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Abnormal latent inhibition and impulsivity in *coloboma* mice, a model of ADHD

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Abstract

Attention deficit hyperactivity disorder (ADHD) is characterized by hyperactivity, inattention, and impulsivity. The *coloboma* mouse model of ADHD exhibits profound hyperactivity. To determine whether *coloboma* mice exhibit other signs of ADHD, we assessed latent inhibition as a test of attention, and impulsivity in a delayed reinforcement paradigm. Latent inhibition was present in control mice but was disrupted in *coloboma* mice. *Coloboma* mice also exhibited impaired performance on the delayed reinforcement task and were not able to wait as long as control mice to obtain the greater reinforcer. Because norepinephrine mediates hyperactivity in *coloboma* mice, we examined the role of norepinephrine in disrupted latent inhibition and impulsivity. Reduction of norepinephrine with DSP-4 (N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine hydrochloride) restored latent inhibition but did not ameliorate impulsivity. In summary, *coloboma* mice exhibit hyperactivity, inattention as determined by latent inhibition, and impulsivity, and norepinephrine mediates hyperactivity and inattention but not impulsivity in these mice.

Keywords

attention deficit hyperactivity disorder; impulsivity; norepinephrine; DSP-4; latent inhibition; delayed reinforcer; conditioning; *coloboma*; animal model; SNAP-25

Introduction

Attention deficit hyperactivity disorder (ADHD) is characterized by hyperactivity, inattention, and impulsivity. Although ADHD is a common pediatric neuropsychiatric disorder (Olsson, 1992, Faraone et al., 2003), the etiology is unknown. It is almost certain that multiple factors, both genetic and environmental, contribute to the expression of ADHD (Biederman et al., 2002, Biederman, 2005, Faraone et al., 2005, Hudziak et al., 2005). Consequently, ADHD is a heterogeneous disorder with variability in both behavioral expression and drug responses observed among patients, as documented in DSM-IV (Frances et al., 1994). The complexity and heterogeneity of the disorder make ADHD difficult to study in humans. Therefore, animal models are useful for understanding the mechanisms underlying ADHD and for developing new pharmacological therapies.

There are several animal models that bear a phenotypic, pathophysiological, or etiological resemblance to ADHD and, therefore, are considered valid models of ADHD. Face validity, the phenotypic reproduction of the disorder, is observed in all current models of ADHD. All models, including juvenile rats with neonatal 6-hydroxydopamine lesions, dopamine transporter (DAT) knockout or knockdown mice and Naples high-excitability rats, exhibit hyperactivity (Shaywitz et al., 1976b, Sadile et al., 1993, Luthman et al., 1997, Gainetdinov et al., 1999, Zhuang et al., 2001). Others, including spontaneously hypertensive rats, exhibit all 3 core symptoms of ADHD—hyperactivity, inattention, and impulsivity (Sagvolden, 2000, Sagvolden et al., 2005). Construct validity, the resemblance of pathophysiology or pharmacological response to the human disorder, is also observed in most animal models of ADHD. For example, just as psychostimulants ameliorate the symptoms of ADHD in humans, they also reduce the hyperactivity observed in rats treated neonatally with 6-hydroxydopamine, spontaneously hypertensive rats, and DAT knockout mice (Shaywitz et al., 1976a, Heffner and Seiden, 1982, Myers et al., 1982, Luthman et al., 1989, Sagvolden et al., 1992, Gainetdinov et al., 1999, Davids et al., 2002). Because ADHD is a multigenic disorder with no single gene identified as a major risk factor, etiological validity is difficult to establish in models of ADHD. However, the DAT mutant models may have etiological validity inasmuch as a recent meta-analysis of genetic association studies in humans suggests that a polymorphism in the gene encoding DAT is a modest risk factor in ADHD (Faraone et al., 2005).

Because ADHD is a heterogeneous disorder, it is essential that each animal model is fully developed. The hyperactive mouse mutant *coloboma* is another model of ADHD. The hyperactivity exhibited by *coloboma* mice is robust and a direct result of their genetic mutation: a semidominant deletion mutation that includes the *Snap25* gene (Hess et al., 1996). This deletion causes a 50% reduction in the expression of SNAP-25, a presynaptic protein involved in calcium-triggered neurotransmitter release (Hess et al., 1992). Identification of the *Snap25* gene defect in these mice provided a candidate gene for studies in humans that revealed an association between *SNAP25* and ADHD (Barr et al., 2000, Brophy et al., 2002, Mill et al., 2002, Kustanovich et al., 2003). Although such studies show associations and not direct causal relationships between a genetic locus and a disorder, the association between *SNAP25* and ADHD suggests that *coloboma* mice have etiological validity. Moreover, *coloboma* mice, like many ADHD patients, exhibit decreased locomotor activity in response to amphetamine and, therefore, the model has construct validity (Hess et al., 1996). Although *coloboma* mice have construct and etiological validity as a model of ADHD, it is unclear whether *coloboma* mice also have face validity for all three signs of ADHD, not just hyperactivity. Here we evaluate latent inhibition and impulsivity in *coloboma* mice. Furthermore, because norepinephrine concentrations are increased in *coloboma* mice compared to control littermates, and depletion of norepinephrine ameliorates the hyperactivity (Jones et al., 2001, Jones and Hess, 2003), we also investigate the role of norepinephrine in these behaviors.

