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Research report

Genetic and dopaminergic modulation of reversal learning in a touchscreen-based operant procedure for mice

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Abstract

Mice are uniquely suited as experimental subjects for various approaches to the study of the molecular and genetic basis of behavior, and there has been a corresponding explosion in the use of mice in behavioral neuroscience. Rats and monkeys, however, remain the preferred species for high-order cognitive models largely due to the unavailability of valid, reliable and translatable endpoint measures of behavior in the mouse. Here we present further development and validation of a touchscreen-based operant method for measuring cognition that is comparable to methods used in other species and human patients. C57BL/6J mice were found to show good performance on visual discrimination and reversal learning using this method. Demonstrating the sensitivity of the paradigm to genetic factors, C57BL/6J and DBA/2J mice exhibited marked differences in discrimination and reversal learning. Systemic treatment with the selective D1-like agonist, SKF81297, produced an impairment in the early phase of reversal learning, but did not alter visual discrimination, in C57BL/6J mice. The same treatment impaired spatial working memory on the T-maze delayed alternation task, but did not alter control measures of behavior including motivation and locomotor activity. These data demonstrate the sensitivity of visual discrimination and reversal learning measured by this method to genetic factors and pharmacological challenge, and thereby provide an extension and further validation of the method for measuring cognition in mice. When combined with emerging molecular techniques uniquely suited to this species such as genetic engineering and RNA modification this paradigm could provide a powerful new tool for behavioral neuroscience.

Keywords: Mouse; Discrimination; Reversal; Cognition; Strain; Gene; Dopamine; SKF81297; D1 receptor

1. Introduction

Mice are uniquely suited as experimental subjects for various approaches to the study of the molecular and genetic basis of behavior, such as genetic engineering and quantitative trait loci analysis. As a result there has been a recent explosion in the use of mice in behavioral neuroscience, which in turn has spurred the need for valid and reliable endpoint measures of brain functions including behavior in the mouse [16]. This issue is particularly pertinent to paradigms measuring cognition, which have a longer tradition of use in the rat than the mouse. A small but growing literature has demonstrated that mice are capable of performing complex behaviors that model human cognitive functions ranging from attention, impulse-control, and working memory [3,26,31,33].

Bussey, Saksida, Rothblat and colleagues have recently described an automated instrumental learning procedure in which mice readily acquired stimulus-reward contingencies by nosepoking at visual stimuli presented on a touch-sensitive monitor [5,8]. This paradigm is similar to that previously used in rats [6,7,9] and monkeys [18] and has features comparable to automated systems used in evaluating cognitive function in human patient groups [42]. Thus it has considerable potential for mouse-to-human translational studies of brain function and neuropathology.

A form of cognition commonly assessed in animals is reversal learning. The ability to respond adaptively to a reversal in reward contingency of a learned discrimination pair measures

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the behavioral flexibility to inhibit previously rewarded behavior [11,27] As such, reversal learning may assay features of certain neuropsychiatric conditions characterized by cognitive inflexibility, impulsivity, and risk-taking, such as schizophrenia and drug addiction [11]. In this context, previous research has demonstrated that reversal learning in rats and monkeys [7,18,27,32] recruits brain regions implicated in the pathophysiology of these neuropsychiatric conditions [34]. Common variants of reversal learning in rodents include odor discrimination reversals [29,43], left/right lever reversals [19], spatial reversal on a T-maze [2], and discrimination reversals using visual stimuli [5,6,8,9]. Of these, the latter is most similar to that used with nonhuman primates and humans and is therefore highly conducive to cross-species comparisons.

The main aim of the present study was to further evaluate the utility of a touchscreen-based operant system for measuring cognitive function in mice, via assessment of visual discrimination and reversal learning. We first developed an in-house procedure for measuring visual discrimination and reversal learning in C57BL/6J inbred mice, the most commonly used mouse strain in behavioral neuroscience. Next, to test for possible strain differences in visual discrimination and reversal learning, we compared the performance of C57BL/6J with another popular mouse strain, DBA/2J. Differences between these two strains would provide a lead into studies aimed at identifying genetic factors underlying these behaviors because of the availability of C57BL/6J × DBA/2J recombinant inbred strains [13].

