at 94 C, 30 s at 55 C, 45 s at 72 C) using Taq DNA polymerase (Promega, Madison, WI, USA). For all strains except CAST/EiJ, PCR products were sequenced directly. For the CAST/EiJ strain, the PCR product was cloned into the PGEM-T easy vector (Promega, Madison, WI, USA). Five bacterial clones were picked from LB plates and grown into LB broth overnight. Plasmid DNA was purified with Qiagen miniprep (Qiagen, Inc.) and sequenced with Sp6 and T7 primers.

Behavioral testing

Chronic studies Mice were treated for 30 days with 0, 10, or 30 mg/kg/day citalopram beginning on day 0. Mice were tested for locomotor activity to determine whether any drug-induced changes in activity levels were responsible for observed behavioral effects in the FST. Due to large numbers of mice, 3 days (days 21–23) were required for locomotor testing. Mice were placed gently into the corner of the open field, and locomotor activity was recorded for 10 min. The open field was cleaned with a 0.1% bleach solution between each mouse. Then, half of the mice from each group were pretested in the FST on day 26, and the other half were pretested on day 28. All mice were tested in the

FST 24 h after the pretest. Mice were tested in the same pseudorandom order on each day. On day 30, mice were sacrificed without any drug washout period for determination of serum citalopram levels.

Subchronic studies Mice were treated for 9 days with 0 or 20 mg/kg/day citalopram. On day 7, mice were tested for locomotor activity. On days 8 and 9, mice were pretested and tested in the FST, respectively. All behavior tests were performed identically for chronic and subchronic studies. Only strains that exhibited an antidepressant-like response to chronic citalopram treatment in the FST were evaluated for response to subchronic citalopram treatment.

Statistical analysis

For all studies, ANOVAs were applied to each measure of the FST (immobility, climbing, and swimming) and the open field test (total distance traveled) for each strain. For each measure, two-way ANOVAs were applied with drug treatment as a between-subject factor and time bin (4 × 1 min bin for FST; 2 × 5 min bin for open field) as a within-subjects factor. No significant interactions with time bin were confirmed by post hoc tests, and therefore, none are reported. Significant main effects and interactions were resolved using Newman–Keuls post hoc tests. For the analysis of FST measures, one-tailed tests were applied because behavioral changes in only one direction (reduced immobility, or increased swimming or climbing) reflect drug-induced antidepressant-like effects. For the FST, animals with mean immobility values greater or less than 2 standard deviations from the group mean were removed from analysis.

Baseline behavior (behavior of control-treated mice) of the inbred strains was also compared separately. One-way ANOVA was applied to immobility in the FST and total distance traveled in the open field test with strain as a between-subjects factor. Significant main effects were resolved using Newman–Keuls post hoc tests. Pearson correlation was performed to examine a potential correlation between mean baseline locomotor activity and baseline immobility between the strains. Mann–Whitney *U* was performed to examine a potential relationship between mean baseline immobility and antidepressant-like response to chronic citalopram in the FST. Lastly, Mann–Whitney *U* was performed to examine a potential relationship between mean plasma citalopram levels and antidepressant-like response to chronic citalopram in the FST. A response to chronic citalopram in the FST was defined as either a significant reduction in immobility or increase in swimming or climbing. Significance was set at $p<0.05$.

Results

Effects of chronic citalopram treatment

The effects of chronic (~4 weeks) citalopram treatment on immobility, swimming, and climbing behavior in the FST are shown in Fig. 2. The inbred strains that showed significant reductions in immobility were BALB/cJ [$F(2, 32)=2.53$; $p<0.05$] and SEA/GnJ [$F(2, 26)=7.46$; $p<0.005$]. A/J mice showed a strong trend for citalopram-induced reduction in immobility [$F(2, 32)=2.31$; $p=0.06$]. Planned Newman–Keuls post hoc comparisons revealed that chronic citalopram treatment reduced immobility at both the 10- and 30-mg/kg/day doses in A/J mice. Newman–Keuls post hoc tests also showed that chronic citalopram treatment reduced immobility at both doses in SEA/GnJ mice and at the 30-mg/kg/day dose in BALB/cJ mice. In contrast, chronic citalopram did not reduce immobility in C57BL/6J, C57BL/10J, CAST/EiJ, or SM/J mice. Ten of 262 mice total had mean immobility values greater or less than 2 standard deviations from the mean and were removed from analysis. Specifically, each of the following groups had one outlier: A/J, 0 mg/kg/day; BALB/cJ, 30 mg/kg/day; C57BL/6J, 0 and 10 mg/kg/day; CAST/EiJ, 0 and 10 mg/kg/day; BALB/cByJ, 0, 10, and 30 mg/kg/day; and SEA/GnJ, 10 mg/kg/day.

Both doses of chronic citalopram treatment significantly increased climbing in BALB/cByJ mice [$F(2, 30)=3.93$; $p<0.05$]. No significant increases in climbing were found in other mouse strains tested. Finally, chronic citalopram treatment did not significantly increase swimming behavior in any strains.

