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Brain Monoamines and Antidepressant-like Responses in MRL/MpJ Versus C57BL/6J Mice

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Abstract

The MRL/MpJ mouse demonstrates enhanced wound healing and tissue regeneration and increased neurotrophic mobilization to chronic antidepressant drug treatments. This study compared brain monoamine systems between MRL/MpJ and C57BL/6J mice as a potential basis for strain differences after chronic antidepressant treatment. MRL/MpJ mice had significantly higher tissue levels of serotonin and dopamine in multiple brain regions. Microdialysis studies demonstrated that baseline levels of extracellular serotonin did not differ between strains. However, acute administration of the selective serotonin reuptake inhibitor citalopram produced an increase in extracellular serotonin in the ventral hippocampus of MRL/MpJ mice that was twice as large as achieved in C57BL/6J mice. The greater effects in MRL/MpJ mice on 5-HT levels were not maintained after local perfusion of citalopram, suggesting that mechanisms outside of the hippocampus were responsible for the greater effect of citalopram after systemic injection. The density of serotonin and norepinephrine transporters in the hippocampus was significantly higher in MRL/MpJ mice. In addition, the expression of 5-HT_{1A} mRNA was lower in the hippocampus, 5-HT_{1B} mRNA was higher in the hippocampus and brain stem and SERT mRNA was higher in the brain stem of MRL/MpJ mice. The exaggerated neurotransmitter release in MRL/MpJ mice was accompanied by reduced baseline immobility in the tail suspension test and a greater reduction of immobility produced by citalopram or the tricyclic antidepressant desipramine. These data suggest that differences in the response to acute and chronic antidepressant treatments between the two strains could be attributed to differences in serotonin or catecholamine transmission.

Keywords

C57BL/6J; MRL/MpJ; citalopram; desipramine; tail suspension test; serotonin; norepinephrine; *in vivo* microdialysis; hippocampus

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1. Introduction

MRL/MpJ (MRL) mice are an inbred strain known to display extraordinary wound healing and dramatic tissue regeneration in response to different types of injury when compared to most other mouse strains, such as C57BL/6J (B6) mice (Clark et al., 1998; Leferovich et al., 2001). Recently, another set of phenotypic differences between MRL and B6 mice were identified, including enhanced responsiveness to the proneurogenic, neurotrophic and behavioral effects of chronic antidepressant treatments (Balu et al., 2009; Hodes et al., 2010). Similarly, MRL mice showed a greater increase of hippocampal cell proliferation than B6 mice after chronic wheel running that lead to improved spatial memory (Thuret et al., 2009). There are a number of reasons for examining genetic variations and physiological mechanisms common between tissue repair mechanisms and the neurotrophic response to antidepressant treatments. Major depressive disorder has been associated with evidence for tissue injury in the brain, especially the hippocampus (MacQueen and Frodl, 2011; Sheline et al., 1999) and with cytokines, which mediate neuroinflammation (Capuron and Miller, 2011). Finally, chronic antidepressant treatments augment mechanisms involved in neuronal plasticity in the hippocampus, such as neurotrophins and neurogenesis, that can counteract these pathological features of depression (Duman and Monteggia, 2006; Wager-Smith and Markou, 2011).

A primary pharmacological effect of most antidepressants is their alteration of serotonin (5-HT) and noradrenergic transmission by inhibiting uptake mechanisms by their respective presynaptic transporters (Frazer, 1997; Sanchez and Hyttel, 1999). 5-HT and norepinephrine (NE) are known mediators of the inflammatory and cell proliferative stages of wound healing (Gosain et al., 2006; Malinin et al., 2004) and produce similar increases in neurotrophic mobilization and hippocampal neurogenesis following chronic administration of antidepressants (Balu and Lucki, 2009; Vaidya et al., 2007). Various behavioral tests in rodents are also used to evaluate compounds for potential antidepressant activity (Crowley and Lucki, 2005). One such paradigm, the tail suspension test (TST), has been shown to be sensitive to numerous antidepressants from different pharmacological classes, including those that augment 5-HT and NE transmission (Steru et al., 1985) and therefore has good predictive validity (Cryan et al., 2005). However, inbred and outbred mouse strains show large differences of performance on the TST (Crowley et al., 2005; Cryan et al., 2005).

The purpose of this study was to examine whether endogenous variations in brain monoamine systems between MRL and B6 mice could contribute to any phenotypic differences observed in their response to acute and chronic treatment with antidepressants. Therefore, tissue monoamine content and monoamine transporter densities and gene expression were compared between strains and in vivo microdialysis was used to confirm functional differences. Behaviorally, MRL and B6 mice were also compared in the TST to acute administration of two pharmacologically distinct antidepressant drugs. These studies established endogenous differences in monoamine transmission between MRL and B6 mice that could mediate their behavioral phenotypes and pharmacological sensitivity to antidepressant drugs.