We next assessed the sensitivity of the paradigm to pharmacological insult. A growing corpus of data supports an important role for the dopamine system in modulating cognition; more specifically, dopaminergic neurotransmission mediated through the D1-like (i.e. D1, D5) receptor subfamily [23,24,44]. For example, loss of D1-like receptor function has been associated with working memory deficits in humans, non-human primates, and rodents [30,35], for review see [41]. However, although there is evidence that dopamine function supports some aspects of reversal learning (for review see [11]), a specific role of D1-like receptors in reversal learning has not been elucidated. In the present study we tested the effects of systemic treatment with the selective D1-like receptor agonist, SKF81297, on visual discrimination and reversal learning in C57BL/6J using the touchscreen-based system. Because of the novelty of the paradigm, we also conducted an experiment as a positive control for the effects of D1-like receptor agonist on a more well-established task for cognitive flexibility [2] that has been previously shown to be impaired by the drug [30], T-maze delayed alternation working memory.

2. Materials and methods

2.1. Subjects

Subjects were male C57BL/6J and DBA/2J mice obtained from The Jackson Laboratory (Bar Harbor, ME) aged 8–12-weeks old at the start of behavioral testing. Mice were housed in groups of 4–5/cage in a temperature- and humidity-controlled vivarium under a 12 h light/dark cycle (lights on 06:00 h). Mice were maintained on a restricted diet and kept at 85% of free-feeding body weight during behavioral testing in order to ensure sufficient motivation to work for food rewards. Mice were fed upon return to the home cage after testing. Testing was conducted during the light phase of the light/dark cycle after mice were acclimated to the testing room for 1 h. All experimental procedures were approved by the National Institute on Alcohol Abuse and Alcoholism Animal Care and Use Committee and strictly followed the NIH guidelines 'Using Animals in Intramural Research'.

2.2. Touchscreen-based operant system

The apparatus was a modified version of that previously described [5,6,8,9]. The operant chamber measuring 21.6 cm × 17.8 cm × 12.7 cm (model # ENV-307W, Med Associates, St. Albans, VT) was housed within a sound and light-attenuating box (Med Associates, St. Albans, VT). 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 pellet tray located at one end of the chamber. At the opposite end of the chamber there was a touch-sensitive screen ('touchscreen') (Light Industrial Metal Cased TFT LCD Monitor, Craft Data Limited, Chesham, U.K.), a houselight, and a tone generator. The touchscreen was covered by a black Plexiglas panel that had 2 cm × 5 cm windows separated by 0.5 cm and located at a height of 6.5 cm from the floor of the chamber. Stimuli on the touchscreen were visible through the windows (1 stimulus/window) (Fig. 1A). Stimulus presentation was controlled

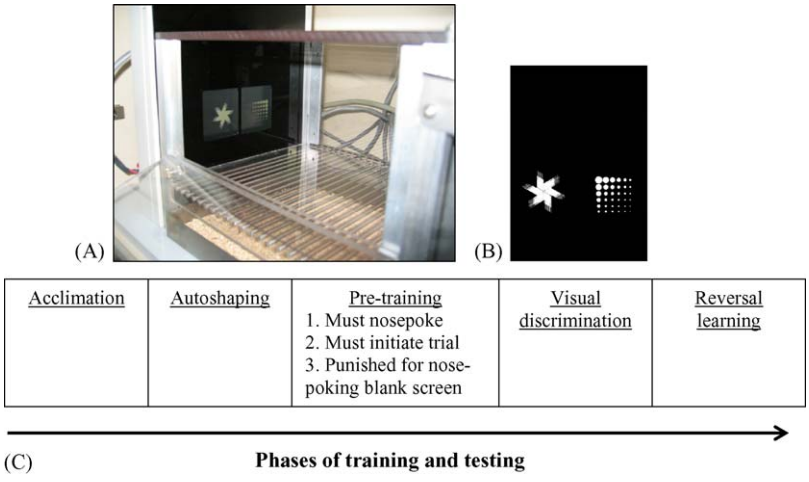

Fig. 1. Apparatus and testing protocol for the touchscreen-based operant procedure. (A) A mouse's eye view of the touchscreen. (B) Stimuli used for visual discrimination and reversal learning. (C) Schematic description of the phases of training and testing.

by custom software ('MouseCat', L.M. Saksida). The stimuli were those used previously [8] and were equiluminescent (Fig. 1B). Nosepokes at the stimuli were automatically detected by the touchscreen and recorded by the 'MouseCat' software.