Materials and methods

Mice

Coloboma (*Cm/+*) mice and control (*+/+*) C3H/HeSnJ littermates (Jackson Laboratories, Bar Harbor, Maine) were bred and housed in group cages at Johns Hopkins University vivarium (lights on at 7:00 and off at 21:00). Testing for locomotor activity and latent inhibition was performed at Johns Hopkins University. For impulsivity tests, a group of mice was moved to Pennsylvania State Hershey Medical Center vivarium, where they were housed under a 12 hr light/dark cycle (lights on at 7:00). Both male and female mice were used for all experiments. Standard laboratory rodent food was available *ad libitum* throughout all experiments. Water was also available throughout locomotor testing, but, for latent inhibition and impulsivity testing, water access was restricted to the morning test session and an additional drinking

session in the afternoon. During experiments involving water deprivation, mice were weighed daily; all mice maintained greater than 90% of their body weight. Experiments were performed in compliance with The Guide for Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committees at Johns Hopkins University and Pennsylvania State University.

Many behavioral tasks are not appropriate for *coloboma* mice. Because *coloboma* mice are extraordinarily active, these mutants are only ~75% normal mouse body weight, so tests using food deprivation/reinforcement, where mice are generally maintained at ~85% body weight, were inappropriate. Further, the hyperactivity itself confounds some tasks. Therefore, the behavioral tests designed for *coloboma* mice did not require food reinforcement and were not disrupted by locomotor hyperactivity.

Locomotor activity

Individual, automated photocell activity cages (29.2 x 50.5 cm) with twelve 2-cm-high infrared beam detectors arranged in a 4 x 8 grid were used to measure locomotor activity (San Diego Instruments, San Diego, CA). Eight *coloboma* mice and 8 control mice, 2 months of age, were habituated to locomotor activity chambers for more than 4 hours prior to testing. Changes in beam status were assessed 18 times per second, and beam breaks were recorded and compiled over 2 hours during the light cycle (9:00 to 11:00) and dark cycle (21:00 to 23:00). Mice had *ad lib* access to both food and water; water was supplied in small glass Petri dishes on the cage floor. Data were collapsed over time and analyzed using Student's *t*-tests.

Two-bottle preference tests

To insure that control and *coloboma* mice were able to discriminate between water and the flavored solutions used in the latent inhibition experiments, 2-bottle preference tests were performed. A cohort of 8 *coloboma* mice and 7 control mice, 3 months of age, had access to water and 0.008 M HCl for 30 min each morning for 4 consecutive days under the same deprivation schedule used in the latent inhibition experiment described below. The cohort then participated in another taste preference test: water vs. 0.8 mM quinine. Consumption data from Days 2–4 of each test were analyzed using 2-factor ANOVAs.

Latent inhibition

Latent inhibition was assessed in a conditioned taste aversion paradigm. Latent inhibition occurs when a stimulus is repeatedly presented without reinforcement or consequence and is then used as a conditioned stimulus (CS) in a conditioning paradigm (Lubow and Moore, 1959). The prior exposure to the stimulus weakens the association of the stimulus with a subsequent unconditioned stimulus (US). The slowed acquisition of the CS-US association in normal animals or humans repeatedly preexposed to the CS without US is latent inhibition. In contrast, subjects quick to make CS-US pairings after repeated noncontingent exposure to the CS are considered impaired in this paradigm. Latent inhibition methods were adapted from Reilly, *et al.* 1993 and Palsson *et al.* 2005.

Twenty *coloboma* mice and 20 control mice, 2–5 months of age, were water-deprived overnight starting at 17:00 the day before the experiment began. For 6 days, mice had access to unflavored distilled water for 30 minutes each morning (9:30–10:00). Then, for 4 days (experiment Days 1–4), the CS preexposed group had access to 0.008 M HCl, while the nonpreexposed group had access to unflavored water for 30 minutes in the morning. On Day 5, the Conditioning day, all mice had access to 0.008 M HCl for 30 minutes in the morning, and, immediately afterward, all mice were injected subcutaneously with 0.15 M LiCl in a volume of 10 mL/kg. On Day 6, mice had access to unflavored water in the morning. On Day 7, the Test day, all mice had access to 0.008 M HCl for 30 minutes in the morning. In addition to the morning drinking

sessions, mice had access to unflavored distilled water for 1 hour each afternoon (16:00–17:00) throughout the experiment. Consumption was measured by weighing water bottles before and after each drinking session. Consumption of HCl during the Test trial (after the aversive LiCl pairing) is expressed as a percentage of consumption of HCl during the Conditioning trial (before the LiCl pairing). Data were analyzed using 2-factor ANOVAs and Student's *t*-tests.

DSP-4 challenge

To test the effects of norepinephrine reduction on latent inhibition, 21 *coloboma* mice and 20 control mice (3–9 months of age) were injected subcutaneously (10 mL/kg) with 50 mg/kg DSP-4 (N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine hydrochloride) (Sigma, St. Louis, MO, USA) freshly prepared in 0.9% NaCl. In the central nervous system, systemically administered DSP-4 is transported specifically into noradrenergic axons via the reuptake transporter and produces a prolonged reduction in noradrenergic innervation attributable to the degeneration of locus coeruleus axons in both rats and mice (Ross, 1976, Jonsson et al., 1981, Fritschy and Grzanna, 1989, Wolfman et al., 1994, Fornai et al., 1996). The peripheral effects of this drug are transient with recovery within a week (Jonsson et al., 1981). We have previously demonstrated that this dose of DSP-4 effectively reduces both norepinephrine concentrations and locomotor hyperactivity in *coloboma* mice (Jones and Hess, 2003).

