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Pharmacological or Genetic Inactivation of the Serotonin Transporter Improves Reversal Learning in Mice

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Growing evidence supports a major contribution of cortical serotonin (5-hydroxytryptamine, 5-HT) to the modulation of cognitive flexibility and the cognitive inflexibility evident in neuropsychiatric disorders. The precise role of 5-HT and the influence of 5-HT gene variation in mediating this process is not fully understood. Using a touch screen–based operant system, we assessed reversal of a pairwise visual discrimination as an assay for cognitive flexibility. Effects of constitutive genetic or pharmacological inactivation of the 5-HT transporter (5-HTT) on reversal were examined by testing 5-HTT null mice and chronic fluoxetine-treated C57BL/6J mice, respectively. Effects of constitutive genetic loss or acute pharmacological depletion of 5-HT were assessed by testing Pet-1 null mice and para-chlorophenylalanine (PCPA)–treated C57BL/6J mice, respectively. Fluoxetine-treated C57BL/6J mice made fewer errors than controls during the early phase of reversal when perseverative behavior is relatively high. 5-HTT null mice made fewer errors than controls in completing the reversal task. However, reversal in Pet-1 null and PCPA-treated C57BL/6J mice was not different from controls. These data further support an important role for 5-HT in modulating reversal learning and provide novel evidence that inactivating the 5-HTT improves this process. These findings could have important implications for understanding and treating cognitive inflexibility in neuropsychiatric disease.

Keywords: antidepressant, executive function, gene, reversal, serotonin

Introduction

There remains an urgent need for novel therapeutic treatments that better alleviate the profound prefrontal cortex (PFC)–mediated cognitive–executive deficits that characterize neuropsychiatric disorders ranging from schizophrenia and drug abuse to obsessive compulsive disorder (OCD) and depression (Carter et al. 2008). A growing corpus of data provides strong evidence that dysfunction of the monoamine serotonin (5-hydroxytryptamine, 5-HT) contributes to the pathophysiology of cognitive–executive symptoms found in these disorders (Chamberlain et al. 2006).

Consistent with such a role, preclinical research has shown that 5-HT disruptions produce impairments in various measures of executive function including assays for impulse control (Robbins and Arnsten 2009). For example, depletion of brain 5-HT (via intracerebroventricular infusions of the 5-HT neurotoxin 5,7-dihydroxytryptamine (5-7-DHT) or systemic treatment with the 5-HT2C receptor antagonist, SB 242084, increases impulsivity in rats (Winstanley et al. 2006; Dalley et al. 2008). These data are generally in line with an influential finding by Linnoila and colleagues in humans demonstrating an inverse correlation between cerebrospinal fluid 5-HT metabolite levels and measures of impulsivity in humans (Linnoila et al. 1983; Chamberlain et al. 2006).

Previous studies in humans and rodents have also assessed the role of 5-HT in modulating "cognitive flexibility." Cognitive flexibility is broadly defined as the capacity for modifying behavior in the face of changing environmental demands and is mediated by the PFC across species (notably the orbitofrontal and ventromedial regions) (Schoenbaum and Shaham 2008; Holmes and Wellman 2009). Various experimental procedures have been developed to test cognitive flexibility in humans, nonhuman primates, and rodents (reviewed in Brigman et al. forthcoming). One commonly employed measure of cognitive flexibility assesses the ability to shift responding for reward after a learned stimulus–reward contingency is changed. These tasks are broadly divided into 2 categories. Set-shifting tasks require a shift in response from a cue in one stimulus dimension to a novel cue in a previously irrelevant dimension. By contrast, reversal learning tasks require a shift in responding from a previously rewarded to a previously unrewarded cue in the same stimulus dimension. Operant-based reversal learning tasks have demonstrated potential as a simple but reliable and readily translatable assay for cognitive flexibility in experimental animals (Clark et al. 2004; Brigman et al. forthcoming).

In humans, some but not all studies have found that reducing brain 5-HT by removal of the 5-HT precursor tryptophan from the diet impairs reversal learning in various tasks (Park et al. 1994; Rogers et al. 1999; Evers et al. 2005; Talbot et al. 2006) (for review, see Clark et al. 2004). In rats, one study found that tryptophan depletion failed to affect spatial reversal (van der Plasse and Feenstra 2008), whereas another showed that treatment with the 5-HT synthesis inhibitor para-chlorophenylalanine (PCPA) impaired reversal in an attentional set-shifting task (Lapiz-Bluhm et al. 2009). In addition, an elegant series of studies by Clarke et al. (2004, 2005, 2007) have shown that 5-7-DHT ablation of 5-HT in the orbitofrontal cortex impairs reversal of a pairwise visual discrimination on a touch screen–based apparatus in Marmoset monkeys.

In parallel with these pharmacological and lesion studies, there is growing evidence that genetic variation in endogenous 5-HT function affects PFC-mediated behavioral processes including the regulation of higher order executive functions

(Hariri and Holmes 2006; Holmes 2008). The 5-HT reuptake regulating 5-HT transporter (5-HTT) has been the most intensively studied in this regard. Variation in the gene encoding the 5-HTT (*SLC6A4*) is associated with risk for mood and anxiety disorders (Caspi and Moffitt 2006; Uher and McGuffin 2008) and functional alterations in corticostriatal circuitry mediating executive functions including cognitive flexibility (Hariri and Holmes 2006; Canli and Lesch 2007). Of particular relevance to the present study, 5-HTT gene variation correlates with differences in object reversal in nonhuman primates (Izquierdo et al. 2007; Vallender et al. 2008) and modifies the effects of tryptophan depletion on reversal (Finger et al. 2007) and ecstasy abuse on decision making (Roiser et al. 2006) in humans.

The role of 5-HT and the 5-HTT in mediating cognitive flexibility remains to be fully clarified. Two particularly important issues are 1) whether increasing levels of brain 5-HT by blocking the 5-HTT can facilitate cognitive flexibility and 2) whether genetically driven variation in 5-HT and the 5-HTT function affects flexibility in the same manner as pharmacological manipulations. In the present study, we examined the effects of various pharmacological and genetic 5-HT and 5-HTT manipulations on a touch screen–based visual reversal task in mice.