2.3. Visual discrimination and reversal learning

The phases of training and testing are shown schematically in Fig. 1C. Mice were trained 5–6 days/week.

2.3.1. Acclimation

Mice were first acclimated to the 14 mg pellet food reward by provision of ~10 pellets/mouse in the home cage for 1–3 days. Mice were then acclimated to the operant chamber and to eating out of the pellet tray by being placed in the chamber for 30 min with pellets freely available in the tray. Upon reaching a criterion of eating 10 pellets within 30 min, mice were moved on to autoshaping.

2.3.2. Autoshaping

For autoshaping, two stimuli were presented on the touchscreen (one per window) for 10 s (inter-trial interval = 15 s). In this phase, the mouse was not yet required to respond to the stimuli; instead the disappearance of the stimuli coincided with the presentation of a 2 s 65 dB auditory tone, illumination of the pellet tray, and provision of a single pellet. The mouse was required to eat the pellet in the pellet tray (detected as a single nosepoke in the tray) in order for the next trial to commence. Upon reaching a criterion of eating 30 pellets within 30 min, the mouse moved on to pretraining.

2.3.3. Pretraining

The pretraining phase consisted of three stages: (1) a stimulus appeared pseudorandomly in one of the two windows and remained on the screen until the mouse nosepoked the stimulus to obtain a reward; (2) the mouse had to initiate the appearance of the stimulus by a head entry into the pellet tray and then nosepoke the stimulus to obtain the pellet; (3) a response at the blank window during presentation of the stimulus resulted in a 5 s (house) lights out punishment during which initiation of a new trial was not possible and subsequent presentation of the same left–right configuration of stimuli, a "correction trial", presented repeatedly until the correct stimulus was chosen. Each stage consisted of 30-trial test sessions with a 5 s inter-trial-interval (ITI). Upon reaching a criterion of 90% correct choices (excluding correction trials) the mouse moved on to the visual discrimination task.

2.3.4. Visual discrimination

Mice were presented with two novel stimuli. A response to one of the two stimuli (the S+) resulted in a single food pellet reward, and a response at the other stimulus (the S−) resulted in a 5 s (house) lights out punishment. The stimuli remained on the screen until a response was made. Designation of the stimulus as the S+ was counterbalanced across mice. Left–right presentation of the S+ was pseudo-randomized. There were 30 trials per session and a 5 s ITI. On reaching a criterion of an average of 85% correct (excluding correction trials) over two consecutive sessions, mice were moved on to reversal learning.

2.3.5. Reversal learning

For reversal, the reinforcement contingencies were reversed such that a nosepoke on the previous S+ was no longer rewarded but instead given a 5 s (house) lights out punishment, and a nose poke on the previously designated S− was now rewarded with a pellet.

The primary dependent measure of visual discrimination and reversal learning was percent correct choices. A number of auxiliary dependent variables were also obtained: number of correction trials, test time, reaction time to stimuli, and latency to retrieve pellet.

2.4. Baseline visual discrimination and reversal learning in C57BL/6J mice

Twenty-three C57BL/6J were tested on visual discrimination and reversal learning.

2.5. Strain differences in visual discrimination and reversal learning

Seventeen C57BL/6J and 14 DBA/2J mice were tested on visual discrimination and reversal learning.

2.6. Effect of SKF81297 on visual discrimination and reversal learning in C57BL/6J mice

The effects of treatment with the D1-like receptor agonist SKF81297 were tested in C57BL/6J mice. In each experiment, SKF81297 (Sigma, St. Louis, MO) was dissolved in a 0.9% physiological saline vehicle and injected intraperitoneally in a volume of 10 ml/kg body weight. For visual discrimination, 21 mice were treated with vehicle, 100 or 250 μg/kg SKF81297 5 min before each discrimination session until they reached criterion. For reversal learning, in the C57BL/6J mice that had undergone baseline visual discrimination and reversal learning described above, the reward contingencies were again reversed and mice were treated with vehicle, 100 or 250 μg/kg SKF81297 5 min before each reversal session. Doses were selected on the basis of previously reported impairing effects on T-maze working memory in mice [30].