Few effects of chronic citalopram treatment were found on FST behavior during the pretest (Fig. 3). Chronic citalopram treatment reduced immobility [$F(2, 24)=7.76$; $p<0.01$] at the 10- and 30-mg/kg/day doses during pretesting in SEA/GnJ mice. No other effects were observed.

Mice showed increases in immobility from pretest to test day (Fig. 4). Specifically, all strains showed increased mean immobility values on test day relative to pretest day following control treatment, except for CAST/EiJ mice which showed virtually no immobility. In control-treated animals, ANOVA revealed a main effect of day [$F(1, 7)=83.58$; $p<0.0001$], strain [$F(7, 69)=12.60$; $p<0.0001$], and an interaction of day and strain [$F(7, 69)=4.15$; $p<0.001$]. Post hoc tests revealed that control-treated C57BL/6J [$F(1, 19)=5.59$; $p<0.05$], C57BL/10J [$F(1, 21)=3.18$; $p<0.05$], A/J [$F(1, 20)=22.46$; $p<0.01$], BALB/cByJ [$F(1, 19)=4.12$; $p<.05$], and SEA/GnJ mice [$F(1, 16)=8.93$; $p<0.05$] showed significantly higher immobility values on test day than during the pretest. Control-treated BALB/cJ mice showed a strong trend for increased immobility [$F(1, 21)=$

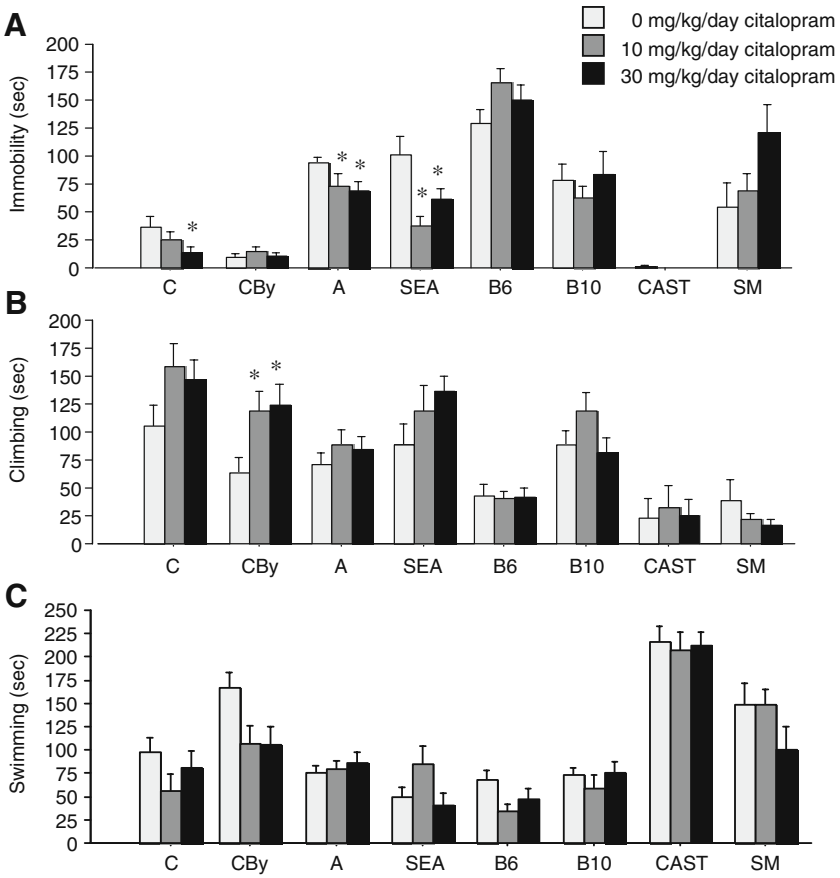

Fig. 2 Effects of ~4 weeks of treatment with citalopram on FST behavior. **a** Total immobility is shown for BALB/cJ (C) (n=35), BALB/cByJ (CBy) (n=33), A/J (A) (n=35), SEA/GnJ (SEA) (n=29), C57BL/6J (B6) (n=34), C57BL/10J (B10) (n=35), CAST/EiJ (CAST) (n=23), and SM/J (SM) (n=28) mice. **b** Total climbing is shown. **c** Total swimming is shown. Values are means±SEM. *Asterisk* indicates significant difference from control-treated animals within the same strain

2.89; p=0.05], and CAST/EiJ and SM/J mice showed no significant effects of day.

The effects of chronic citalopram treatment on total locomotor activity in the open field are shown in Fig. 5. Chronic citalopram treatment significantly altered locomotor activity in SEA/GnJ [F(2, 26)=6.37; p<0.05] and C57BL/610 [F(2, 32)=5.22; p<0.05] mice. Post hoc Newman–Keuls tests indicated that both the 10- and 30-mg/kg/day citalopram doses decreased locomotor activity in SEA/GnJ mice, while only the 10-mg/kg/day dose reduced locomotion in C57BL/10J mice. No effects of chronic citalopram treatment were found on locomotion in BALB/cJ, BALB/cByJ, A/J, C57BL/6J, CAST/EiJ, or SMJ mice.