Materials and Methods

Subjects

5-HTT null mutant mice were generated as previously described (Bengel et al. 1998) and backcrossed onto a C57BL/6J background for >10 generations. Pet-1 null mutant mice were generated as previously described (Hendricks et al. 2003) and backcrossed onto a C57BL/6J background for 10 generations. To avoid potential phenotypic abnormalities resulting from genotypic differences in maternal behavior and early life environment in these mice (Carroll et al. 2007; Millstein and Holmes 2007), wildtype (WT), heterozygous (HET) and knockout (KO) mice were generated from HET × HET matings for both mutant lines. The effects of fluoxetine, PCPA, and N-(2-Chloroethyl)-N-ethyl-2-bromobenzylamine hydrochloride (DSP-4) were tested in C57BL/6J mice (i.e., same background as the 2 mutant lines) obtained from The Jackson Laboratory (Bar Harbor, ME). Mice were housed 1–3/cage in a temperature- and humidity-controlled vivarium under a 12 h light:dark cycle (lights on 0600 h) (note, there was no systemic relationship between single-housing and genotype, and data analysis revealed no effect of housing on behavior). With the exception of the Pet-1 null mutant line, for which males and females were tested, all mice were males and aged between 3 and 4 months of age. The number of mice used in each experiment is given in the figure legends. Experimental procedures were approved by the National Institute on Alcohol Abuse and Alcoholism Animal Care and Use Committee and were treated in accordance to the National Institutes of Health guidelines "Using Animals in Intramural Research."

Apparatus

The touch screen–based operant apparatus and procedure for testing visual discrimination and reversal were as previously described (Izquierdo, Wiedholz, et al. 2006; Brigman et al. 2008, 2009; Hefner et al. 2008; Karlsson et al. 2009). An operant chamber measuring 21.6 × 17.8 × 12.7 cm (model # ENV-307W, Med Associates, St Albans, VT) was housed within a sound and light attenuating box (Med Associates). The grid floor of the chamber was covered with solid Plexiglas to facilitate ambulation. A pellet dispenser delivering 14 mg dustless pellets (#F05684, BioServ, Frenchtown, NJ) into a magazine was located at one end of the chamber. At the opposite end of the chamber, there was a touch-sensitive screen (Light Industrial Metal Cased TFT LCD Monitor, Craft Data Limited, Chesham, UK), a house light, and a tone generator. The touch screen was covered by a black Plexiglas panel that had 2 × 5 cm windows separated by 0.5 cm and located at a height of 6.5 cm from the floor of the chamber. Stimuli presented on the screen were controlled by custom software ("MouseCat," L.M. Saksida) and visible through the windows (1 stimulus per window). Nosepokes at the stimuli were detected by the touch screen and recorded by the software.

Discrimination and Reversal

Pairwise visual discrimination and reversal learning was assessed as previously described. Mice were first slowly reduced and then maintained at 85% free-feeding body weight. Prior to testing, mice were acclimated to the 14-mg pellet food reward by provision of ~10 pellets per mouse in the home cage for 1–3 days. Mice were then acclimated to the operant chamber and to eating out of the pellet magazine by being placed in the chamber for 30 min with pellets available in the magazine. Mice eating 10 pellets within 30 min were moved onto autoshaping. Autoshaping consisted of visual stimuli (shape randomly varied) being presented in the touch screen windows (1 per window) for 10 s (intertrial interval [ITI] 15 s). The disappearance of the stimuli coincided with delivery of a single pellet food reward, concomitant with presentation of stimuli (2-s 65 dB auditory tone and illumination of pellet magazine) that served to support instrumental learning. Pellet retrievals from the magazine were detected as a head entry and triggered the next trial. To encourage screen approaches and touches at this stage, nosepokes at the touch screen delivered 3 pellets in the magazine.

Mice retrieving 30 pellets within 30 min were moved onto pretraining. During pretraining, mice first obtained rewards by responding to a visual stimulus (shape randomly varied) that appeared in 1 of the 2 windows (spatially pseudorandomized) and remained on the screen until a response was made ("respond" phase). Mice retrieving 30 pellets within 30 min were next required to initiate each new trial with a head entry into the pellet magazine. In addition, responses at a blank window during stimulus presentation now produced a 5-s timeout (signaled by extinction of the house light) to discourage indiscriminate screen responding ("punish" phase). Incorrect responses were followed by correction trials in which the same stimulus and spatial configuration were presented until a correct response was made. Mice making ⩾75% (excluding correction trials) of their responses at a stimulus-containing window over a 30-trial session were moved onto discrimination.

Two novel approximately equiluminescent stimuli were presented in spatially pseudorandomized manner over 30-trial sessions (15 s ITI). Responses at 1 stimulus (correct) resulted in reward; responses at the other stimulus (incorrect) resulted in a 5-s timeout (signaled by extinction of the house light) followed by a correction trial. Stimuli remained on screen until a response was made. Designation of the correct and incorrect stimulus was counterbalanced across genotype and drug treatment group. Performance criterion was an average of 85% correct (excluding correction trials) over 2 consecutive days.

After attaining discrimination criterion, the designation of the same discriminated stimuli as correct versus incorrect was reversed and performance tested over 30-trial daily sessions to a criterion of an average of 85% correct (excluding correction trials) over 2 consecutive days. Multiple reversals were not tested. In our laboratory, training and testing through reversal typically takes 35 daily sessions in C57BL/6J mice.

The dependent variable for autoshaping and pretraining was trials to criterion for each phase. The dependent variables for discrimination and reversal were trials, errors, and correction errors to criterion and average reaction time and reward retrieval latency. In order to examine early and late reversal learning, we separately analyzed trials, errors, and correction errors for sessions where performance was below 50% and performance from 50% to criterion, as previously described (Brigman et al. 2008). To further examine perseverative responding during reversal, we calculated a perseveration index (=average number of correction errors committed per error committed) (Brigman et al. 2008). Group differences on these measures were analyzed using analysis of variance followed by Newman Keuls post hoc tests (to compare 5-HTT or Pet-1 genotypes) or Student's *t*-test (to compare drug treatments).