2.7. T-maze delayed alternation working memory task

The apparatus and procedure was a modified version of that previously described [39]. The apparatus was constructed of black, opaque Plexiglas. The runway arm was 70 cm × 10 cm × 9 cm and the goal arms at the top of the runway were each 34.5 cm × 10 cm × 9 cm. Located at the end of each goal arm was a removable guillotine door that prevented the mouse from viewing the food before making its choice. The start box was a 10 cm-long portion of the runway arm (distal to the goal arms) sectioned-off by another removable guillotine door. Extra-maze cues were kept constant throughout training.

Nineteen C57BL/6J mice were first acclimated to the 'froot loops' food reward by provision of 3–4 froot loops/mouse in the home cage for 1–3 days. Each mouse was then acclimated to the T-maze, to being handled and to eating in the T-maze, by being exposed daily to the T-maze littered with quarter pieces of froot loops whilst being intermittently picked up by the experimenter. Upon reaching a criterion of eating 10 pieces of food within 8 min whilst being picked up five times, the mouse was moved onto training.

The mouse was given 11-trial daily test sessions. On the first trial of each session, both goal arms were baited with the reward. A trial began with the mouse positioned in the start box. The guillotine door was then raised and the mouse was allowed to traverse the length of the runway, choose a baited arm and eat the reward. The mouse was then returned immediately to the start box for the next trial and the maze was wiped down with 70% ethanol to prevent olfactory cues from guiding arm selection on the next trial. On each of the next 10 trials, only the goal arm that was not chosen on the previous trial was baited. A visit to the same goal arm as the previous trial was scored as incorrect and a visit to a different goal arm was scored as correct. Criterion was set at an average of 85% correct across two consecutive sessions. Upon reaching criterion at a given interval, the working memory load was increased on subsequent sessions by increasing the inter-trial interval (ITI) by 5 s up to a maximum of 15 s. On reaching a criterion of 60–80% correct over two consecutive sessions, each mouse was tested with either vehicle or 100 μg/kg SKF81297 on the following session. Treatments were administered i.p. 5 min before testing.

2.8. Open field test locomotor activity and anxiety-like behavior

The open field test was conducted as previously described [4]. The apparatus was a 40 cm × 40 cm × 35 cm square arena (50 lx) constructed of white Plexiglas. The mouse was placed in the perimeter and allowed to explore the apparatus for 30 min. Total distance traveled and average movement velocity in the whole arena, and percent time spent in the center (20 cm × 20 cm) was measured by the Ethovision videotracking system (Noldus Information Technology Inc., Leesburg, VA). Treatments were administered i.p. 5 min before testing.

2.9. Statistical analyses

Differences between mouse strains on discrimination and reversal learning were assessed by *t*-tests and differences between drug groups on these tasks were assessed by one-way analysis of variance (ANOVA). Effects of drug were analyzed using one-way ANOVAs for each of the first three sessions. Drug effects for the first session of reversal learning were analyzed using a repeated-measures ANOVA on the first three blocks of 10 trials. The effects of drug on delayed alternation in the T-maze were assessed using ANOVA, with repeated-measures for pre- versus post-drug performance. A probability level of ≤ 0.05 was used for measure of statistical significance. All data were analyzed using Statview software (SAS Institute, Cary, NC).

3. Results

3.1. Visual discrimination and reversal learning in C57BL/6J mice

Consistent with previous data [8], C57BL/6J mice showed acquisition of a visual discrimination problem. Because there was no significant effect of stimulus type (i.e. assignment of S+) on the rate of learning, data were collapsed across this variable for all analyses. Our main measure of learning was sessions to criterion; repeated-measures analyses across session were not performed due to attrition of the best performers early in learning. On average, mice progressed from chance-level performance to criterion within 10.0 ± 1.5 sessions. Acquisition curves for the first 20 sessions are shown in Fig. 2A.

As expected, reversing the reinforcement contingencies of the stimuli caused performance to drop to well below chance, to an average of 24.9% correct. As shown in Fig. 2B, performance then progressively increased over the subsequent 20 sessions, such that criterion was attained within 19.5 ± 1.7 sessions. Mean sessions to criterion for discrimination and reversal learning conditions are shown in Fig. 2C.