2. Materials and methods

2.1. Animals

Adult male B6 and MRL (Jackson Laboratories, Bar Harbor, ME, USA) were 8-10 weeks old at the beginning of all studies. The animals were housed in groups of five in polycarbonate cages and maintained on a 12-h light/dark cycle (lights on at 07:00 hours) in a temperature (22°C)- and humidity-controlled colony. The animals were given free access to food and water. Animal procedures were conducted in accordance with the guidelines

published in the NIH Guide for Care and Use of Laboratory Animals and all protocols were approved by the University of Pennsylvania Institutional Animal Care and Use Committee.

2.2. Tissue monoamine analysis

Mice were decapitated and their brains quickly removed for dissection. The hippocampus, frontal cortex, amygdala, and brain stem were dissected, flash frozen and stored at -80°C until preparation for analysis using high performance liquid chromatography (HPLC) with electrochemical detection.

Tissue samples were homogenized in 0.1 N perchloric acid with 100 μM EDTA (15 $\mu\text{l}/\text{mg}$ of tissue) using a tissuemizer (Tekmar, Cleveland, OH, USA). Samples were centrifuged at 15,000 rpm (23,143 g) for 15 min at $2-8^{\circ}\text{C}$. The supernatants were filtered using Costar Spin-XTM centrifugal filters (Fisher Scientific, Pittsburgh, PA, USA) and then split into two aliquots. Samples (12 μl) were injected by an autosampler (Sample Sentinel, Bioanalytical Systems, West Lafayette, IN, USA) and analyzed in separate assays for tissue content of 5-HT, 5-hydroxyindoleacetic acid (5-HIAA) and dopamine (DA) or for NE.

The HPLC separation for 5-HT consisted of a PM80 solvent delivery system and a 10 μl sample loop linked in series to a reversed phase microbore column (ODS 3 μm , $100^{\circ}-1$ mm; Bioanalytical Systems). The mobile phase for the separation of 5-HT consisted of 12.42 mM citric acid (Sigma), 39.85 mM NaPO_4 monobasic (Fluka, Buchs SG, Switzerland), 0.25 mM EDTA (Fluka), 0.737 mM 1-decanesulfonic acid (Sigma), 10 mM NaCl (Fluka), 0.2% triethylamine (Sigma), 16.5% methanol (Fisher Scientific) adjusted to a pH of 4.1. The flow rate through the system was 60 $\mu\text{l}/\text{min}$, and the detector was set at a potential of +0.60 V relative to a Ag/AgCl reference electrode.

The HPLC separation for NE and DA consisted of a PM80 solvent delivery system and a 10- μl sample loop linked in series to a reversed phase microbore column (ODS 5 μm , $150^{\circ}-1$ mm; Bioanalytical Systems). The mobile phase for the separation of NE and DA consisted of 14.5 mM NaH_2PO_4 (Fluka), 30 mM sodium citrate (Fluka), 27 μM disodium EDTA (EMD Chemicals, Gibbstown, NJ, USA), 10 mM diethylamine HCL (Sigma), 1.95 mM 1-decanesulfonic acid (Sigma), 8% acetonitrile (Fisher Scientific), 1% tetrahydrofuran (Fluka) adjusted to pH 3.4. Mobile phase was pumped through the system at 80 $\mu\text{l}/\text{min}$, and the detector was set at a potential of +0.65 V relative to a Ag/AgCl reference electrode.

Standard concentrations of 5-HT, NE and DA were prepared before injection of tissue samples. Tissue concentrations of monoamines were determined using a linear regression analysis of the peak heights obtained from a range of standards and expressed as pg/mg tissue.

2.3. Microdialysis procedures

Microdialysis probes were custom-made and surgically implanted as described previously (Knobelman et al., 2000). Mice were anesthetized with chloral hydrate (400 mg/kg, i.p.). The probe was implanted in the ventral hippocampus at the following coordinates: AP -2.8, ML $\pm$ 3.2, and DV -5.0 mm from bregma (Franklin and Paxinos, 1997) using a stereotaxic instrument. Following surgery, the mice were placed into a 21.5 cm high, clear polycarbonate cylindrical *in vivo* microdialysis apparatus with a counterbalance arm holding a liquid swivel (Instech Laboratories, Plymouth Meeting, PA) and allowed to recover overnight.

Microdialysis experiments started 17–20 h after surgery. Dialysate samples were collected into polypropylene microcentrifuge vials at 20-min intervals. Four fractions were collected to measure baseline values before systemic administration of citalopram (20 mg/kg).