To serve as controls for the DSP-4 experiment, 16 control mice and 16 *coloboma* mice were injected subcutaneously with saline; saline-treated mice were age- and sex-matched to the DSP-4-treated mice. Drug-treated mice were tested for latent inhibition alongside saline-treated mice using the methods described above, except that 0.8 mM quinine was used as the tastant instead of 0.008 M HCl, and LiCl was administered intraperitoneally instead of subcutaneously to insure that the behavioral responses were independent of both tastant and route of administration. None of the 73 mice tested in this experiment were used in the latent inhibition experiment where HCl was the tastant. All mice were injected with DSP-4 simultaneously to provide consistent lesions. Mice were then tested in 4 cohorts due to logistical considerations arising from the large number of mice needed to complete this experiment; all experimental conditions were represented in each cohort. Consequently, there was variability between cohorts in the time between DSP-4 treatment and latent inhibition testing: Mice were tested 2 weeks to 2 months after DSP-4 administration. However, postmortem neurochemical analysis of forebrain tissue confirmed that norepinephrine was similarly reduced in all groups, and the small standard errors in the latent inhibition results demonstrate that performance was consistent among groups (see Results). Data were analyzed using 2-factor ANOVAs and Student's *t*-tests. DSP-4-treated and saline-treated mice were euthanized by carbon dioxide inhalation. Brains were removed, dissected rapidly over ice, and stored at –80°C until preparation for HPLC.

Impulsivity. Apparatus

Mice were trained in one of four identical modular operant chambers (MED Associates, Inc., St. Albans, VT, USA), measuring 8.5 x 7.5 x 5.0 inches (length x width x height). Front, back, and top walls were made of clear polycarbonate, while the side walls (including the test wall) were made of aluminum. The grid floors consisted of 24 stainless steel rods, -inch thick and spaced -inch apart (center to center). Each chamber was equipped with 3 retractable Lixit tubes (nozzles supplying water or tastants) that could enter the chamber through holes ¾-inch in diameter and spaced 3 inches apart (center to center). In the extended position, the tip of the Lixit tube was aligned in the center of the hole, flush with the right end wall. A contact lickometer circuit was used to monitor and automatically record licks.

Training

The impulsivity procedure was based on the established concept of greater vs. lesser reinforcers (Evenden and Ryan, 1996). *Coloboma* mice and control mice, 12 months of age, were water-deprived overnight, beginning at 17:00 the day before the start of training. Subsequently, mice had access to water during the morning test session and from 15:00–17:00. Training occurred for 4 days. The morning test session consisted of 6 trials and lasted about 30 min. Water, the more preferred reinforcer, was available for 3 licks at the beginning of each trial to cue the start of the trial and to insure that the mice were attending to the task. Immediately upon fulfillment of the 3-lick requirement, a second Lixit containing 1 mM quinine, the less preferred reinforcer, became available. The water Lixit continued to be available. Upon selection of one Lixit, which was defined as 20 licks on either Lixit, the opposite Lixit retracted, and the chosen Lixit was available for an additional 5 sec. Then the chosen Lixit retracted and a 60-sec intertrial interval began. If a Lixit was not selected within 120 sec of the start of the trial, the trial ended and the intertrial interval commenced. Water and quinine were counterbalanced such that water was on the left side of the test wall for half of each genotype and on the right side of the test wall for the other half. Water preference was defined as selection of the water Lixit in 70% or more of completed trials. Mice that did not meet this criterion were excluded from analysis; 7 of 10 control mice and 4 of 9 *coloboma* mice met this criterion. With the exception of one mouse where no clear preference was established, excluded mice exhibited a preference for water over quinine but did not meet the 70% exclusion criterion.

Testing

During the impulsivity test, 1 mM quinine was available immediately, while access to water was increasingly delayed. Immediately upon fulfillment of 3 licks on water at the beginning of each trial, the water Lixit retracted and 1 mM quinine became available. The delay for access to water increased over sessions as follows: 2, 4, 6, 8, 12, 16, and 20 sec. Therefore, mice had a choice between the less preferred immediate reinforcer (quinine) and the more preferred delayed reinforcer (water). Lixit selection was defined as 20 licks on either Lixit. Upon selection of a Lixit, the opposite Lixit retracted, and the chosen Lixit was available for an additional 5 sec. Like training, impulsivity testing consisted of 6 trials per session, one session per day. Mice were tested for 2–3 days per delay until a stable average preference was achieved. Mice were observed during testing, and sessions in which licks were inaccurately recorded were excluded; approximately 6% of sessions were excluded.

Impulsivity data are reported as percent of completed trials in which water was selected over 1 mM quinine and vice versa. The indifference point was used as an indicator of impulsivity. The indifference point is the point at which the value of quinine is equal to that of water and there is no clear preference for either quinine or water. The indifference point for each mouse represents the delay at which the mouse's average water preference dropped to or below 50%. Indifference points were compared using a Student's *t*-test.

DSP-4 challenge

After completion of impulsivity testing in drug-naïve mice, the same group of mice was injected subcutaneously with 50 mg/kg DSP-4 to test the effects of norepinephrine reduction on impulsivity. Beginning 9 days after injection, mice were retrained for 8 days with no delay and retested. Mice were tested for 2–3 days per delay to achieve a stable average preference; sessions in which licks were inaccurately recorded were excluded. To determine the effect of DSP-4 on performance by each genotype, water preference data over delays were compared before and after DSP-4 administration using ANOVA with repeated measures.