No significant relationship was found between baseline immobility and baseline locomotor activity between the strains, as indicated by Pearson's correlation (Fig. 6). ANOVA revealed significant inter-strain differences in both baseline immobility [F(7, 75)=14.03; p<0.0001] and locomotion [F(7, 82)=19.64; p<0.0001]. Newman–Keuls post hoc tests indicated the following significant differences in baseline immobility: C57BL/6J and SEA/GnJ>SM/J, BALB/cJ, BALB/cByJ, and CAST/EiJ; A/J and C57BL/10J>BALB/cJ, CBy, and CAST/EiJ; and SM/J > BALB/cByJ and CAST/EiJ and for locomotor activity: SM/J and CAST/EiJ>SEA/GnJ, BALB/cByJ, C57BL/6J, C57BL/10J, and BALB/cJ>A/J. Lastly, Mann–Whitney U found no relationship between baseline immobility values and the response to chronic citalopram treatment between the strains.

Effects of subchronic citalopram treatment

The effects of subchronic (~1 week) treatment with 20 mg/kg/day citalopram on FST behavior are shown in Fig. 7. SEA/GnJ and A/J strains showed antidepressant-like effects of subchronic citalopram treatment. SEA/GnJ mice showed reduced immobility [F(1, 25)=3.28; p<0.05] and increases in swimming [F(1, 25)=4.63; p< .05]. A/J mice showed reduced immobility [F(1, 26)=5.24; p<0.05] with no changes in swimming or climbing. Subchronic citalopram treatment did not alter FST behavior in BALB/cJ, or BALB/cByJ mice. Three of 114 mice total had mean immobility values greater or less than 2 standard deviations from the mean and were removed from analysis. Specifically, one outlier was removed from each of the following groups: A/J, 20 mg/kg/day; SEA/GnJ, 0 mg/kg/day; and SEA/GnJ, 20 mg/kg/day.

No effects of subchronic citalopram treatment were found on FST behavior during the pretest (Fig. 8). Several strains

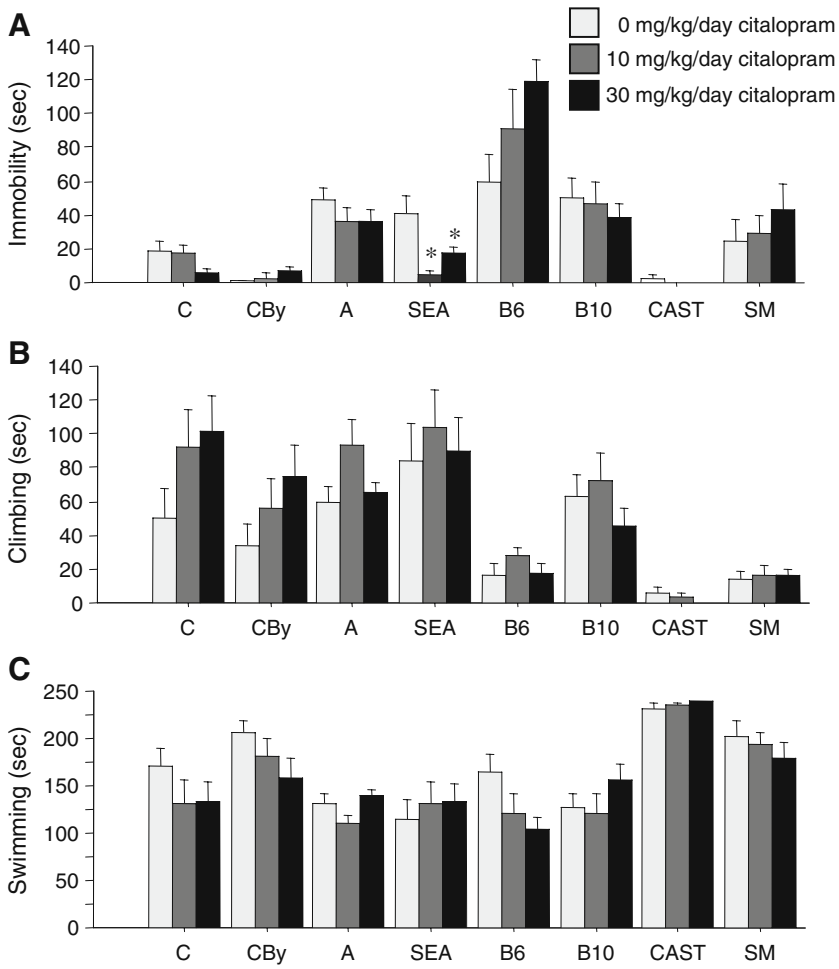

Fig. 3 Pretest data for the FST are shown for mice receiving ~4 weeks of treatment with citalopram. **a** Total immobility, **b** total climbing, and **c** total swimming are shown. Values are means±SEM. *Asterisk* indicates significant difference from control-treated animals within the same strain

showed increases in immobility from the pretest (day 1) to test day (day 2; Fig. 9). In control-treated animals, ANOVA found a main effect of strain [$F(3, 46)=18.50$; $p<0.0001$] and an interaction of day and strain [$F(3, 46)=3.76$; $p<0.01$]. Post hoc tests revealed that control-treated BALB/cByJ mice showed significantly higher immobility values on test day compared to pretest day [$F(1, 14)=10.42$; $p<0.01$]. However, control-treated BALB/cJ mice showed the opposite effect with higher immobility on pretest day [$F(1, 11)=4.81$; $p<0.05$]. No effects of chronic citalopram on FST behavior during the pretest were found (Fig. 3).