Samples were collected for three additional hours after systemic drug challenge to compare the effects of fluoxetine on extracellular 5-HT levels between B6 and MRL mice. To examine the effects of citalopram given directly into the hippocampus, the perfusion media was changed from aCSF to citalopram (1 mM) after the collection of baseline samples using a liquid switch. Samples were stored at -80°C until analyzed using a Bioanalytical Systems 460 High Pressure Liquid Chromatograph by a BAS Sample Sentinel Refrigerated Microsampler set to a 12 µl injection volume as described previously (Kreiss and Lucki, 1994). The 5-HT from chromatographs of dialysate samples were identified by comparing their elution times with those of reference standards. The amount of 5-HT in each dialysate sample was quantified from their respective peak heights using a linear regression analysis of the peak heights obtained from a series of reference standards.

At the completion of the experiment, brains were removed, placed in cold isopentane and frozen at -80°C. The brains were then sectioned (35 µm) with a refrigerated cryostat, stained with Neutral Red, and the tissue examined for the location of the dialysis probe. No animals were excluded for improper probe placement.

2.4. Radioligand binding for SERT and NET

Binding methodology was adapted from previous studies (Dewar et al., 1991; Tejani-Butt, 1992). Tissues were homogenized in 50 mM Tris buffer (pH 7.4 at 24° C) containing 400 mM NaCl and 5mM KCl and centrifuged twice at $35,000 \times g$ for 10 minutes in fresh buffer. The homogenates were added to tubes containing 10 nM [3H]nisoxetine or 300 pM [3H]paroxetine for measurements of the NE transporter or the 5-HT transporters, respectively. Incubations were carried out for 4 h at 4° C and bound transporters were separated from free ligand by filtration over Whatman GF/C filters wet with 0.5% polyethylenimine and mounted on a Brandell Cell Harvester (Gaithersburg, MD). The filters were then counted in a liquid scintillation counter. Nonspecific binding to the NE or 5-HT transporter was determined in the presence of 10 µM desipramine or 30 µM citalopram, respectively, and specific binding was defined as the difference between total binding and nonspecific binding.

2.5. Quantitative real-time PCR

Mice were sacrificed and the hippocampal tissue was dissected from the surrounding cortex by sight. For sections of the brainstem, including the raphe nuclei, the whole brain was extracted and placed immediately in a mouse brain block (Kopf Brain Blocker, Ireland) on ice. Tissue slices through the brainstem were obtained and a round 1 mm punch taken from the region containing the raphe and stored immediately at -70 degrees until use. RNA was isolated using the RNAqueous-4PCR kit for isolation of DNA-free RNA (Ambion, Applied Biosystems, Austin, TX) following the manufacturer's instructions. 500 ng of RNA was used to synthesize cDNA using the Superscript Vilo cDNA synthesis kit (Invitrogen, Carlsbad, CA). All reactions were performed using a master mix of SYBR green (Applied Biosystems, Austin, TX) and 300 nM OligoDt primers (final concentration, Operon, Huntsville, AL). Reactions (25 µl) were run using the Stratagene MX3000 and MXPro QPCR software. Cycling parameters were 95° C for 10 min, followed by 40 cycles of 95° C (30 sec) and 60° C (1 min), followed by a melting curve analysis. Reactions were performed in triplicate and the median cycle threshold was used for analysis and normalized to TATA binding protein (TBP). Primers for SERT: Forward 5'-GCTGAGCTGACTTGGATA-3', Reverse 5'-ACAGACGTTACAGACCTAA-3'. Primers for 5-HT_{1A}: Forward 5'-CTGTTTATCGCCCTGGATG-3', Reverse 5'-ATGAGCCAAGTGAGCGAGAT-3'. Primers for 5-HT_{1B}: Forward 5'-TTCTTCATCATCTCCCTGGTG-3', Reverse 5'-AGCGTATCAAGTTTGTGGACG-3'.

2.6. Tail suspension test

The tail suspension test (TST) was conducted using procedures described previously that measured the response to antidepressant drugs (Crowley et al. 2005). Mice were suspended by their tail with tape to a vertical aluminum bar connected to a strain gauge for 6 min. Mice were positioned so that the base of their tail was aligned with the bottom of the bar in order to decrease the propensity for mice to climb their tail during the test, particularly with B6 mice (Mayorga and Lucki, 2001). The TST measured the duration of behavioral immobility using an automated device to measure movements by the mouse over time (Med Associates, St. Albans, VT). The total duration of immobility was calculated as the time the force of the mouse's movements was below a preset threshold.

Mice were administered 0.9% NaCl (saline), desipramine HCl (Sigma St. Louis MO; 10, 20 mg/kg, n=10/group), or citalopram hydrobromide (Anawa Zurich; 10, 20 mg/kg, n=10/group) by intraperitoneal injection 30 min prior to all behavioral testing. The doses were calculated according to the base weight of the drug and injected in a volume of 10 ml/kg.