HPLC analysis

To insure that DSP-4 had effectively reduced brain norepinephrine concentration, HPLC analysis was performed on forebrain samples. Forebrain was defined as the region anterior to the superior colliculus. The entire forebrain was homogenized in 0.1 M NaOAc (pH 3.5) at 10 μ L/mg and then sonicated. A 500 μ L aliquot of each forebrain homogenate was centrifuged at 14,000 $\times$ g for 10 min at 4°C. Supernatant was filtered through 0.45 μ m microspin centrifuge tubes by centrifuging at 14,000 $\times$ g for 2 minutes at 4°C. A 20 μ L aliquot was analyzed by HPLC with electrochemical detection (ESA, Chelmsford, MA) to determine norepinephrine concentration in brain tissue. Monoamines were separated on an HPLC equipped with a C18, MD-150 column (150 mm length $\times$ 3 mm i.d.; ESA, Chelmsford, MA) and electrochemical detectors at potentials of 150, 250, 450, 550 mV. Mobile phase contained 75 mM sodium dihydrogen phosphate, 1.7 mM 1-octanesulfonic acid sodium salt, 25 μ M EDTA, and 8% acetonitrile (pH 2.9). Flow rate was 0.6 mL/min. Monoamines were identified by retention time, and concentration was based upon peak height compared to that of a known standard. Data were analyzed using ANOVAs.

Statistical analysis

StatView® software version 5.0.1 (SAS Institute, Inc.) was used for all statistical analyses.

Results

Coloboma mice are hyperactive

Consistent with previous results (Hess et al., 1992), *coloboma* mice were significantly more active than control littermates during both the light (9:00–11:00; Student's *t*-test, $t(14) = 3.38$, $p < 0.005$) and dark (21:00–23:00) cycles (Student's *t*-test, $t(14) = 3.99$, $p < 0.005$; Figure 1). On average, *coloboma* mice were 2–3 times more active than control mice. There was no effect of gender on the level of locomotor hyperactivity exhibited by *coloboma* mice. We have previously demonstrated that both habituation and circadian rhythms are intact in *coloboma* mice (Hess et al., 1992). Tests of latent inhibition and impulsivity were performed during the light cycle (9:00–11:00) because the logistical constraints of these assays were not compatible with work under red light conditions.

Coloboma and control mice can distinguish flavored solutions from water

To first determine if the *coloboma* mouse mutation disrupted the ability to distinguish tastants—an ability essential for conditioned taste aversion—mice were challenged in 2-bottle preference tests. All mice showed a strong preference for water over 0.008 M HCl (Figure 2a, 2-factor ANOVA, main effect of tastant, $F_{1,26} = 285.64$, $p < 0.0001$) and for water over 0.8 mM quinine (Figure 2b, 2-factor ANOVA, main effect of tastant, $F_{1,26} = 131.49$, $p < 0.0001$), suggesting that both control and *coloboma* mice were clearly able to distinguish the flavored solutions from water. There was no effect of genotype on HCl consumption ($F_{1,26} = 1.40$, NS) or quinine consumption ($F_{1,26} = 0.25$, NS) and no significant genotype $\times$ tastant interaction effect for the HCl ($F_{1,26} = 1.54$, NS) or quinine ($F_{1,26} = 4.16$, NS) preference tests. Male and female *coloboma* mice exhibited similar preferences.

Coloboma mice exhibit altered latent inhibition

Mice were water-deprived, and half of the control and *coloboma* mice were preexposed to dilute HCl, a sour-tasting but innocuous solution, for 4 days while the other half were not preexposed and drank water. During the Conditioning trial, all mice consumed the HCl solution and then immediately received an aversive pairing with LiCl. The Test trial assessed HCl consumption 2 days after the aversive pairing. For this test, mice were offered only HCl, not a choice between water and HCl. Because latent inhibition is a within-genotype test, mice need

only to perceive the tastant, as demonstrated in Figure 2; relative preferences of control and *coloboma* mice for water and HCl are irrelevant to latent inhibition.

Nonpreexposed control mice consumed ~55% less HCl during the Test trial than during the Conditioning trial (Figure 3a); these mice learned the association between HCl and LiCl during the Conditioning trial. Preexposed control mice consumed significantly more HCl than nonpreexposed control mice during the Test trial (Figure 3a, Student's *t*-test, $t(18) = 2.76$, $p < 0.05$). Preexposed control mice consumed nearly as much HCl during the Test trial as they did during the Conditioning trial. The failure to reduce consumption during the Test trial after preexposure to the CS indicates latent inhibition.

Like nonpreexposed control mice, nonpreexposed *coloboma* mice consumed significantly less HCl during the Test trial than during the Conditioning trial (Figure 3a, Student's *t*-test, $t(18) = 2.16$, $p < 0.05$). However, unlike preexposed control mice, preexposed *coloboma* mice consumed significantly less HCl during the Test trial than during the Conditioning trial (Student's *t*-test, $t(18) = 3.71$, $p < 0.005$). Indeed, the percent HCl consumption by nonpreexposed and preexposed *coloboma* mice during the Test trial was comparable (Student's *t*-test, $t(18) = 0.86$, NS). There was no effect of gender on percent HCl consumption by preexposed *coloboma* mice.