Subchronic citalopram treatment significantly altered locomotor activity only in BALB/cByJ mice [$F(1, 27)=4.55$; $p<0.05$]. No effects of subchronic citalopram treatment on locomotion were found in BALB/cJ, A/J, or SEA/GnJ mice (Fig. 7).

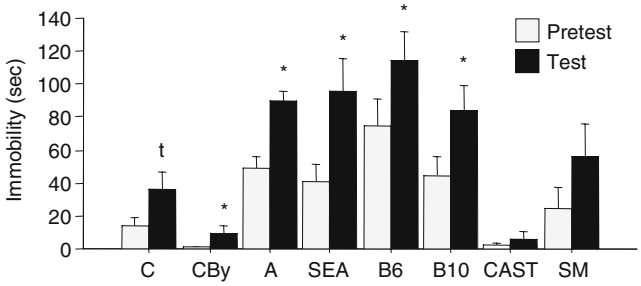

Fig. 4 Immobility values during pretesting and testing following chronic control treatment are shown. Values are means±SEM. *Asterisk* indicates significant difference from pretesting within the same strain

Plasma citalopram levels

Mean plasma citalopram levels for the eight strains ranged from 5.9 to 100.64 nM for the 10-mg/kg/day dose and 3.5 to 1,441.7 nM for the 30-mg/kg/day dose (Table 1). For either dose of citalopram, Mann–Whitney *U* found no relationship between plasma citalopram levels and the antidepressant-like response to chronic drug treatment in the FST.

C1473G genotype in eight inbred strains

We found that the closely related BALB/cJ, BALB/cByJ, A/J, and SEA/GnJ strains all carry the 1473G allele, while

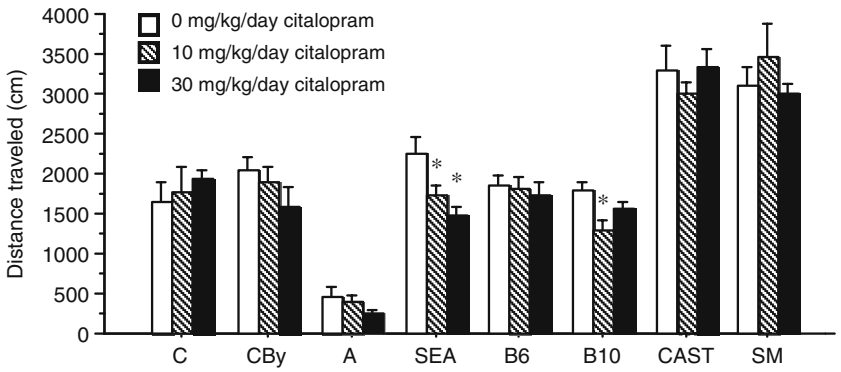

Fig. 5 Total distance traveled in the open field is shown for eight inbred mouse strains treated chronically with 0, 10, or 30 mg/kg/day citalopram. Values are means±SEM. * indicates significant difference from control-treated animals within the same strain

the distantly related C57BL/6J, C57BL/10J, CAST/EiJ, and SMJ strains all carry the 1473C allele of Tph2 (Table 2).

Discussion

Here, we found that BALBc/J and the closely related BALB/cByJ, A/J, and SEA/GnJ strains show antidepressant-like responses to chronic (~4 weeks) citalopram treatment in the FST, while the distantly related C57BL/6J, C57BL/10J, SM/J, and CAST/EiJ strains do not. Furthermore, the BALB/cJ and BALB/cByJ strains showed no antidepressant response to subchronic (~1 week) citalopram treatment, and SEA/GnJ mice exhibited an attenuated response. Citalopram-induced changes in motor activity did not underlie observed behavioral effects in the FST. Baseline levels of immobility were unrelated to the antidepressant response to chronic citalopram treatment in the FST and baseline levels of locomotion in the open field. We also found that the citalopram-responsive strains carry the lower-functioning 1473G allele in Tph2, while the citalopram-nonresponsive strains carry the common 1473C allele. The present findings suggest that BALB/cJ and related strains exhibit an antidepressant to chronic SSRI treatment with a time course that mimics the clinical therapeutic response, and this behavioral trait is likely heritable.