2.7. Locomotor activity

Animals were placed in empty mouse cages with no bedding for 30 min. Their behavior was videotaped from above. Digital video output was analyzed by an IBM-compatible computer running SMART II Video Tracking System software (San Diego Instruments). Locomotor activity was defined as the distance traveled over the 30-min test period. Saline, fluoxetine or desipramine were administered 30 min prior to behavioral testing. This procedure is sensitive to measuring locomotor activity increased by the administration of d-amphetamine (unpublished data).

2.8. Elevated zero maze

The zero maze (Stoelting, Wood Dale, IL) was elevated 24 in from the ground and consisted of two open areas (wall height, 0.5 inches) and two closed areas (wall height, 12 in). Mice were acclimated to the room for 1 h before testing. At the start of the test, each mouse was placed in the closed area. The duration of testing was 5 min. The Viewpoint Tracking System (Viewpoint, Champagne au Mont d'Or, France) was used to video record and measure the time spent in the open areas and the number of entries into each area. The test was performed in dim lighting.

2.9. Statistical analysis

Tissue content of monoamines and metabolites and elevated zero maze data were compared between strains using unpaired two-tailed Student's t-test. Immobility times and distances traveled, between saline-injected controls and antidepressant-treated animals for each strain, were compared using one-way analysis of variance (ANOVA). For microdialysis studies, baseline values were defined as the 3 values obtained immediately prior to injection or infusion. Two-way ANOVA with repeated measures over time was used to compare extracellular 5-HT levels between mouse strains. Dunnett *post-hoc* analysis was used to compare time points after injection or infusion to baseline, and Dunn's multiple range test to compare values between strains. Values of $p < 0.05$ were considered significant.

3. Results

3.1. Monoamine and catecholamine tissue content in B6 and MRL mice

The tissue content of 5-HT, 5-hydroxyindoleacetic acid (5-HIAA), NE, and DA from several brain regions associated with depression was measured by HPLC (Table 1). MRL mice had significantly higher tissue levels of 5-HT than B6 mice in the hippocampus (25%; $t(15) =$

2.48, $p = 0.03$), frontal cortex (35%; $t(15) = 5.01$, $p < 0.001$), amygdala (30%; $t(15) = 4.36$, $p < 0.001$), and brain stem (20%; $t(15) = 2.78$, $p = 0.01$). MRL mice also had significantly higher levels of the major 5-HT metabolite, 5-HIAA, than B6 mice in the frontal cortex (60%; $t(16) = 6.37$, $p < 0.001$), amygdala (55%, $t(16) = 4.93$, $p < 0.001$), and brain stem (25%; $t(15) = 2.24$, $p = 0.04$). The ratio of 5-HIAA/5-HT, which is used as an index of 5-HT turnover, was significantly different between the two strains only in the frontal cortex (B6: 0.28 ± 0.01 , MRL: 0.33 ± 0.01 ; $t(15) = 3.01$, $p = 0.004$), indicating that the differences in content were not due to altered metabolism. MRL mice had significantly higher levels of tissue DA content than B6 mice in the hippocampus (63%; $t(15) = 2.70$, $p = 0.02$), frontal cortex (290%; $t(16) = 2.91$, $p = 0.01$), and brain stem (40%; $t(16) = 3.38$, $p = 0.004$). Finally, there were no differences in NE tissue content in any of the examined brain regions.

3.2. Extracellular 5-HT levels in the hippocampus of B6 and MRL mice following citalopram

Microdialysis was used to measure extracellular levels of 5-HT in the hippocampus at baseline and in response to an acute challenge with the SSRI citalopram (20 mg/kg). Baseline values did not differ significantly between the MRL (0.43 ± 0.07 pg) and B6 (0.54 ± 0.10 pg) strains. Overall ANOVA indicated significant main effects of strain ($F(1,11) = 10.82$, $p < 0.01$), time ($F(11,121) = 22.03$, $p < 0.001$) and interaction between strain and time ($F(11, 121) = 4.55$, $p < 0.001$). Citalopram (20 mg/kg, i.p.) increased hippocampal 5-HT levels dramatically in both strains immediately following the injection (Fig. 1; panel A). The MRL mice showed a significantly greater response to citalopram, increasing 5-HT levels 7-9 times higher than baseline, in contrast to the more modest 3-fold increase shown by B6 mice (Fig. 1; panel A). The significant differences between strains persisted throughout the course of sampling for 3 hours after injection (200-360 min).