Effects of Genetic or Pharmacological Inactivation of the 5-HTT

Phenotype of 5-HTT Null Mutant Mice

5-HTT KO, HET, and WT mice were assessed for discrimination and reversal as described above.

Effects of Chronic Fluoxetine Treatment in C57BL/6J Mice

C57BL/6J mice were trained to discrimination criterion as above and then provided with 160 mg/L fluoxetine hydrochloride (LKT Laboratories Inc., St Paul, MN) in (their only source of) drinking water. This concentration was chosen based on previous data from our laboratory (Karlsson et al. 2008; Norcross et al. 2008). Mice drank an average daily dose of 15.1 ± 0.58 mg/kg in the current experiment. Nontreated controls were matched with the fluoxetine-treated group for number of trials to discrimination criterion and received water alone. To allow the drug to achieve steady-state levels and to mimic the clinical situation in which therapeutic effects emerge after chronic treatment, mice were administered fluoxetine for 2 weeks prior to reversal and were then maintained on drug throughout reversal testing. Given the long interval between discrimination and reversal, mice were given discrimination refresher sessions to ensure retention of discrimination performance at criterion levels before reversal testing.

As a positive control for the behavioral effects of chronic fluoxetine treatment, mice were tested in the forced swim test for antidepressant-related effects (Porsolt et al. 1977; Cryan and Holmes 2005) after completing operant testing (and while still on drug). Mice were gently lowered into a Plexiglas cylinder (20 cm diameter) filled halfway with water (24 ± 1 °C) for a 6-min trial, as previously described (Boyce-Rustay and Holmes 2006). Immobility (cessation of limb movements except minor involuntary movements of the hind limbs) was measured by observing mice once every 5 s and scoring immobility as present or absent. Data were expressed as a percentage of total observations during the period.

Effects of Genetic Deficiency or Pharmacological Depletion of 5-HT

Phenotype of Pet-1 Null Mutant Mice

Pet-1 KO, HET, and WT mice were assessed for discrimination and reversal using the procedure described above for 5-HTT null mutant mice. The Pet-1 ETS domain factor controls the developmental differentiation of the 5-HT neurons (Hendricks et al. 2003). Pet-1 KO mice have a 70% loss of 5-HT neurons and an 89% decrease in cortical and hippocampal 5-HT tissue content (Hendricks et al. 2003).

Effects of PCPA (or DSP-4) Treatment in C57BL/6J Mice

C57BL/6J mice were trained to discrimination criterion as above and 24 h later injected intraperitoneally (i.p., 10 mL/kg body weight dissolved in a saline vehicle) with 250 mg/kg of the 5-HT synthesis inhibitor PCPA methyl ester hydrochloride (Fratta et al. 1973) (Sigma-Aldrich, St Louis, MO). Treatment was repeated once daily for 3 days. The dose and treatment regimen has been shown to markedly deplete 5-HT in C57BL/6J mice in our laboratory (Boyce-Rustay et al. 2008). Nontreated controls were matched for trials to discrimination criterion and injected daily with saline. Reversal testing began 24 h after the final injection and was limited to the first 6 sessions in order to focus the analysis on the sub-50% "perseverative" phase (which we hypothesized to be most sensitive to 5-HT depletion) and to limit testing to a time period before significant recovery of brain 5-HT levels.

To confirm 5-HT depletion, mice were sacrificed after the sixth reversal session for high-performance liquid chromatography (HPLC) analysis of brain monoamine content. Briefly, mice were sacrificed via cervical dislocation and decapitation, and tissue from the medial PFC (mPFC) (comprising prelimbic, infralimbic, and posterior medial orbital cortices) and (dorsal) hippocampus were dissected on ice. Samples were homogenized in 800 mL of 0.1 M perchloric acid containing 1% ethanol and 0.02% ethylenediaminetetraacetic acid (EDTA) and centrifuged for 20 min at 3000 × *g*. Thirty microliters of the homogenate was used for catecholamine analysis by HPLC using a Luna 5 μ C18(2), 250 × 2.0 mm column (Phenomenex 00G-4252-B0, Torrance, CA) held at 30 °C, a Waters Corporation (Milford, MA) 717plus autosampler at 4 °C, 510 pump at 0.4 mL/min, and amperometric electrochemical detector (EiCOM CB100) set at Eox. 0.82 V. The mobile phase contained 2.8 g 1-heptanesulfonic acid sodium salt, 0.17 g EDTA, 20 mL triethylamine, dissolved in 2.2 L water, pH adjusted to 2.5 with 13 mL 85% phosphoric acid, plus 90 mL acetonitrile. The detector output was recorded and analyzed with Waters Empower 2 Chromatography Data Software. Data were expressed as percentage of change from vehicle control.

Given the known role of the norepinephrine (NE) system in modulating reversal (Dalley et al. 2004; Seu et al. 2009), we also tested the effects of NE depletion. C57BL/6J mice were trained to discrimination criterion and, 24 h later, injected i.p. (10 mL/kg body weight dissolved in a saline vehicle) with 40 mg/kg of the tyrosine hydroxylase inhibitor *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine hydrochloride (DSP-4) (Jonsson et al. 1981) (Sigma-Aldrich). Reversal testing began 8 days later. This dose and treatment test interval has been shown to deplete NE in C57BL/6J mice in our laboratory (Boyce-Rustay et al. 2008). Nontreated controls were matched for number of trials to discrimination criterion and injected with saline. Reversal testing was again limited to the first 6 sessions for the same reasons described for 5-HT depletion. To confirm NE depletion, mice were sacrificed after the sixth reversal session for HPLC analysis of brain monoamine content, as above.

Results

Effects of Pharmacological or Genetic Inactivation of the 5-HTT

Effects of Chronic Fluoxetine in C57BL/6J

Prior to treatment, groups showed a similar number of trials to discrimination criterion (water = 247 ± 30, fluoxetine = 277 ± 43) and to reattain criterion after fluoxetine treatment (water = 2.1 ± 0.3 refresher trials; fluoxetine = 1.7 ± 0.3). Following treatment, there was no significant difference between treatment groups for trials (Fig. 1*A*), errors (Fig. 1*B*), or correction errors (Fig. 1*C*) to reach reversal criterion. Groups did not differ in perseveration index (water = 4.0 ± 0.7, fluoxetine = 3.7 ± 0.6), trials omitted (water = 60 ± 17, fluoxetine = 49 ± 12), stimulus reaction time (water = 4.7 ± 0.5 s, fluoxetine = 4.9 ± 0.7), or reward retrieval latency (water = 1.8 ± 0.1 s, fluoxetine = 1.8 ± 1.8).