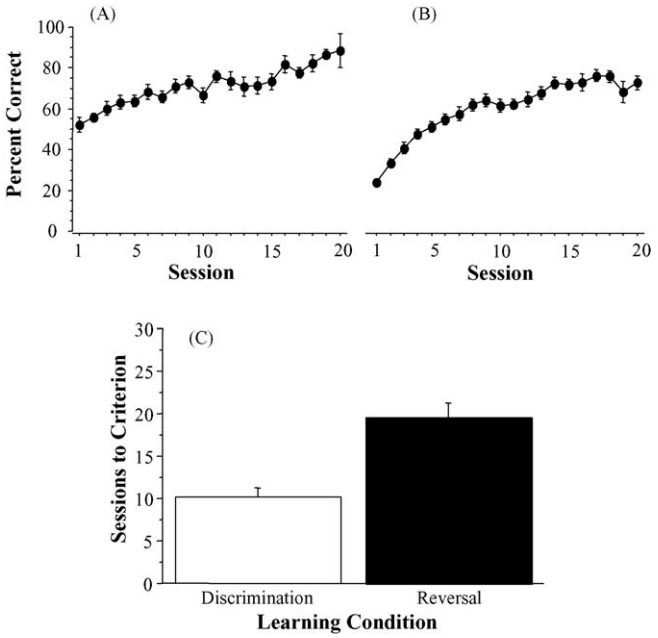

Fig. 2. Visual discrimination and reversal learning in C57BL/6J mice. (A) Acquisition curve during the first 20 sessions of visual discrimination. (B) Acquisition curve during the first 20 sessions of reversal learning. (C) Average number of sessions to reach criterion. $n = 23$. Data in Figs. 2–5 are means ± S.E.M.

3.2. Strain differences in visual discrimination and reversal learning

There were marked differences in visual discrimination and reversal learning between C57BL/6J and DBA/2J mice. DBA/2J mice learned the initial visual discrimination pair at a significantly faster rate than C57BL/6J mice ($t(29) = 3.3$, $p < 0.01$), reaching criterion within 4.8 ± 0.6 sessions, as opposed to 10.7 ± 1.6 sessions in C57BL/6J mice (Fig. 3A). The DBA/2J strain was quicker to finish sessions and retrieve the pellet from the magazine than C57BL/6J strain, while there were no differences in number of correction trials received or latency to respond to the stimulus (Table 1). DBA/2J mice showed faster reversal learning ($t(14) = 4.0$, $p < 0.01$), reaching criterion of 85% correct within 5.0 ± 1.8 sessions, as compared to 19.2 ± 2.5 sessions in C57BL/6J mice (Fig. 3B). To account for the possibility that faster reversal learning in the DBA/2J strain was due to the performance of fewer trials in discrimination learning compared to C57BL/6J mice, both strains were matched for experience in initial learning. Only mice learning in 6–10 sessions were included in the analysis. DBA/2J mice showed faster reversal learning even in this more restricted analysis ($t(12) = 2.6$, $p < 0.03$), reaching the 85% criterion within 7.1 ± 2.0 sessions as compared to 17 ± 3.2 sessions in the C57BL/6J strain. There were no strain differences in auxiliary measures of behavioral performance during reversal learning (Table 1).

Table 1
Auxiliary measures of visual discrimination and reversal learning performance in C57BL/6J and DBA/2J mice

	Visual discrimination		Reversal	
	C57BL/6J	DBA/2J	C57BL/6J	DBA/2J
Number of correction trials	20.1 ± 2.7	13.2 ± 1.7	42.9 ± 3.4	32.1 ± 3.7
Test time (min)	24.1 ± 1.7	15.2 ± 1.1*	25.7 ± 2.9	26.3 ± 3.2
Reaction time to stimuli (s)	13.8 ± 1.9	9.6 ± 0.9	6.5 ± 1.4	9.3 ± 1.4
Latency to retrieve pellet (s)	3.1 ± 0.5	1.6 ± 0.3*	1.7 ± 0.6	1.7 ± 0.5

DBA/2J 6J mice were significantly quicker to complete test sessions and were significantly slower to retrieve the pellet than C57BL/6J mice during visual discrimination, but not reversal learning. Neither the number of correction trials received, nor reaction time to the respond to the stimuli were different between strains during either visual discrimination or reversal learning. Data are means ± S.E.M. averaged across all sessions taken to reach criterion. $n = 14$–17/strain.