In order to determine whether the different responses were due to variables intrinsic to the hippocampus rather than systemic factors, extracellular levels of 5-HT were also measured after the infusion of citalopram (1 mM) directly into the hippocampus through the dialysis probe (Fig. 1, panel B). Baseline values did not differ significantly between the MRL (0.97 ± 0.03 pg) and B6 (0.79 ± 0.07 pg) strains. Overall ANOVA indicated a significant effect of time ($F(11,176) = 17.28$, $p < 0.001$) and interaction between strain and time ($F(11, 176) = 3.07$, $p < 0.001$), and a trend for a significant effect of strain ($F(1,16) = 3.18$, $p = 0.09$). Citalopram infusion increased hippocampal 5-HT levels in B6 mice to about 3-4 fold over baseline achieved after 40 min of infusion and this level was maintained for the duration of the experiment. Citalopram increased hippocampal 5-HT levels to a peak of 7-fold above baseline after 60 min of infusion. After this initial effect, the difference between strains decreased over time, suggesting local adaptation to the higher 5-HT levels in MRL mice. The increase of 5-HT in MRL mice was significantly greater than B6 mice at 2 sampling intervals (240 and 280 min) shortly only after the start of the citalopram infusion.

3.3. 5-HT and NE transporters density in B6 and MRL mice

Homogenate binding with [3 H]paroxetine and [3 H]nisoxetine was used to measure the density of 5-HT and NE transporters in the hippocampus, frontal cortex and brainstem, respectively. As shown in the upper panel of Fig. 2, the MRL mice displayed 30% higher levels of [3 H]paroxetine binding than the B6 mice in the hippocampus ($p = 0.001$), demonstrating a significant increase in the basal amount of 5-HT transporters in this region. Similarly, a 12% significant increase in [3 H]nisoxetine was measured in hippocampal homogenates from MRL mice relative to B6 mice (Fig. 2, lower panel). These effects were region specific in that there were no differences in SERT or NET binding densities in the frontal cortex or the brainstem between the strains.

3.4. Levels of gene expression in B6 and MRL mice

To gain insight into strain differences in gene expression, mRNA levels were measured from the hippocampus (Fig. 3A) and brainstem tissue punches for SERT, 5-HT_{1A}, and 5-HT_{1B} receptors. In the hippocampus, MRL mice showed a significant 66% reduction in 5-HT_{1A} mRNA expression compared to B6 mice ($t(13) = 4.036$, $p < 0.01$, Fig. 3A). In contrast, MRL mice showed a striking 4-fold increase in 5-HT_{1B} mRNA expression compared to B6 mice ($t(14) = 4.058$, $p < 0.01$).

Strain differences in gene expression were also found in the brainstem section (Fig. 3B). In particular, MRL mice showed a two-fold increase of SERT mRNA expression ($t(11) = 6.584$, $p < 0.001$) and a small increase in 5-HT_{1B} receptor expression ($t(30) = 2.773$, $p < 0.01$) compared to B6 mice. In contrast, there was no significant difference in 5-HT_{1A} mRNA expression ($p > 0.05$).

3.5. Behavioral responses of B6 and MRL mice to citalopram

At baseline, MRL mice had a lower level (70-75%) of TST immobility than the B6 mice (Fig 4A). The response of MRL mice to citalopram was much greater than that observed in B6 mice (Fig 4A-4B). Citalopram dose-dependently reduced the time spent immobile in MRL mice by 75% and 85%, respectively ($F_{(2,24)} = 13.26$, $p < 0.001$). In contrast, citalopram produced only a 25% and 20% reduction in immobility at 10 and 20 mg/kg, respectively, in B6 mice ($F_{(2,23)} = 8.62$, $p = 0.002$).

Baseline locomotor activity did not differ significantly between the two strains of mice (Fig. 4C). Citalopram produced elevations of locomotor activity in both strains of mice (Fig. 4C, D). Citalopram dose-dependently increased locomotion in B6 mice by 25% and 45%, respectively ($F_{(2,26)} = 18.83$, $p < 0.001$). However, in the MRL mice, only the 10 mg/kg dose caused a significant elevation (36%) in locomotor activity ($F_{(2,26)} = 6.55$, $p = 0.005$).

3.6. Behavioral responses of B6 and MRL mice to desipramine

A similar pattern of response to the TST between strains was observed following an acute desipramine challenge. Desipramine at doses of 10 and 20 mg/kg reduced immobility in the MRL mice by 65% and 50%, respectively (Fig 5A, B; $F_{(2,36)} = 8.65$, $p < 0.001$). In contrast, B6 mice demonstrated a more subdued response in the TST to desipramine (Fig 5A, B; $F_{(2,25)} = 8.54$, $p = 0.001$). Although the 10 mg/kg dose produced a significant reduction (30%) in immobility, the 15% reduction by 20 mg/kg desipramine did not differ significantly from saline.