Although there were no effects of genotype when the 2 phases of the task were combined, analysis of reversal performance on the <50% and ⩾50% phases separately revealed that fluoxetine-treated mice committed significantly fewer trials ($t = 2.77$, degrees of freedom [df] = 19, $P < 0.05$) (Fig. 1*D*) and made significantly fewer errors ($t = 2.63$, df = 19, $P < 0.05$) (Fig. 1*E*) and correction errors ($t = 2.37$, df = 19, $P < 0.05$) (Fig. 1*F*) during the <50% phase than water-treated controls. Groups did not significantly differ in perseveration index (water = 4.9 ± 1.1, fluoxetine = 3.6 ± 0.3), trials omitted (water = 60 ± 17, fluoxetine = 49 ± 12), stimulus reaction time (water = 6.4 ± 0.8 s, fluoxetine = 7.1 ± 1.0), or reward retrieval latency (water = 2.0 ± 0.2 s, fluoxetine = 2.1 ± 0.4).

Trials (Fig. 1*D*), errors (Fig. 1*E*), and correction errors (Fig. 1*F*) during the ⩾50% phase did not significantly differ between treatment groups. Perseveration index (water = 1.6 ± 0.2, fluoxetine = 1.5 ± 0.1), trials omitted (water = 0.0 ± 0.0, fluoxetine = 0.0 ± 0.0), stimulus reaction time (water = 3.3 ± 0.3 s, fluoxetine = 4.0 ± 0.6), and reward retrieval latency (water = 1.7 ± 0.1 s, fluoxetine = 1.9 ± 0.2) also failed to differ during the ⩾50% phase.

After the completion of reversal, fluoxetine-treated mice showed significantly reduced immobility relative to untreated controls in the forced swim test (water = 65.0 ± 4.0%, fluoxetine = 46.0 ± 6.0%).

Phenotype of 5-HTT Null Mutants

There was no significant effect of genotype on discrimination performance, as measured by number of trials, errors, or

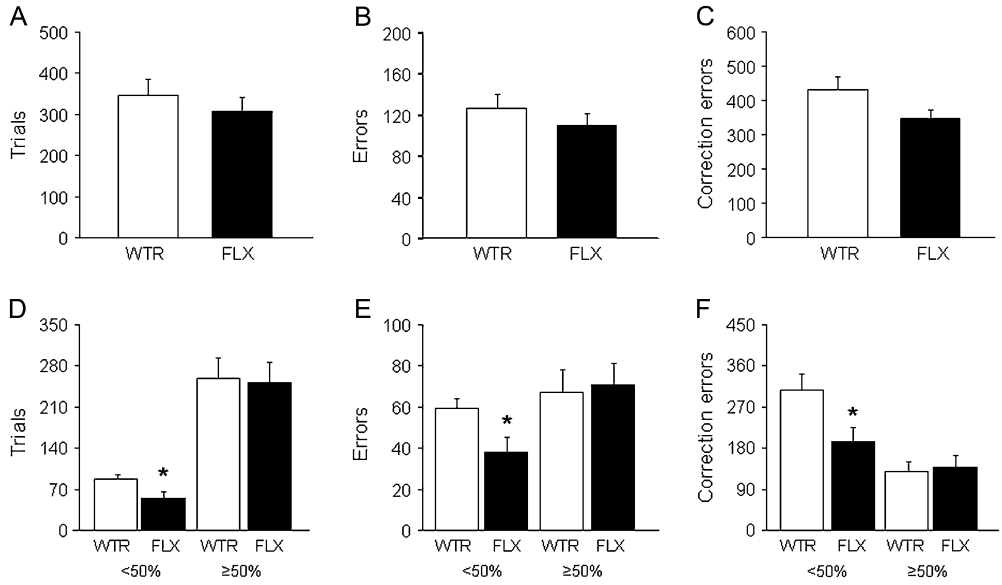

Figure 1. Effects of chronic fluoxetine in C57BL/6J mice. Fluoxetine- and water-treated mice did not significantly differ in trials (*A*), errors (*B*), or correction errors (*C*) to reversal criterion. Fluoxetine-treated mice made significant fewer trials (*D*), errors (*E*), and correction errors (*F*) during the <50% correct reversal phase, but not ≥50% correct phase, relative to water-treated controls. WTR, water; FLX, fluoxetine. Data are mean ± standard error of the mean. $n = 10$ per treatment. $^*P < 0.05$ versus water treated.

correction errors to criterion (Table 1). However, there was trend for lower scores on all 3 of these measures in HET and KO relative to WT, although this was not close to statistical significance (main effect of genotype for all measures: $P = 0.17$). Genotypes did not differ on trials omitted, stimulus reaction time, or reward retrieval latency during discrimination (Table 1).

There was a significant effect of genotype for the number of errors ($F_{1,20} = 3.97$, $P < 0.05$) and correction errors ($F_{1,20} = 5.16$, $P < 0.05$) and a borderline significant effect of genotype for trials ($F_{1,20} = 3.01$, $P = 0.07$) to reversal criterion. Post hoc analysis showed that HET and KO did not significantly differ from WT in trials to reach criterion (Fig. 2*A*) but made significantly fewer errors (Fig. 2*B*) and correction errors (Fig. 2*C*) in reaching criterion. Genotypes did not significantly differ in perseveration index (WT = 3.2 ± 1.1, HET = 2.6 ± 0.2, KO = 2.5 ± 0.3), trials omitted (WT = 66 ± 42, HET = 46 ± 11, KO = 43 ± 15), stimulus reaction time (WT = 9.3 ± 1.3 s, HET = 10 ± 1.6, KO = 10 ± 1.5), or reward retrieval latency (WT = 2.0 ± 0.2 s, HET = 2.2 ± 0.2, KO = 2.2 ± 0.3). Analysis of reversal performance on the <50% and ≥50% phases found that genotypes did not significantly differ in trials (Fig. 2*D*), errors (Fig. 2*E*), or correction errors (Fig. 2*F*) (or any other measure) during either phase. Finally, when genotypes were compared for performance over a fixed number of trials, when all mice were still on task (=300 trials), as recently described by Rudebeck and Murray (2008), there were again no differences (errors: WT = 9.3 ± 1.3 s, HET = 10 ± 1.6, KO = 10 ± 1.5; correction errors: WT = 9.3 ± 1.3 s, HET = 10 ± 1.6, KO = 10 ± 1.5).