The ability of *coloboma* mice to associate HCl with LiCl despite repeated, nonreinforced preexposures suggests disrupted latent inhibition. During the Conditioning trial, there was no difference in the amount of HCl consumed among test groups, regardless of genotype and prior exposure (Figure 3b, 2-factor ANOVA, main effect of genotype, $F_{1,36} = 0.64$, NS; main effect of exposure, $F_{1,36} = 0.40$, NS). There was also no difference in consumption during the Test trial between nonpreexposed control and *coloboma* mice (Student's *t*-test, $t(18) = 1.25$, NS).

To determine if the disruption of latent inhibition in *coloboma* mice was dependent on the tastant per se or if the lack of latent inhibition was a general deficit independent of tastant, the same experiment was performed using quinine as the tastant. This group of mice also served as the control group for the DSP-4-treated mice (described below) and was, therefore, injected with saline prior to the test. Preexposed saline-treated control mice consumed significantly more quinine than nonpreexposed saline-treated control mice during the Test trial (Figure 4a, Student's *t*-test, $t(14) = 7.26$, $p < 0.0001$), indicating latent inhibition. In contrast, the percent quinine consumption by nonpreexposed and preexposed saline-treated *coloboma* mice during the Test trial was comparable (Student's *t*-test, $t(14) = 0.79$, NS), similar to the disruption in latent inhibition observed using HCl as tastant. A 2-factor ANOVA revealed a significant genotype x exposure interaction effect (Figure 4a, $F_{1,28} = 6.35$, $p < 0.05$).

During the Conditioning trial, there was no difference in the amount of quinine consumed among test groups, regardless of genotype and prior exposure (Figure 4c, 2-factor ANOVA, main effect of genotype, $F_{1,28} = 0.30$, NS; main effect of exposure, $F_{1,28} = 2.28$, NS). There was no difference in consumption during the Test trial between nonpreexposed saline-treated control and *coloboma* mice (Student's *t*-test, $t(14) = 1.15$, NS).

Norepinephrine reduction restores latent inhibition in *coloboma* mice

Consistent with previous results (Jones and Hess, 2003), compared to control mice, norepinephrine concentrations were significantly increased in *coloboma* mice (Table 1, ANOVA with post-hoc Scheffé, $F_{3,66} = 74.44$, $p < 0.01$). Both systemic and central reduction of norepinephrine with noradrenergic neurotoxin DSP-4 significantly decreased locomotor activity of *coloboma* mice but not control mice (Jones and Hess, 2003), further implicating norepinephrine in hyperactivity of *coloboma* mice. To determine whether norepinephrine also plays a role in inattention of *coloboma* mice, latent inhibition was tested in DSP-4-treated

control and *coloboma* mice. The DSP-4-treated mice were tested simultaneously with the saline-treated mice described above.

DSP-4 treatment did not change control mouse responses. Similar to results of saline-treated control mice, quinine consumption by DSP-4-treated nonpreexposed control mice during the Test trial was >60% lower than during the Conditioning trial (Figure 4b), suggesting that DSP-4 treatment did not affect aversive conditioning in control mice. The percent quinine consumption during the Test trial by DSP-4-treated nonpreexposed control mice was significantly lower than that of DSP-4-treated preexposed control mice (Figure 4b, Student's *t*-test, $t(18) = 3.41$, $p < 0.005$). In contrast to the interaction effect that was observed in saline-treated mice, DSP-4-treated *coloboma* mice responded similarly to preexposure compared to control mice and no interaction effect was observed (2-factor ANOVA, $F_{1,37} = 0.004$, NS). In fact, after DSP-4-treatment, the performance of *coloboma* mice was virtually indistinguishable from that of control mice, whereby DSP-4-treated preexposed *coloboma* mice drank significantly more than DSP-4-treated nonpreexposed *coloboma* mice (Figure 4b, Student's *t*-test, $t(19) = 2.63$, $p < 0.05$). There was no effect of gender on percent quinine consumption by preexposed, DSP-4-treated *coloboma* mice or by preexposed, saline-treated *coloboma* mice.

Postmortem neurochemical analysis of forebrain tissue demonstrated a significant reduction in norepinephrine concentration in DSP-4-treated control and *coloboma* mice compared to saline-treated mice (Table 1, ANOVA with post-hoc Scheffé, $F_{3,66} = 74.44$, $p < 0.0001$ for both genotypes). Dopamine concentrations were not significantly different in DSP-4-treated control and *coloboma* mice compared to saline-treated controls (post-hoc Scheffé test, $p > 0.05$); however, a significant effect was noted on ANOVA (Table 1; $F_{3,66} = 6.05$, $p < 0.005$) due to a significant difference between saline-treated control mice and DSP-4-treated *coloboma* mice (post-hoc Scheffé test, $p < 0.005$). The biological significance of this statistic is not clear, particularly as we and others have previously demonstrated that brain dopamine concentrations are not affected by DSP-4 treatment (Jonsson et al., 1981, Fornai et al., 1996, Jones and Hess, 2003), and there was clear overlap between individual sample values in each group.