Contrary to the effects of citalopram, desipramine caused a dose-dependent reduction in locomotor activity in both strains (Fig 5A, B). In B6 mice, desipramine at 10 and 20 mg/kg reduced locomotor activity by 30% and 40%, respectively ($F_{(2,34)} = 22.20$, $p < 0.001$). In MRL mice, only the higher dose significantly reduced locomotor activity (30%) ($F_{(2,27)} = 5.79$, $p = 0.008$).

3.7. Elevated zero maze

In the elevated zero maze, a test used to measure anxiety-like behavior, there were no differences between the two strains in the amount of time spent in the open areas (B6: 121 ± 19 sec; MRL: 89 ± 24 sec; $t(15) = 0.67$, $p = 0.52$) or in the number of entries to the open areas (B6: 19.7 ± 3.1 ; MRL: 17.4 ± 4.7 ; $t(15) = 0.42$, $p = 0.68$).

4. Discussion

MRL mice demonstrate increased cell proliferation, neurotrophic mobilization and behavioral responses following chronic antidepressant treatments when compared with B6 mice (Balu et al., 2009; Hodes et al., 2010; Thuret et al., 2009). The MRL mouse is well-known for their extraordinary wound healing and tissue regeneration after injury compared with B6 mice (Heber-Katz et al., 2004). This study considered the role of differences in brain monoamines between these strains because most conventional antidepressant drugs produce their principal pharmacological effects by augmenting transmission of brain 5-HT and NE and prolonged depression has also been associated with brain tissue injury and increased neuroinflammation (Wager-Smith and Markou, 2011). MRL mice showed higher levels of 5-HT in all brain regions examined, higher tissue levels of dopamine in the hippocampus, frontal cortex, and brain stem, but no differences in NE content. MRL mice also showed higher levels of SERT and NET in the hippocampus, a brain region associated with antidepressant activity. These widespread differences in brain monoamine concentrations and turnover could underlie the enhanced responsiveness of MRL mice to antidepressant drugs (Balu et al., 2009) and other phenotypes such as wound healing (Gosain et al., 2006; Malinin et al., 2004), in comparison with B6 mice.

To demonstrate directly that antidepressants produced a greater pharmacological effect in MRL mice, the response to the SSRI citalopram on extracellular 5-HT levels in the hippocampus was measured using *in vivo* microdialysis. Systemic administration of citalopram produced a response that was approximately twice as large in MRL mice as compared to B6 mice. Because it is well known that systemic administration of SSRIs produce a maximal increase in extracellular levels of 5-HT of only 2-4 fold in terminal regions in rodents (Bel and Artigas, 1992; Hjorth et al., 2000; Knobelmann et al., 2001b; Malagie et al., 2001), the difference between strains obtained at 20 mg/kg citalopram likely represents a difference in the magnitude of the maximal response to citalopram and not a difference in potency. An increase in SERT binding sites and SERT mRNA in MRL mice may contribute to the strain differences.

Citalopram was perfused locally into the hippocampus to determine whether the strain differences were due to factors intrinsic to the hippocampus. Although the perfusion of citalopram initially increased extracellular 5-HT more in MRL mice, the large difference between strains minimized rapidly with continued perfusion. Thus, different response patterns were produced between systemic and local citalopram. It is possible that compensatory mechanisms (e.g., 5-HT_{1B} terminal autoreceptors; (Knobelmann et al., 2001a)) intrinsic to the hippocampus that regulate 5-HT may have contributed to diminishing the strain differences over time. However, the different response patterns between methods of citalopram administration likely points to mechanisms outside of the hippocampus being responsible for the greater effect of citalopram after systemic injection. Extracellular 5-HT levels in the hippocampus are regulated by 5-HT mechanisms derived from other regions, such as 5-HT_{1A} somatodendritic autoreceptors (Knobelmann et al., 2001b; Knobelmann et al., 2000) or 5-HT_{2C} receptors (Cremers et al., 2007), but also by other neurotransmitters, such as norepinephrine and glutamate (Gobert et al., 1997; Imre et al., 2006). These transmitters mechanisms may contribute to circuitry that enhanced 5-HT transmission in the brain of MRL mice, but were not examined directly in the present study.

Behavioral studies also demonstrated a larger behavioral response to acute citalopram and desipramine in the TST for MRL mice. Although drug-induced increases of locomotor activity can complicate interpretation of this test (Cryan et al., 2005), the alterations in locomotor response to antidepressant treatments did not mirror each other in the two strains. In MRL mice, citalopram dose-dependently reduced immobility by 75% and 85%, while the

10 mg/kg dose increased locomotion by 35% and the 20 mg/kg dose did not significantly alter activity. In B6 mice, both doses of citalopram reduced immobility by approximately 25%, while it dose-dependently increased locomotion by 25% and 47%. The increased locomotor effects of citalopram agree with previously published studies (Brocco et al., 2002; Crowley et al., 2005) and may have contributed to its effects in the TST. On the other hand, desipramine also dose-dependently reduced immobility but reduced locomotion in both strains. Overall, these data suggest that the antidepressant-like effects of citalopram and desipramine in the TST are not correlated with changes in locomotor behavior.