A general observation was that the number of trials, errors, and correction errors required to reach discrimination and reversal criteria was higher in this experiment (and to lesser extent the Pet-1 experiment), regardless of genotype, than in C57BL/6J mice, both in this study (fluoxetine experiment) and previously in our laboratory (Izquierdo, Wiedholz, et al., 2006; Brigman et al. 2009). The reasons for this are currently unclear but may stem from subtle differences in genetic background (despite repeated backcrossed to C57BL/6J) or early postnatal experience (e.g., influence of HET mothers; Millstein and Holmes 2007; Carola et al. 2008).

Table 1
Autoshaping, pretraining, and discrimination performance in 5-HTT null mutant mice

	WT	HET	KO
Autoshaping sessions	1.9 ± 0.6	2.3 ± 0.9	2.0 ± 0.6
Pretraining			
Respond phase sessions	4.4 ± 1.6	3.4 ± 0.4	3.6 ± 1.1
Punish phase sessions	6.8 ± 1.8	6.1 ± 1.9	7.3 ± 1.9
Discrimination			
Total trials	613.0 ± 104.2	389.1 ± 73.0	456.6 ± 157
Total errors	184.3 ± 36.3	111.3 ± 25.1	133.9 ± 47.2
Total correction errors	412.1 ± 88.2	225.9 ± 37.6	261.1 ± 81.8
Total trials omitted	22.4 ± 7.7	4.6 ± 3.6	17.9 ± 10.2
Average stimulus reaction time (s)	8.0 ± 1.0	6.0 ± 1.0	8.2 ± 0.4
Average reward retrieval latency (s)	2.7 ± 0.5	2.8 ± 0.8	2.5 ± 0.4

Genotypes did not significantly differ in autoshaping, pretraining, or discrimination performance, although there was a nonsignificant trend for HET and KO mice to require fewer trials make fewer errors to reach criterion than WT controls. n = 8–10 per genotype. Data are means ± standard errors of the mean.

Effects of Pharmacological Depletion or Genetic Deficiency of 5-HT

Effects of PCPA (or DSP-4) Treatment in C57BL/6J

Prior to PCPA treatment, groups showed a similar number of trials to discrimination criterion (vehicle = 311 ± 50, PCPA = 272 ± 47). Following treatment, there was no significant effect of treatment on trials (Fig. 3*A*), errors (Fig. 3*B*), or correction errors (Fig. 3*C*) over the 6 reversal sessions. Groups did not significantly differ on perseveration index (vehicle = 3.8 ± 0.4, PCPA = 4.5 ± 0.7), trials omitted (vehicle = 19 ± 7, PCPA = 9 ± 4), stimulus reaction time (vehicle = 5.0 ± 0.9 s, PCPA = 5 ± 0.7), or reward retrieval latency (vehicle = 2 ± 0.1 s, PCPA = 2 ± 0.2). Groups had attained similar levels of percent correct

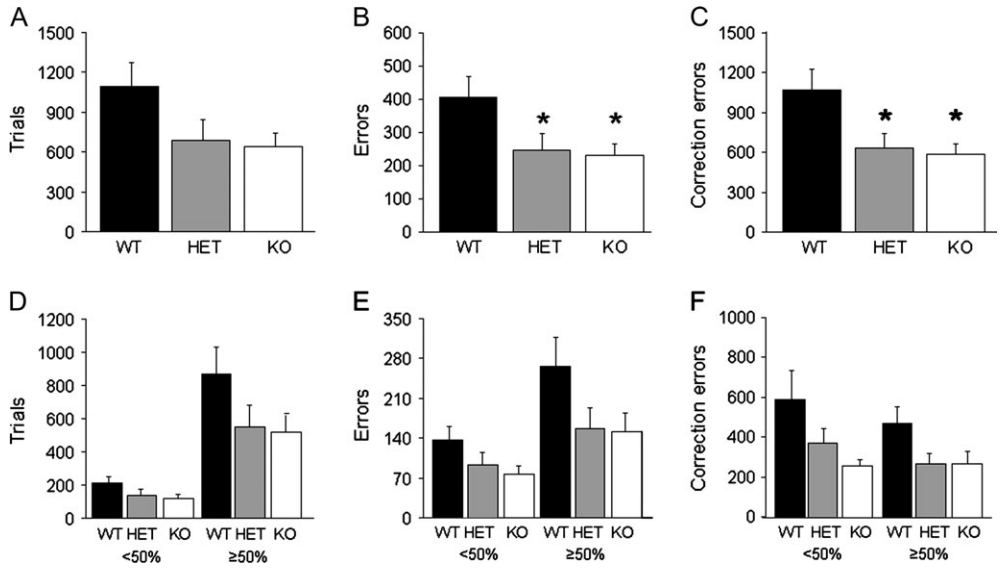

Figure 2. Phenotype of 5-HTT null mutant mice. 5-HTT KO and HET showed a trend for fewer trials (*A*) and made significantly fewer errors (*B*) and correction errors (*C*) to reversal criterion, as compared with WT controls. Genotypes did not significantly differ in trials (*D*), errors (*E*), or correction errors (*F*) when separately examining the <50% correct and ≥50% correct reversal phases. Data are mean ± standard error of the mean. $n = 8$–10 per genotype. *$P < 0.05$ versus WT.