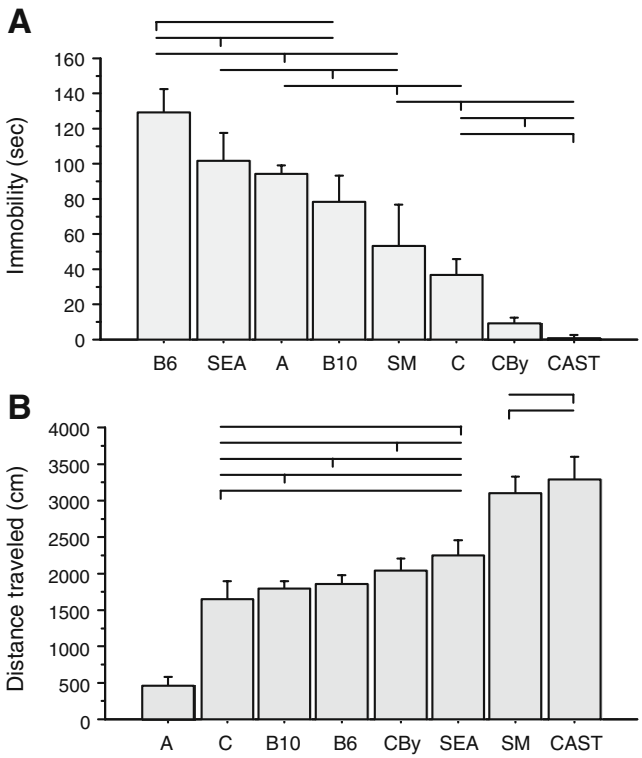

Fig. 6 Baseline values for immobility in the FST (**a**) and total distance traveled in the open field (**b**) for eight inbred mouse strains. Values are means±SEM. *Lines* indicate strains that do not differ statistically significantly from one another

Animal models exhibiting sensitivity to antidepressant drugs and their time course of therapeutic action represent powerful and much needed tools for studying the mechanism of action of antidepressants. Few animal models sensitive to chronic, but not short-term, antidepressant treatment exist that are reliable and easy to use (Berton and Nestler 2006; Cryan et al. 2002). We previously reported that BALB/cJ mice respond to chronic, but not subchronic, treatment with fluoxetine in the open field, novelty-induced hypophagia test, and FST (Dulawa et al. 2004). Here, we have identified three additional strains that respond to chronic treatment with an SSRI in the FST: BALB/cByJ, A/J, and SEA/GnJ. This effect was the weakest in the A/J strain (p=0.06), for which planned post hoc comparisons revealed significant effects of chronic citalopram. While the BALB/c and BALB/cByJ substrains have been suggested to be nearly identical genetically (Petkov et al. 2004), we previously identified several DNA copy number variants and large phenotypic differences between these two substrains (Velez et al. 2009). The BALB/cJ and BALB/cByJ strains did not exhibit an antidepressant-like response to subchronic (~1 week) citalopram treatment in the FST (Fig. 7). SEA/GnJ mice responded to subchronic citalopram treatment, although this response was smaller in magnitude compared to the robust