Coloboma mice are impulsive

To determine whether *coloboma* mice are impulsive, mice were tested in a delayed reinforcer paradigm. Water-deprived mice chose between two immediately-available reinforcers: water and dilute quinine, a bitter, nonpreferred tastant. After establishing a preference for water, an increasing delay was imposed on water over days, while quinine continued to be available immediately. Water preference by control mice gradually decreased as the delay for water increased (Figure 5a), whereas *coloboma* mice lost their preference for water as soon as a delay was imposed (Figure 5b); two-factor repeated measures ANOVA revealed a significant genotype x delay interaction effect ($F_{7,63} = 3.58$; $p < 0.005$; Power = 0.96). Control mice reached their indifference point at an average delay of 9.1 ± 1.1 seconds and then developed a preference for quinine with the increasing delay for water. *Coloboma* mice reached their indifference point at an average delay of 4.5 ± 1.1 seconds. There was a significant effect of genotype on indifference point (Student's *t*-test, $t(9) = 2.92$; $p < 0.05$). As the delay for water increased, *coloboma* mice did not develop a preference for quinine. Participation by control and *coloboma* mice was comparable: There was no difference in overall consumption by control and *coloboma* mice throughout the impulsivity experiment (Figure 5c, 2-factor ANOVA with repeated measures, main effect of genotype, $F_{1,9} = 1.07$, NS). Therefore, the discrepancy in indifference points between the genotypes and the lack of preference by *coloboma* mice with increasing delays for access to water were not caused by differences in participation between control and *coloboma* mice.

Norepinephrine reduction does not correct impulsivity in *coloboma* mice

After DSP-4 treatment, the water preference curve of control mice shifted to the left (Figure 6a, 2-factor ANOVA with repeated measures, treatment x delay interaction effect, $F_{7,91} = 2.23$, $p < 0.05$). An improvement in impulsivity after DSP-4 treatment would have resulted in a rightward shift in the water preference curve of *coloboma* mice. However, the water preference curve of *coloboma* mice was unchanged by DSP-4 treatment (Figure 6b, 2-factor ANOVA with repeated measures, main effect of treatment, $F_{1,5} = 0.002$, NS). As in the previous impulsivity experiment, there was no difference in overall consumption by control and *coloboma* mice throughout the impulsivity experiment (Figure 6c, 2-factor ANOVA with repeated measures, main effect of genotype, $F_{1,10} = 2.04$, NS), indicating that the lack of preference by *coloboma* mice after they reached their indifference point was not caused by decreased participation. Further studies are needed to determine the effect of gender on indifference points of *coloboma* mice before and after DSP-4 treatment.

Discussion

ADHD is characterized by 3 core features: hyperactivity, inattention, and impulsivity. *Coloboma* mice exhibit hyperactivity, but other behavioral attributes of ADHD were not previously explored in *coloboma* mice. Here we demonstrate a disruption in latent inhibition and an inability to perform a delayed reinforcer paradigm, suggesting that *coloboma* mice exhibit additional behavioral defects consistent with ADHD.

Control mice exhibited normal latent inhibition; *coloboma* mice did not. Latent inhibition was disrupted in *coloboma* mice when HCl was used as the CS and when quinine was used as the CS, demonstrating that the disruption is consistent and independent of the tastant used as a CS. However, because *coloboma* mice exhibit profound hyperactivity, confounders such as motivation or participation, which might bias results, must be considered to explain the disruption. There was no difference in consumption of either HCl or quinine among test groups, regardless of genotype and prior exposure, suggesting that the hyperactivity of *coloboma* mice did not alter participation (CS intake) and that neither genotype showed a differential preference or aversion to the tastants. These results also suggest that neophobia did not affect consumption because consumption by nonpreexposed control and *coloboma* mice was comparable to that by preexposed mice. Further, *coloboma* mice were clearly capable of forming the CS-US association, as demonstrated by the nonpreexposed groups, suggesting that the failure of latent inhibition is not likely attributable to deficits of associative learning.

Latent inhibition reflects the ability to filter historically irrelevant stimuli (CS), thus impairing the acquisition of a subsequent CS-US association. It is thought that by reducing the salience of the CS during preexposure, normal subjects direct attention away from the CS or devote less attention to the CS (Mackintosh, 1975, Lubow, 1976, Lubow, 2005). A disruption in latent inhibition, therefore, suggests a defect in selective attention. Latent inhibition may also be explained by associative processes, whereby, during preexposure, normal subjects learn to associate the CS with a lack of US. The “CS-noUS” association must be unlearned and replaced by a CS-US association during conditioning, accounting for the slowed CS-US acquisition (Weiner, 1990, Hemsley, 1993). In this case, disruption of latent inhibition reflects a pathologically enhanced propensity to substitute or switch the previously learned noncontingent association with the appropriate CS-US response. While neither theory completely accounts for the observed phenomenon (Escobar et al., 2002), with either interpretation, a disruption in latent inhibition is consistent with the inattention/impulsivity characteristic of ADHD.

Latent inhibition occurs in humans and rodents and appears to be governed by similar mechanisms across species (Alek et al., 1975, Hellman et al., 1983, Weiner et al., 1984, Matzel,

1988, Weiner et al., 1988, Gray et al., 1992, Shalev et al., 1998, Salgado et al., 2000, Braunstein-Bercovitz et al., 2001, Meyer et al., 2004), suggesting that this is an appropriate test for *coloboma* mice in this context. Although latent inhibition is apparently a universal phenomenon across species, the expression of latent inhibition is influenced by both environmental and genetic factors (Gould and Wehner, 1999), again supporting the utility of this test for mutant mice. Further, from their work in mice, Gould and Wehner (1999) suggest that latent inhibition may be a useful tool for screening ADHD. Indeed, in two separate studies, Lubow and colleagues (1993, 2005) demonstrated an impairment in latent inhibition in ADHD-affected children. However, interpretation of these studies is perhaps not so straightforward. The results from control groups were sometimes not entirely distinct from those from affected groups, and the complex behavioral tasks employed in these studies suggest that the conditioning paradigms themselves were likely assessing more than a simple attentional integrity. Possible confounds of these studies notwithstanding, the results from ADHD-affected groups are intriguing and deserve continued study.