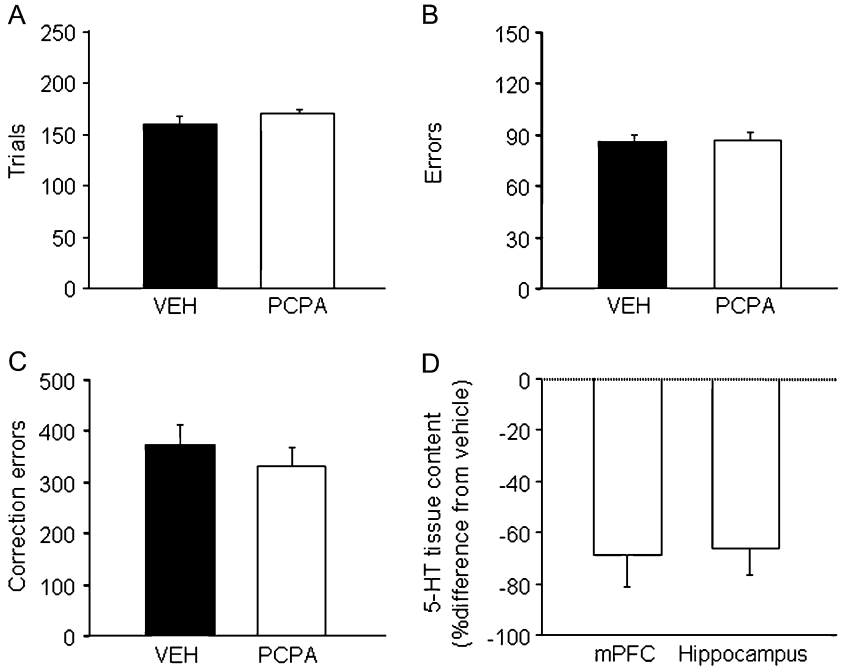

Figure 3. Effects of PCPA treatment in C57BL/6J mice. PCPA- and vehicle-treated mice did not significantly differ in trials (*A*), errors (*B*), or correction errors (*C*) during 6 reversal sessions (approximating to <50% correct phase). 5-HT tissue content in the mPFC and hippocampus (*D*) was significantly lower after the sixth reversal session in PCPA-treated mice relative to and vehicle-treated controls. VEH, vehicle. Data are mean ± standard error of the mean. $n = 10$ per treatment. *$P < 0.05$ versus vehicle treated.

performance by the final reversal session (vehicle = 57 ± 5% correct, PCPA = 58 ± 16%).

HPLC analysis confirmed that PCPA treatment reduced levels of 5-HT in mPFC and hippocampus relative to vehicle-treated controls (Fig. 3*D*). PCPA treatment did not alter NE content in mPFC (+1.9 ± 4.9% of vehicle) and modestly reduced hippocampal NE content (–21.5 ± 13.5% of vehicle).

Prior to DSP-4 treatment, treatment groups showed a similar number of trials to discrimination criterion (vehicle = 361 ± 38 trials to criterion, DSP-4 = 294 ± 73 trials). Following treatment, there was no significant effect of treatment on trials, errors, or correction errors (Table 2). Groups did not significantly differ on perseveration index, trials omitted, stimulus reaction time, or reward retrieval latency (Table 2). DSP-4– and vehicle-treated mice had attained similar levels of percent correct performance by the sixth session (vehicle = 52 ± 6% correct, DSP-4 = 59 ± 5%). HPLC analysis confirmed that DSP-4–treated mice had significantly reduced NE tissue content in mPFC and hippocampus relative to vehicle-treated controls, although 5-HT content was unaltered (Table 2).

Table 2
Reversal performance and forebrain NE depletion after DSP-4 treatment in C57BL/6J mice

	Vehicle	DSP-4
Reversal performance		
Total trials	171 ± 5	157 ± 8
Total errors	94 ± 4	90 ± 4
Total correction errors	364 ± 34	400 ± 34
Perseveration index	3.7 ± 0.4	4.7 ± 0.6
Total trials omitted	9.2 ± 5.0	23.5 ± 7.8
Average stimulus reaction time (s)	3.8 ± 0.4	4.3 ± 0.5
Average reward retrieval latency (s)	2.0 ± 0.1	2.1 ± 0.2
NE tissue content		
mPFC (ng/g)	395.4 ± 68.5	222.9 ± 30.3 (−43.6%)*
Hippocampus (ng/g)	412.3 ± 35.3	238.0 ± 33.0 (−42.3%)*
5-HT tissue content		
mPFC (ng/g)	397.8 ± 46.4	397.8 ± 73.6 (−5.9%)
Hippocampus (ng/g)	448.6 ± 55.2	423.8 ± 30.1 (−4.6%)

DSP-4 treatment did not significantly affect any measure of reversal performance, despite significantly reducing tissue content of NE (but not 5-HT) in the mPFC and hippocampus. $n = 10$ per treatment. Data are means ± standard errors of the mean.
*$P < 0.05$ versus vehicle.

Table 3
Autoshaping, pretraining, and discrimination performance in Pet-1 null mutant mice

	WT	HET	KO
Autoshaping sessions	1.0 ± 0.0	1.0 ± 0.0	1.1 ± 0.1
Pretraining			
Respond phase sessions	3.9 ± 0.4	3.3 ± 0.3	3.0 ± 0.3
Punish phase sessions	4.9 ± 0.4	5.9 ± 0.5	4.3 ± 0.6
Discrimination			
Total trials	359.2 ± 49.5	227.2 ± 31.5	413.6 ± 1129
Total errors	113.5 ± 18.9	69.8 ± 13.8	124.5 ± 35.0
Total correction errors	255.3 ± 46.5	175.5 ± 39.7	288.0 ± 63.5
Total trials omitted	13.3 ± 3.6	8.8 ± 4.3	6.0 ± 2.1
Average stimulus reaction time (s)	6.5 ± 0.5	7.7 ± 1.6	5.1 ± 0.5
Average reward retrieval latency (s)	2.8 ± 0.3	2.6 ± 0.2	2.1 ± 1.3

Genotypes did not significantly differ in autoshaping, pretraining, or discrimination performance. $n = 8$–12 per genotype. Data are means ± standard errors of the mean.

Phenotype of Pet-1 Null Mutants

There was no significant effect of genotype on trials, errors, or correction errors to discrimination criterion (Table 3). However, there was a nonsignificant trend for lower scores on all 3 measures in HET relative to WT. Genotypes did not differ on trials omitted, stimulus reaction time, or reward retrieval latency during discrimination (Table 3).