The greater behavioral response of MRL mice to antidepressants that activate 5-HT and NE neurotransmission is consistent with the differences in SERT and NET and 5-HT levels between strains. The antidepressant-like effect of SSRIs, such as fluoxetine or citalopram, is believed to be due to the enhancement of 5-HT transmission because depleting 5-HT by inhibiting synthesis completely blocked the acute antidepressant-like effects of the SSRIs on behavior (Gavioli et al., 2004; O'Leary et al., 2007). The antidepressant effects of NRIs, including desipramine, are due to augmentation of central catecholamine signaling (Cryan et al., 2004; Gavioli et al., 2004; O'Leary et al., 2007). Although no differences in NE tissue content were detected in any of the examined brain regions between strains, it is possible that differences in the increase of extracellular NE in response to desipramine could underlie their behavioral differences. In addition, higher tissue levels of DA suggest that this monoamine could underlie behavioral differences between strains. Extracellular DA levels in the frontal cortex have been shown to be increased by desipramine in rats (Tanda et al., 1994), but it is unclear how this is related to antidepressant behavioral effects.

Baseline immobility values are generally not predictive of the sensitivity of that strain to the behavioral effects of antidepressants in the TST (Crowley et al., 2005). This idea was reinforced by data from this study. At baseline, B6 mice displayed TST immobility values that were 3-times greater than MRL mice. Yet, despite the lower immobility scores, the MRL mice showed larger responses to antidepressant treatment than B6 mice. B6 mice have previously been shown to have higher baseline immobility values in the TST and the forced swim test compared to other mouse strains and these differences in immobility did not correspond with differences in locomotor activity or increased responsiveness to antidepressants (Crowley et al., 2005; Lucki et al., 2001).

Pharmacological effects produced by chronic administration may be more important for understanding the clinical effects of antidepressants because antidepressants need to be given chronically before they are therapeutic in depression. Chronic administration of fluoxetine to MRL mice, but not B6 mice, produced robust increases of cell proliferation, higher BDNF levels, and behavior effects in tests of neohypophagia (Balu et al., 2009), and higher tissue levels of 5-HT may underlie their greater responsiveness. The MRL strain is suited to determine more precisely how chronic antidepressant treatments cause changes in hippocampal cell proliferation, neurotrophin levels and behavior.

5. Conclusion

The MRL mouse strain, as compared to the B6 strain, was exceptionally responsive to the neuropharmacological and behavioral effects of SSRIs, such as fluoxetine and citalopram. The strain differences were associated with higher tissue levels of 5-HT and DA in the MRL mouse, altered density of 5-HT and NE transporters, and greater increases in extracellular 5-HT levels produced by SSRIs. Although increased release of 5-HT may have contributed to increasing cell proliferation in MRL mice, other mechanisms, such as the induction of neurotrophins, are likely involved in sustaining the effects of chronic antidepressant treatment. Because of its greater response to antidepressants, the MRL mouse is a useful

strain for screening antidepressants and understanding the effects of chronic antidepressant treatments.

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Highlights

1. MRL mice showed high tissue levels of serotonin and dopamine than C57BL/6 mice
2. 5-HT synthesis, transporter density and release were also higher in MRL mice
3. MRL showed larger behavioral responses to acute antidepressants
4. Higher serotonin tone contributed to greater antidepressant responses in MRL mice

A. Injection of 20 mg/kg citalopram **B. Infusion of 1 mM citalopram** **Fig. 1.**

The effects of citalopram on extracellular 5-HT levels in the ventral hippocampus of MRL and B6 mice. A) Systemic injection of citalopram (20 mg/kg; at arrow) increased extracellular 5-HT levels in both strains, MRL mice showed a greater increase than B6 mice at all time-points following citalopram administration. There were no differences between mouse strains in baseline 5-HT levels (120-180 min) collected prior to injection (B6: filled square, n = 5; MRL: open square, n = 8). B) Citalopram was perfused directly into the hippocampus (1 mM; starting at the arrow) using a liquid switch. Citalopram increased extracellular 5-HT levels in both strains (after 220 min). Levels of 5-HT were higher in MRL than B6 mice at two time points (240 and 280 min). There were no differences in baseline (120-180 min) 5-HT levels between mouse strains before perfusion (B6: filled

square, $n = 7$; MRL: open square, $n = 11$). Asterisks indicate significant differences between strains: * $p < 0.05$; ** $p < 0.01$.