While the significance of decreased neurotransmission of dopamine, serotonin, and glutamate in *coloboma* mice is unknown (Raber et al., 1997, Jones et al., 2001), a clear role of increased norepinephrine neurotransmission in the *coloboma* mouse phenotype has emerged (Jones et al., 2001, Jones and Hess, 2003). Because a DSP-4-induced reduction in norepinephrine ameliorates hyperactivity in *coloboma* mice (Jones and Hess, 2003), we evaluated whether a reduction in norepinephrine neurotransmission also ameliorates the inattention exhibited by *coloboma* mice. A reduction in norepinephrine by DSP-4 did not change the ability of nonpreexposed mice to make the CS-US pairing, regardless of genotype. DSP-4 treatment did not affect latent inhibition in control mice, consistent with studies demonstrating that a reduction in norepinephrine transmission does not affect latent inhibition in normal animals (Archer et al., 1983, Tsaltas et al., 1984, Mohammed et al., 1986). However, reducing norepinephrine levels restored latent inhibition in *coloboma* mice, suggesting that the increase in norepinephrine expressed by these mice disrupts attentional/associative processes.

Norepinephrine is known to play an important role in the regulation of attention. Electrophysiological recordings in rats and monkeys have revealed characteristic patterns of noradrenergic activity in response to salient environmental stimuli and during tasks that require selective attention (Aston-Jones and Bloom, 1981b, Aston-Jones and Bloom, 1981a, Aston-Jones et al., 1991, Foote et al., 1991, Aston-Jones et al., 1999, Aston-Jones et al., 2000, Brown et al., 2004). Further, chemical activation of the locus coeruleus produces EEG patterns in the forebrain and hippocampus that are characteristic of an alert state (Berridge and Foote, 1991). Psychopharmacological studies have also implicated norepinephrine in attention: Lesioning the dorsal noradrenergic bundle with neurotoxin 6-hydroxydopamine disrupts selective attention in rats (Mason and Iversen, 1978, Mason and Iversen, 1979, Carli et al., 1983). In *coloboma* mice, lesioning the noradrenergic system apparently counteracted pathological elevations in norepinephrine neurotransmission, producing beneficial effects on attention.

To evaluate impulsivity, mice were tested for their ability to resist a less preferred immediate reinforcer to obtain access to a more preferred reinforcer (Evenden and Ryan, 1996). In this delayed reinforcer paradigm, the indifference point was used as an indicator of impulsivity. Control mice exhibited a gradual decrease in water preference with the increasing delay for access to water and reached their indifference point at ~9 seconds. Conversely, the delay for access to water was extremely disruptive to *coloboma* mice regardless of the length of the delay, whereby a preference was never established following the imposition of a delay for water. Despite their atypical delay-discounting curve, *coloboma* mice lost their preference for water at 4.5 seconds, a delay half that of control mice, suggesting that *coloboma* mice are impulsive relative to control mice.

In contrast to latent inhibition, impulsivity was not improved after DSP-4 treatment. A reduction in impulsivity of *coloboma* mice would have been reflected by a rightward shift in the water preference curve—an increase in indifference point—as access to water was increasingly delayed. Instead, the indifference point of *coloboma* mice in the impulsivity test was not changed by DSP-4 treatment. The indifference point of control mice decreased after DSP-4 treatment. The reduction in norepinephrine may have induced impulsivity in control mice. Alternatively, the decrease in the indifference point of control mice may be attributable to learning the experimental paradigm and anticipating the increasing delays. Regardless, results of the DSP-4 study in *coloboma* mice suggest that norepinephrine does not directly mediate impulsivity in *coloboma* mice. However, this study cannot entirely eliminate the possibility that norepinephrine impacts impulsivity of *coloboma* mice. Greater norepinephrine depletion with a higher dose of DSP-4 may reduce impulsivity of *coloboma* mice.

In a study of different mouse strains, Isles *et al.* 2004 found that more hyperactive mouse strains tend also to be more impulsive, suggesting that common genetic factors mediate both hyperactivity and impulsivity. These symptoms occur together not only in ADHD, but also in Tourette syndrome and obsessive-compulsive disorder (Sheppard *et al.*, 1999), suggesting a common influence of genetics in humans, as well. Although *coloboma* mice exhibit both hyperactivity and impulsivity, the symptoms appear to be mediated by different mechanisms. Hyperactivity is mediated by norepinephrine in *coloboma* mice (Jones and Hess, 2003), whereas impulsivity is not, suggesting that while these abnormal behaviors may share a common genetic cause, the pathophysiological basis for the behaviors is probably not so closely linked.

Coloboma mice satisfy multiple validation criteria as a model of ADHD: face validity, construct validity, and etiological validity. Although the *coloboma* mouse model is a valid, useful model of ADHD, this model alone cannot provide a complete understanding of this complex disorder. Because a range of genetic and biological profiles can produce ADHD symptoms, multiple models are desirable. We have demonstrated that norepinephrine mediates the hyperactivity and disrupted latent inhibition in *coloboma* mice. The *coloboma* mouse model will be useful alongside other animal models in understanding the neurochemical and neurobiological mechanisms underlying this complex, heterogeneous disorder.