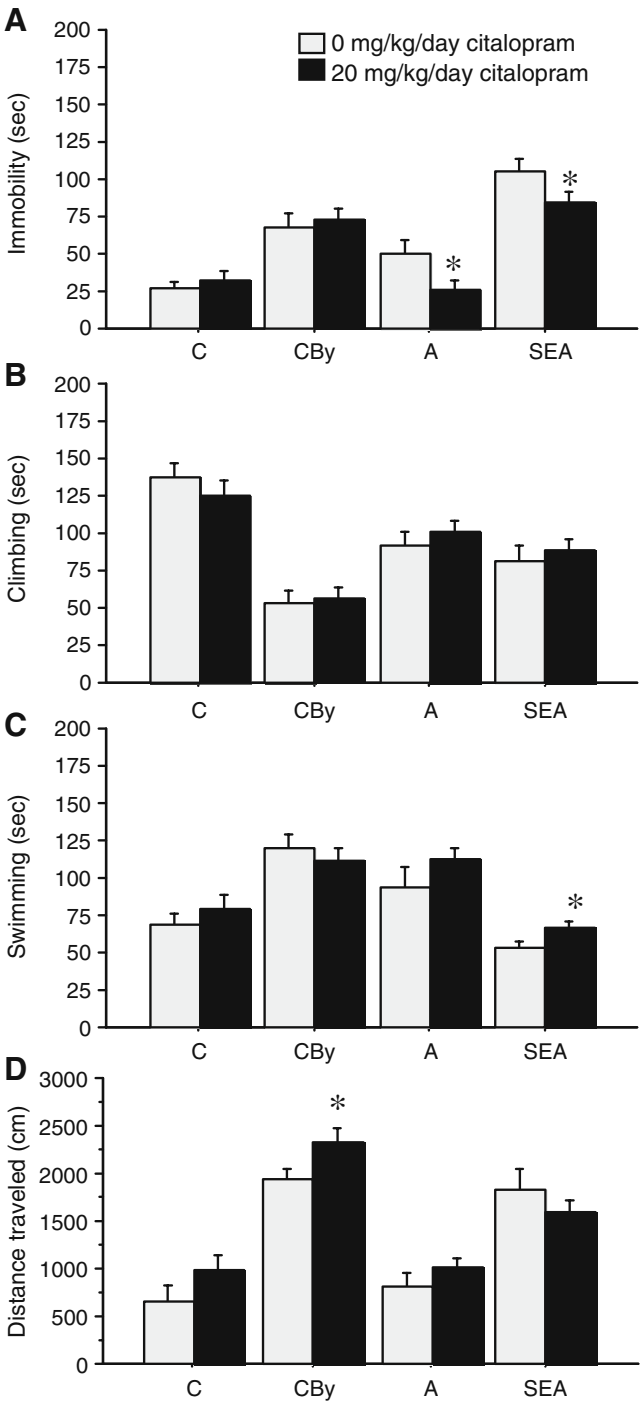

Fig. 7 Effects of subchronic treatment with citalopram on FST behavior. **a** Total immobility, **b** total climbing, and **c** total swimming are shown for BALB/cJ (C) (n=29), BALB/cByJ (CBy) (n=30), A/J (A) (n=28), and SEA/GnJ (SEA) (n=27) mice. **d** Total locomotion in the open field is shown for the same experimental animals. Values are means±SEM. *Asterisk* indicates a significant difference from control-treated animals within the same strain

response observed following chronic treatment (Fig. 2). A/J mice showed small reductions in immobility following subchronic and chronic citalopram treatment that were similar in magnitude. BALB/cJ, BALB/cByJ, and SEA/GnJ mice show an antidepressant-like response to citalopram that mimics the time course of therapeutic action observed in depressed patients. Thus, these strains provide useful tools to study the mechanisms of SSRIs and their therapeutic onset.

The present studies also identify additional inbred strains that do not respond to chronic citalopram treatment: C57BL/6J, C57BL/10J, SM/J, and CAST/EiJ. We previously reported that C57BL/6J, DBA/2J, and 129S6/SvEv-Tac mice do not respond to chronic fluoxetine treatment in the FST (Dulawa et al. 2004). However, our negative findings with the CAST/EiJ strain in the present studies should be interpreted with caution. This strain showed virtually no immobility in the FST (Fig. 2). This floor effect could obscure any potential reduction in immobility induced by citalopram treatment.

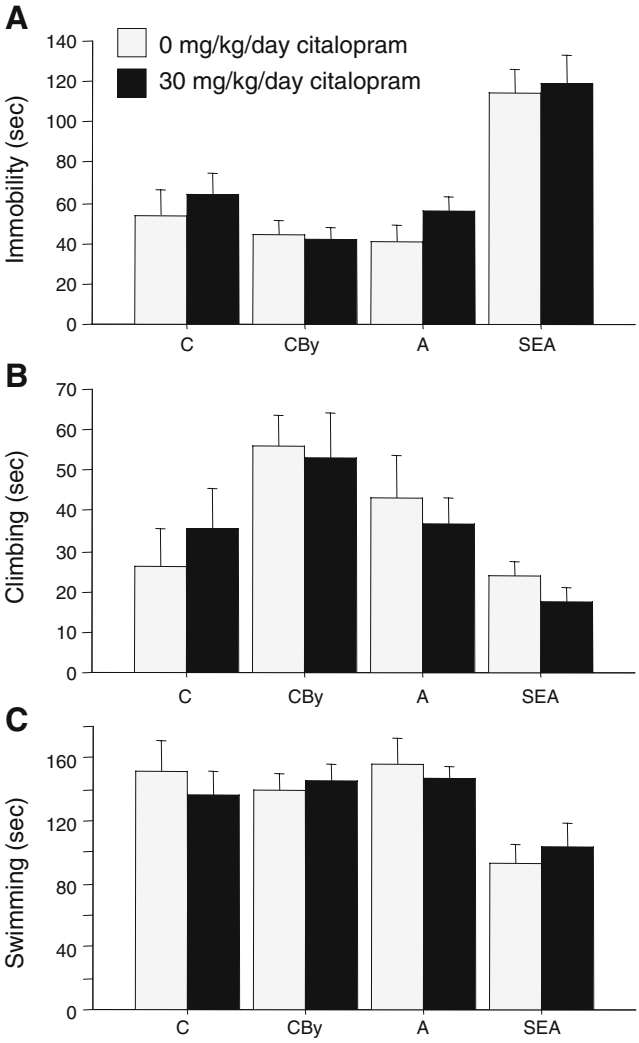

Fig. 8 Pretest data for the FST are shown for mice receiving subchronic treatment with citalopram. **a** Total immobility, **b** total climbing, and **c** total swimming are shown. Values are means±SEM. *Asterisk* indicates significant difference from control-treated animals within the same strain