Fig. 2. Comparison of SERT and NET binding between B6 and MRL mice. The density of SERT was measured using [^3H]-paroxetine (Panel A) and NET was measured using [^3H]-nisoxetine (Panel B) at concentrations producing a maximum level of specific binding in B6 (black bars; $n = 4$) and MRL (open bars; $n = 4$) mice. Bars represent mean specific binding (fmoles/mg protein) $\pm$ SEM. Asterisks indicate significant differences between groups according to two-tailed Student's t -test (** $p < 0.005$).

Fig 3.

Comparison of mRNA expression of SERT, 5-HT_{1A} and 5-HT_{1B} receptors in the hippocampus and brainstem between B6 and MRL mice. A) MRL mice (white bars, n = 7-8) showed increased 5-HT_{1B} gene expression in the hippocampus and brainstem, while showing decreased 5-HT_{1A} mRNA expression in the hippocampus in comparison to B6 mice (black bars, n=6-8 mice per group). Bars represent mean fold change normalized to B6 $\pm$ SEM. Asterisks indicate significant differences between groups according to two-tailed Student's t-test (* $p < 0.05$, ** $p < 0.01$). B) MRL mice (white bars, n = 7-16) showed increases in SERT mRNA expression in the brainstem compared to B6 mice (black bars, n=7-16). Bars represent mean fold change normalized to B6 mice $\pm$ SEM. Asterisks indicate

significant differences between groups according to two-tailed Student's t-test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

Fig. 4.

Effect of citalopram in the TST and on locomotor activity in B6 and MRL mice. Saline or citalopram (CIT: 10 mg/kg, striped bar; 20 mg/kg, black bar) was administered 30 min prior to testing. $N = 9-10$ mice/group. (A) Vertical bars represent the mean amount of time (sec) spent immobile during the 6-min test $\pm$ SEM. (B) The magnitude of change (%) in immobility for each dose of drug compared with absolute saline values. (C) Effect of citalopram on locomotor activity in B6 and MRL mice. Vertical bars represent the mean distance traveled (cm) during the 30 min period $\pm$ SEM. (D) The magnitude of change (%) in locomotion for each dose of drug compared with absolute saline values. Asterisks indicate groups that differed significantly from corresponding controls (* $p < 0.05$, ** $p < 0.005$) according to Dunnett's post-hoc analysis.

Fig. 5.

Effect of desipramine in the TST and on locomotor activity in B6 and MRL mice. Saline or desipramine (DMI: 10 mg/kg, striped bar; 20 mg/kg, black bar) was administered 30 min prior to testing. N = 10-20 mice/group. (A) Values represent the mean amount of time (sec) spent immobile during the 6-min test. (B) Values represent the magnitude of change (%) in immobility for each dose of drug. Asterisk indicates groups that differed significantly from control (** $p < 0.005$) according to Dunnett's post-hoc analysis. (C) Effect of desipramine on locomotor activity in B6 and MRL mice. Values represent the mean distance traveled (cm) during the 30 min period. (D) The magnitude of change (%) in locomotion for each dose of drug compared with absolute saline values. Asterisks indicate groups that differed significantly from corresponding controls (* $p < 0.05$, ** $p < 0.005$) according to Dunnett's post-hoc analysis.

Table 1

Comparison of tissue monoamine content between B6 and MRL mice.

	MRL	B6
Tissue Content (pg analyte / mg tissue)		
Hippocampus		
5-HT	802 ± 51*	641 ± 24
5-HIAA	388 ± 24	330 ± 22
5-HIAA / 5-HT	0.49 ± .03	0.50 ± .03
DA	26 ± 3*	16 ± 3
NE	412 ± 18	376 ± 13
Frontal Cortex		
5-HT	540 ± 22**	400 ± 17
5-HIAA	182 ± 6**	115 ± 8
5-HIAA / 5-HT	0.28 ± .01**	0.33 ± .01
DA	800 ± 174**	274 ± 51
NE	262 ± 21	290 ± 20
Amygdala		
5-HT	948 ± 34**	744 ± 32
5-HIAA	192 ± 11**	124 ± 6
5-HIAA / 5-HT	0.20 ± .01	0.17 ± .01
DA	134 ± 11	204 ± 33
NE	281 ± 11	259 ± 18
Brain Stem		
5-HT	654 ± 31*	554 ± 15
5-HIAA	410 ± 33*	324 ± 15
5-HIAA / 5-HT	0.63 ± .04	0.58 ± .02
DA	38 ± 2**	27 ± 2
NE	401 ± 25	392 ± 17

The amount of analyte (5-HT, serotonin; 5-HIAA, 5-hydroxy-indoleacetic acid; NE, norepinephrine; DA, dopamine) from each region (n = 9/ strain) was quantified by HPLC and normalized to the wet weight of the tissue. Asterisk indicates comparison where the strains differed significantly from each other (* $p < 0.05$, ** $p < 0.005$) according to Student's *t*-test.