There was no significant difference between treatment groups for trials (Fig. 4*A*), errors (Fig. 4*B*), or correction errors (Fig. 4*C*) to reach reversal criterion. Groups did not differ in perseveration index (WT = 3.8 ± 0.6, HET = 3.1 ± 0.2, KO = 2.6 ± 0.4), trials omitted (WT = 79.7 ± 22.6, HET = 47.6 ± 9.0, 40.0 ± 22.6), stimulus reaction time (WT = 5.0 ± 0.7, HET = 7.0 ± 1.0, KO = 4.7 ± 1.0), or reward retrieval latency (WT = 2.4 ± 0.2, HET = 2.1 ± 0.1, KO = 2.4 ± 0.2). Analysis of reversal performance on the <50% and ⩾50% phases found that genotypes did not significantly differ in trials (Fig. 4*D*), errors (Fig. 4*E*), or correction errors (Fig. 4*F*) (or any other measure) during either phase.

Discussion

In the current study, we used a touch screen–based visual reversal task to study the role of 5-HT and the 5-HTT in mediating reversal learning, a form of cognitive flexibility, in mice. The major findings were that 1) chronic treatment with the 5-HTT blocker fluoxetine improved reversal learning, specifically during the early phase of the task, 2) constitutive loss of the 5-HTT also led to improved reversal learning, and 3) neither acute pharmacological depletion of brain 5-HT (or NE) nor constitutive loss of brain 5-HT in Pet-1 null mutant mice demonstrably impaired reversal.

5-HTT KO and HET mice exhibit gene dosage-dependent reduced 5-HT clearance and a corresponding elevation of extracellular 5-HT levels in various cortical, hippocampal, and striatal areas studied (Mathews et al. 2004; Daws et al. 2006). Chronic fluoxetine treatment is also expected to significantly augment extracellular 5-HT levels in these regions in the mouse (Cryan et al. 2004). In this context, improved reversal learning following 5-HTT null mutation or fluoxetine treatment is generally consistent with the observation of an inverse relationship between central 5-HT levels and performance on other forms of executive control, such as impulsivity (Linnoila et al. 1983; Brigman et al. 2008). Although this represents a novel and important finding, a number of caveats should be considered.

First, in contrast to the aforementioned link between 5-HT and impulsivity (Linnoila et al. 1983; Chamberlain et al. 2006), there is little direct evidence linking 5-HT levels with variability in measures of cognitive flexibility in human subjects. That is, there is, to our knowledge, little direct empirical precedent for our current finding that increased 5-HT availability would promote cognitive flexibility in human subjects. Second, the preclinical literature on the effects of genetic and pharmacological 5-HTT inactivation on cognitive flexibility and impulsivity has actually been rather mixed. For example, rats, in which the 5-HTT has been constitutively inactivated by a different gene knockout method (*N*-ethyl-*N*-nitrosourea chemical mutagenesis), show attenuated impulsivity but are normal on a visuospatial reversal task (Homberg et al. 2007). Moreover, rhesus macaques carrying the putatively lesser functioning orthologue of the human 5-HTT–linked polymorphic region (*5HTTLPR*) showed impaired rather than facilitated object reversal (Izquierdo et al. 2007). On the other hand, in agreement with our current data, another laboratory has recently shown that the same variant was associated with improved reversal (Jedema et al. 2009). Similarly, monkeys with a different putatively lesser functioning form of the 5-HTT gene (T1970, G1991, and T2327 haplotype in 3′ untranslated region) also showed improved reversal (Vallender et al. 2008). Taken together with the current data, these findings suggest that, as with other phenotypes such as stress-related behaviors, the penetrance of 5-HTT gene variation on reversal likely depends upon interactions with other factors, including training history, task specifics, genetic background, and environmental factors (Holmes and Hariri 2003; Caspi and Moffitt 2006; Uher and McGuffin 2008).

5-HTT null mutants made fewer errors to reach the final performance criterion for the reversal task (but were statistically equivalent to WT in attaining and retaining the initial discrimination). This phenotype was evident throughout the task, and not restricted to either the relatively early or late phases of reversal, when behavior is relatively dominated by perseveration and learning processes, respectively (Jones and Mishkin 1972; Chudasama and Robbins 2003; Brigman et al. 2008). By contrast, improved reversal performance in fluoxetine-treated C57BL/6J mice was specifically restricted to the earlier, relatively perseverative phase (again, in the absence of any effects on discrimination retention). The lack of fluoxetine's effects on later reversal was unlikely an artifact of

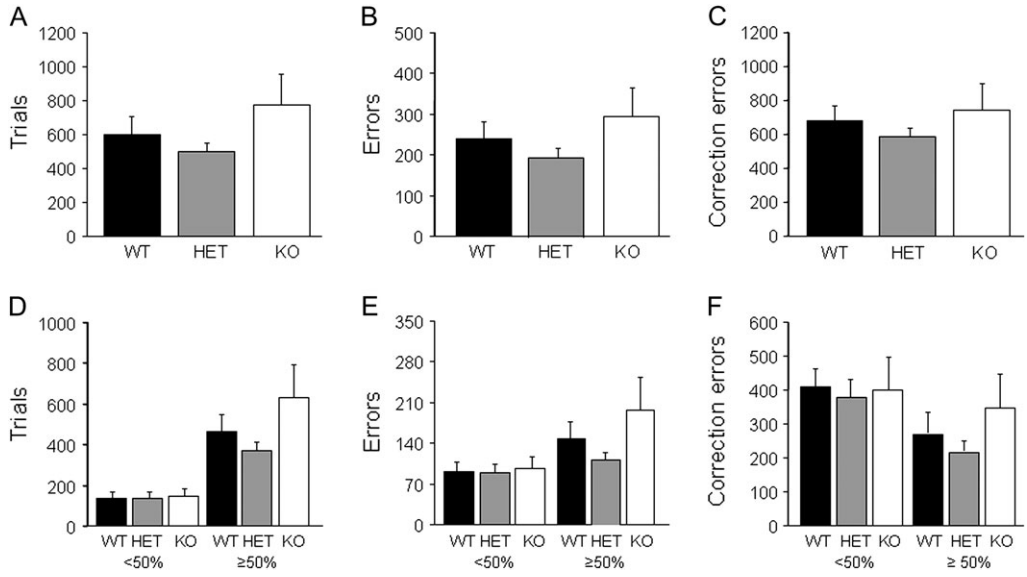

Figure 4. Phenotype of Pet-1 null mutant mice. Genotypes did not significantly differ in trials (*A*), errors (*B*), or correction errors (*C*) to reversal criterion. Genotypes did not significantly differ in trials (*D*), errors (*E*), or correction errors (*F*) when separately examining the <50% correct and ≥50% correct reversal phases. Data are mean ± standard error of the mean. $n = 8$–12 per genotype.