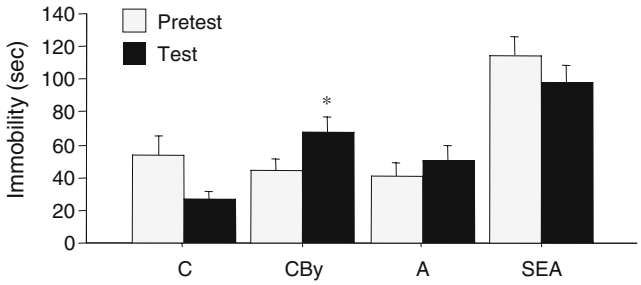

Fig. 9 Immobility values during pretesting and testing following subchronic control treatment are shown. Values are means±SEM. *Asterisk* indicates significant difference from pretesting within the same strain

SSRIs have been reported to increase swimming, but not climbing, behavior in the FST, while noradrenaline reuptake inhibitors have been reported to increase climbing, but not swimming behavior (Detke et al. 1995, 1997). These results have been observed following either acute or chronic treatment with antidepressants in rats (Detke et al. 1997). Our present finding that chronic citalopram treatment significantly increased climbing in BALB/cByJ mice (Fig. 2) conflicts with these reports. Additionally, chronic treatment with the SSRI fluoxetine has been reported to increase swimming behavior in the closely related BALB/cJ mice (Dulawa et al. 2004; Holick et al. 2008). Further studies may be required to resolve this inconsistency.

None of the antidepressant effects of citalopram in the FST resulted from nonspecific increases in motor activity. Although chronic citalopram treatment either reduced immobility or increased climbing behavior in BALB/cJ, BALB/cByJ, A/J, and SEA/GnJ mice (Fig. 2), no increases in locomotor activity were found in the open field in these strains (Fig. 5). In fact, both doses of chronic citalopram treatment reduced locomotion in SEA/GnJ mice. Additionally, there was no relationship between baseline locomotor activity and baseline immobility between the strains (Fig. 6). Following subchronic citalopram treatment, SEA/GnJ mice exhibited small magnitude reductions in immobility and increases in swimming behavior, but no changes in locomotor activity were found (Fig. 7). Thus, FST behavior was independent from general motor activity as assessed by locomotion in the open field in the present studies.

FST studies using mice usually do not include a pretest (Bourin et al. 2005; Lucki et al. 2001; Porsolt et al. 1977), while rat FST studies have typically used a pretest 24 h before testing (Cryan et al. 2002; Porsolt et al. 1977; but see Cryan et al. 2005). Pretesting is performed in rats to induce more immobility, which increases with exposure to FST conditions. Pretesting thus increases sensitivity for detecting the effects of antidepressants such as reduced immobility and/or increases in active behaviors. It is widely thought that only one FST exposure is sufficient to generate an increased and stable immobility readout in mice (Cryan et al. 2002), making pretesting unnecessary. However, our present results show that immobility is significantly increased on test day relative to pretest day in C57BL/6J, C57BL/10J, A/J, BALB/cByJ, and SEA/GnJ mice (Fig. 4). CAST/EiJ and SM/J mice did not show significant increases in immobility after pretesting. BALB/cJ mice showed a strong trend for increased immobility following pretesting in the chronic study, but showed the opposite effect in the subchronic study; the reasons for this inconsistency are unclear. Regardless, the majority of mouse strains tested showed increased immobility on test day relative to pretest day. Thus, the effects of pretesting are strain dependent in mice, and several commonly used inbred strains show increased immobility following pretesting. Furthermore, we found antidepressant effects of chronic citalopram treatment in SEA/GnJ mice during pretesting and testing, but identified antidepressant effects in A/J, BALB/cJ, BALB/cByJ, and SEA/GnJ mice only during testing. These findings suggest that pretesting increases sensitivity for detecting antidepressant effects in mice.

Plasma citalopram levels were highly variable in the present studies, but largely fell within the range found in depressed patients taking therapeutic doses. Thera-

Table 1 Mean plasma citalopram levels (nanomolar)±SEM following chronic citalopram treatment

Strain	10 mg/kg/day	30 mg/kg/day
C	53.8±45.2	1,441.7±1,041.7
CBY	100.6±63.2	39.5±7.0
A	8.4±8.4	3.5±2.2
SEA	12.3±7.6	49.3±28.4
B6	10.8±1.5	130.0±58.9
B10	5.9±1.3	276.3±185.6
CAST	95.7±52.5	264.0±32.4
SM	30.1±22.5	88.8±14.9

Table 2 C1473G genotype and response to chronic citalopram in eight inbred mouse strain

Strain	Genotype	Response
C	G	Y
CBY	G	Y
A	G	Y
SEA	G	Y
B6	C	N
B10	C	N
CAST	C	N
SM	C	N