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Figure 1.

Locomotor activity of control and *coloboma* mice during the light and dark cycles. Mice (n=8/genotype) were habituated to the locomotor chambers for more than 4 hr before the start of the test. There was a significant effect of genotype on locomotor activity (** $p < 0.01$) during both the light and dark cycles. Data represent total beam breaks over 2 hours during the light cycle (9:00-11:00) and dark cycle (21:00-23:00). Data are expressed as means $\pm$ SEM.

Figure 2.

Preferences exhibited by control and *coloboma* mice in 2-bottle tests. *a*, Control mice (n=7) and *coloboma* mice (n=8) both consumed more water than 0.008 M HCl. *b*, Control mice (n=7) and *coloboma* mice (n=8) both consumed more water than 0.8 mM quinine. There was no difference between preferences exhibited by control and *coloboma* mice.

Figure 3.

Latent inhibition in control and *coloboma* mice. Mice were preexposed to 0.008 M HCl for 4 days before challenge with LiCl or were not preexposed and received only water. On the 5th day, all mice were presented with the Conditioning trial: HCl paired with LiCl. Mice were then presented with HCl again two days later during the Test trial. Data represent HCl consumption (mean $\pm$ SEM) during the Test trial (after the aversive pairing), expressed as a percent of consumption before LiCl treatment. *a*, During the Test trial, there was a significant difference in percent HCl consumption between nonpreexposed control mice ($n=10$) and preexposed control mice ($n=10$) ($*p<0.05$). However, there was not a significant difference in percent HCl consumption between nonpreexposed *coloboma* mice ($n=10$) and preexposed *coloboma* mice

(n=10). *b*, There was no difference in HCl consumption by control and *coloboma* mice during the Conditioning trial.

Figure 4.

Effect of 50 mg/kg DSP-4 on latent inhibition in control and *coloboma* mice. *a*, Saline treatment. During the Test trial, there was a significant difference in percent quinine consumption between nonpreexposed control mice ($n=8$) and preexposed control mice ($n=8$) (**** $p<0.0001$). However, there was not a significant difference in percent quinine consumption between nonpreexposed *coloboma* mice ($n=8$) and preexposed *coloboma* mice ($n=8$). *b*, DSP-4 treatment. During the Test trial, the percent quinine consumed by DSP-4-treated nonpreexposed control ($n=9$) and *coloboma* mice ($n=10$) was significantly lower than that consumed by DSP-4-treated preexposed control ($n=11$) and *coloboma* ($n=11$) mice

(** $p < 0.01$ for control mice, * $p < 0.05$ for *coloboma* mice). *c*, There was no difference in quinine consumption by control and *coloboma* mice during the Conditioning trial.

Figure 5.

Water preference with increasing delays as a test of impulsivity in control and *coloboma* mice. Initially, quinine and water were presented simultaneously and then increasing delays were imposed between the presentation of quinine, a nonpreferred tastant, and the presentation of water. *a*, Preference by control mice ($n=7$) gradually switched from water to quinine with the increasing delay. Control mice reached their indifference point, the point at which there was no clear preference for water, at a delay of 9.1 ± 1.1 seconds. *b*, *Coloboma* mice ($n=4$) reached their indifference point at a delay of 4.5 ± 1.1 seconds and did not form a preference at subsequent delays. There was a significant effect of genotype on indifference point ($p < 0.05$). Data represent percent preference for water and for 1 mM quinine. *c*, There was no difference in overall consumption by control and *coloboma* mice throughout the impulsivity experiment. Data are expressed as means $\pm$ SEM.

Figure 6.

Effect of 50 mg/kg DSP-4 on impulsivity in control and *coloboma* mice. Data represent the percent of completed trials where water was selected over 1 mM quinine. *a*, After DSP-4-treatment, the water preference curve of control mice ($n=7$ before DSP-4; $n=8$ after DSP-4) shifted to the left ($p<0.05$). *b*, Performance by *coloboma* mice ($n=4$ before DSP-4; $n=4$ after DSP-4 during 0-12-sec delays; $n=3$ during 16- and 20-sec delays) was unchanged by DSP-4 treatment. *c*, There was no difference in overall consumption by control and *coloboma* mice throughout the impulsivity experiment. Data are expressed as means $\pm$ SEM.

Table 1

Effect of DSP-4 treatment on forebrain catecholamine concentrations.

	Norepinephrine	Dopamine
+/+ Saline	28 ± 0.5	193 ± 4
+/+ DSP-4	21 ± 0.5 ^{***}	184 ± 5
<i>Cm</i> /+ Saline	31 ± 0.5 ⁺	179 ± 4
<i>Cm</i> /+ DSP-4	24 ± 0.5 ^{***}	170 ± 4 ⁺⁺

Values are expressed in ng/mL homogenate.

*** denotes significant difference ($p < 0.0001$) from saline-treated mice.

⁺ $p < 0.01$ and

⁺⁺ $p < 0.005$ denotes significant difference ($p < 0.001$) from saline-treated control mice. Data represent mice treated with saline or DSP-4 in the latent inhibition experiment (n=16 saline-treated control mice, n=16 saline-treated *coloboma* mice, n=19 DSP-4-treated control mice, n=21 DSP-4-treated *coloboma* mice).