tolerance to the drug's behavioral effects, as the drug retained antidepressant-like effects after completion of the reversal task. Thus, these data might suggest that although both 5-HTT manipulations improved cognitive flexibility, the predominant action of chronic pharmacological inhibition specifically appeared to reduce perseveration, whereas constitutive gene deletion had a more generalized effect on the perseverative and learning components of the task.

Reversal performance is the net manifestation of multiple processes, including detection of a change in stimulus–reward, inhibition of a previously learned prepotent response, sensitivity to negative reinforcement following perseverative responding, and learning of a new stimulus–reward contingency (Roberts 2006). Alterations in any one or more of these processes could contribute to the improved reversal we observed. However, given evidence that loss of 5-HTT gene functions is associated with increased anxiety, stress reactivity, and neural response to negative stimuli (Caspi and Moffitt 2006; Hariri and Holmes 2006; Uher and McGuffin 2008), it is tempting to speculate that improved reversal in the 5-HTT null mutants may be driven by heightened sensitivity to negative reinforcement that served to better guide subsequent choices. This could be one aspect of the enhanced performance monitoring posited to underlie the improved performance on a probability discounting task in monkeys (Jedema et al. 2009) and a risky gambling task in humans (Roiser et al. 2006). Additional studies will be needed to more directly probe this hypothesis, for example, by testing whether the mutants fail to show improved reversal on a task in which incorrect responding is not negatively reinforced.

An interesting observation was that although chronic fluoxetine treatment in nonmutant C57BL/6J mice also improved reversal, the profile of effects differed from those seen in the 5-HTT null mutants in that drug effects were specific to the early (relatively preservative) phase of the task. This profile is reminiscent of the type of effect produced by neuronal or 5-HT lesions of the orbitofrontal cortex in nonhuman primate effect (albeit, in this case, impairing rather than facilitating), which also tend to be specific to the perseverative phase (Clarke et al. 2004, 2005, 2007; Murray et al. 2007). Other researchers have suggested that this type of profile primarily reflects alterations in inhibitory response control rather than shifts in perception of stimulus–reward contingency change (Boulougouris et al. 2008). This raises the interesting possibility that increased inhibitory control could underlie the improved reversal performance of fluoxetine-treated mice. This will be another important avenue for future work. Whatever the precise nature of the effects of 5-HTT gene loss and fluoxetine treatment on reversal, the finding that the nature of the 2 effects differed is not unexpected. Genetically driven 5-HTT loss not only impacts 5-HTT function but also produces neurodevelopmental alterations in key (e.g., cortical and amygdala) neuroanatomical nodes within the reversal-mediating circuitry (Esaki et al. 2005; Hariri and Holmes 2006; Wellman et al. 2007), and improved reversal in monkeys was associated with reduced PFC gray matter volume rather than alterations in 5-HTT binding (Jedema et al. 2009).

Previous work has shown that various means of reducing brain 5-HT, including removing dietary tryptophan, 5-7-DHT–induced lesions, and PCPA treatment, impair reversal on various tasks in humans, nonhuman primates, and rats (Rogers et al. 1999; Clarke et al. 2004, 2005, 2007; Lapiz-Bluhm et al. 2009) (for review, see Clark et al. 2004). Although we are unaware of earlier analogous studies in mice, a somewhat unexpected finding of the current study was that neither constitutive genetically driven loss nor acute neurochemical depletion of brain 5-HT produced demonstrable effects on reversal. The reasons for these negative effects remain to be determined. We confirmed that PCPA-treated mice had levels of 5-HT tissue content in the mPFC and hippocampus that were still only approximately 30% of those in controls 6 days after treatment. It does remain possible, however, that the magnitude of PCPA-induced depletion was insufficient to produce significant impairment on our task or that homeostatic alterations in the 5-HT system mitigated the effects of acute depletion.

Compensatory 5-HT alterations could also account for the intact reversal phenotype in the Pet-1 null mutants. This would in-of-itself be a remarkable demonstration of the capacity to mitigate the effects of 5-HT loss, given the life-long loss of near 90% of 5-HT raphe neurons and forebrain 5-HT in these mice (Hendricks et al. 2003). Nonetheless, it should be noted that there are a number of reports that global depletion of 5-HT via dietary tryptophan depletion did not impact reversal in rats (van der Plasse and Feenstra 2008) and has not always produced significant reversal deficits in humans (Park et al. 1994; Evers et al. 2005; Talbot et al. 2006; Finger et al. 2007). A parsimonious explanation for our negative data is that, in contrast to the more marked effects of selective ablation of 5-HT in orbitofrontal cortex (Clarke et al. 2004, 2005, 2007), the net effects of brain-wide loss of 5-HT may be less disruptive in our paradigm.

In summary, the major findings of the current study were that either chronic pharmacological inhibition or constitutive genetic loss of the 5-HTT improved performance on a touch screen–based assay for cognitive flexibility in mice. By contrast, we were unable to detect effects of pharmacological or genetically driven depletion (via Pet-1 KO) of brain 5-HT. Our findings in 5-HTT null mutants add to growing evidence that although loss-of-function 5-HTT gene variation can increase sensitivity to stress, it may be advantageous for certain cognitive processes that benefit from greater performance monitoring and sensitivity to negative feedback. Similar processes may contribute to the improved reversal learning we saw following chronic fluoxetine treatment. Collectively, these findings further support an important role for the 5-HT system in modulating cognitive flexibility, with implications for understanding the pathophysiology and treatment of neuropsychiatric disorders characterized by executive dysfunction, such as OCD and depression.

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Notes

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