peutic doses of citalopram range from 10 to 60 mg/day for depressed patients (Bech et al. 2002). Patients receiving 30–60 mg/day exhibit mean plasma citalopram levels of 28–616 nM (Dufour et al. 1987; Fredricson Overo 1982a; Pedersen et al. 1982). We administered 10–30-mg/kg/day citalopram to mice since 24-mg/kg/day citalopram results in plasma levels toward the middle of this range in mice (383 nM; Honig et al. 2009). Mean plasma levels fell within this range for many of the strains tested (Table 1), although the C57BL/6, C57BL/10, A/J, and SEA/GnJ strains had lower plasma levels at the 10-mg/kg/day dose, as did A/J mice at the 30-mg/kg/day dose. However, citalopram plasma levels below 28 nM likely result from therapeutic doses in humans, since citalopram doses as low as 10 mg/day treat depression (Bech et al. 2002). Similarly, SEA/GnJ and A/J mice had plasma levels below 28 nM (Table 1) and responded chronic citalopram treatment (Fig. 2). The variability in plasma levels observed likely resulted from the administration of citalopram via the drinking water. The half-life of citalopram in plasma is 1.5 h in mice (Fredricson Overo 1982b), which drink in bouts across the circadian cycle (Gannon et al. 1992). Finally, the lack of response to subchronic citalopram treatment (Fig. 7) did not result from lower plasma citalopram levels because steady-state levels of citalopram are achieved in less than 2 days (Honig et al. 2009).

No correlation was found between plasma citalopram levels and the response to chronic citalopram treatment between the strains tested. This lack of relationship between plasma levels and behavioral response likely reflects the importance of other factors besides drug metabolism in determining the antidepressant response to citalopram. Differences in percent 5-HTT occupancy between the strains are also unlikely to explain the observed differences in behavioral response. In humans, therapeutic doses of citalopram (10–60 mg/day) and other SSRIs result in mean 5-HTT occupancies of approximately 75–80% across doses (Erlandsson et al. 2005; Meyer et al. 2001). To our knowledge, the relationship between and citalopram plasma levels and 5-HTT occupancy in mice has not been reported. However, the predicted steady-state plasma concentrations for the SSRIs escitalopram, paroxetine, and sertraline at 80% 5-HTT occupancy are approximately 43–58 nM in male NMRI/BOM mice (Kreilgaard et al. 2008). Each citalopram nonresponsive strain identified here had plasma citalopram levels above this range for at least one dose (Table 1).

The closely related BALB/cJ, BALB/cByJ, A/J, and SEA/GnJ inbred strains share many genomic regions (Petkov et al. 2004). It is therefore likely that polymorphisms shared among these strains underlie their behavioral sensitivity to chronic citalopram treatment. Forward genetic strategies could be used to attempt to identify the genetic variants responsible for this phenotype. Here, we determined genotype at the C1473G allele in Tph2 in the eight inbred strains for several reasons: (1) variants in the Tph2 gene have been associated with the SSRI response in humans (Tsai et al. 2009; Tzvetkov et al. 2008), although further studies will be required to corroborate these findings, and (2) we hypothesized that serotonin deficiency in the 1473G-carrying strains may confer sensitivity to chronic SSRI treatment. Consistent with our hypothesis, we found that the citalopram-responsive BALB/cJ, BALB/cByJ, A/J, and SEA/GnJ strains carry the lower-functioning 1473G allele, while the citalopram-nonresponsive strains C57BL/6J, C57BL/10J, SM/J, and CAST/EiJ carry the higher-functioning 1473C allele. Thus, our present findings identify a correlation between the C1473G allele and response to chronic SSRI treatment. Our findings differ from previous reports suggesting that 1473C-carrying strains (C57BL/6J and 129/Sv) respond to acute SSRI injection in the FST, while 1473C-carrying strains (BALB/c and DBA/2J) do not (Cervo et al. 2005; Guzzetti et al. 2008), although these effects have not always been replicated (Lucki et al. 2001). These opposing findings are consistent with the idea that the behavioral response to acute vs. chronic SSRI treatment in the FST requires different mechanisms (Holick et al. 2008). Although our present results do not causally link the 1473G allele with the response to chronic SSRI treatment, they suggest that this allele and not the 1473C allele might contribute to the therapeutic effects of SSRIs. To determine whether the 1473C allele actually confers sensitivity to chronic citalopram treatment, controlled studies will be required in which the behavioral response to citalopram is evaluated using knockin mice carrying the 1473G or 1473C alleles on the same genetic background. Additionally, the C1473G variant in Tph2 is found in mice, but not humans. The corresponding human variant in Tph2, C1487G, has never been reported in a clinical population to our knowledge (Garriock et al. 2005). Regardless, the murine C1473G variant can be used to assess the effects of variation in Tph2 function on various phenotypes including the response to chronic SSRI treatment.

In conclusion, we show that the closely related BALBc/J, BALB/cByJ, A/J, and SEA/GnJ inbred mouse strains exhibit an antidepressant-like response to chronic citalopram treatment. In BALBc/J, BALB/cByJ, and SEA/GnJ mice, this response mimics the time course of action of SSRIs in depressed patients. Shared genetic factors likely underlie the sensitivity of these related strains to chronic SSRI treatment. We also found that these citalopram-response strains carry the lower-functioning 1473G allele in Tph2, while the nonresponsive strains C57BL/6J, C57BL/10J, SM/J, and CAST/EiJ carry the higher-

functioning 1473C allele. Future studies will be required to identify the genetic variants conferring sensitivity to chronic SSRI treatment in these strains.

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Conflict of interest None of the authors reported biomedical financial interests or potential conflicts of interest.

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