* $p < 0.05$ vs. C57BL/6J

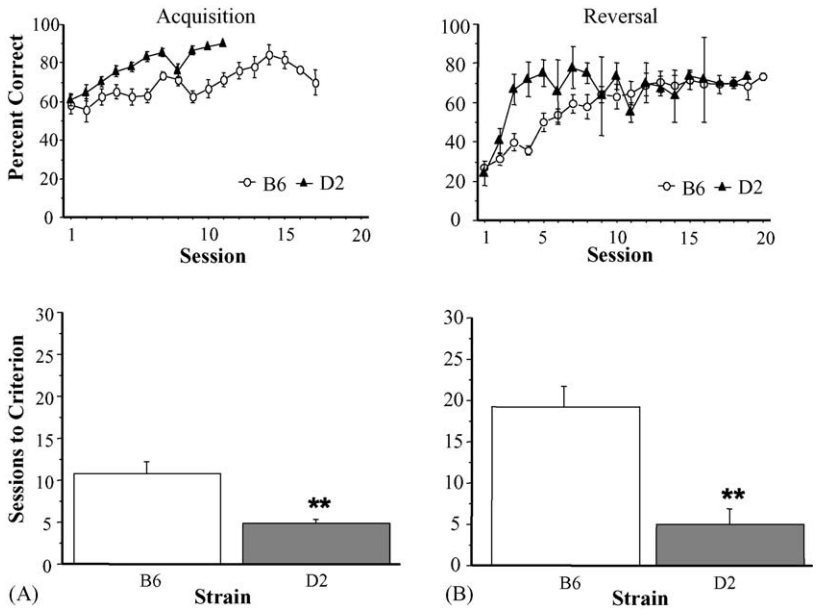

Fig. 3. Visual discrimination and reversal learning differs between the C57BL/6J and DBA/2J strains. (A) Acquisition of initial visual discrimination pair. Top: learning curves in the two strains. Bottom: DBA/2J mice required fewer sessions to reach criterion compared to C57BL/6J mice ($n = 14$–17/strain). (B) Reversal learning. Top: learning curves in the two strains upon reversal or reward contingency. Bottom: DBA/2J mice required fewer sessions to reach criterion compared to C57BL/6J mice ($n = 6$–12/strain). $^{**}p < 0.01$ vs. DBA/2J.

3.3. Effect of SKF81297 on visual discrimination and reversal learning in C57BL/6J mice

Treatment with SKF81297 impaired initial reversal learning. There was a significant effect of drug on percent correct during the first session of reversal learning ($F_{2,20} = 3.78$, $p < 0.05$), though there was no difference in number of sessions to criterion between doses. As shown in Fig. 4A, mice treated with either 100 or 250 μg/kg SKF81297 made significantly fewer correct responses than vehicle-treated controls. A non-significant trend for an impairing effect of drug on performance continued into the second session of reversal and was abolished by the third and subsequent sessions.

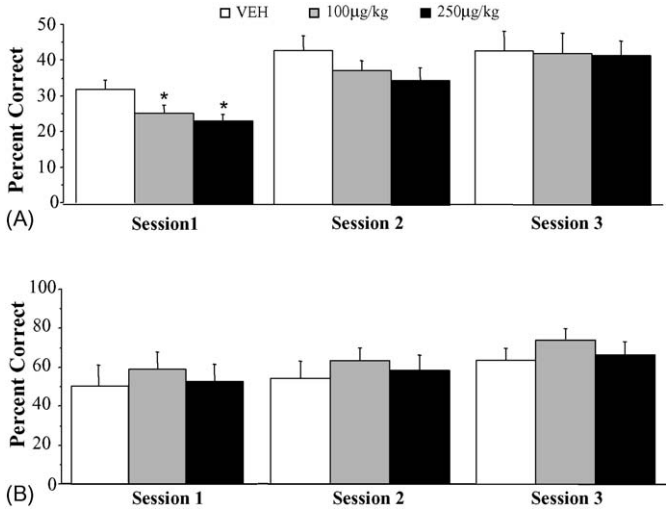

Fig. 4. Treatment with the D1-like agonist SKF81297 impairs initial reversal learning but not visual discrimination. (A) Performance was significantly impaired on the first session, and marginally impaired on the second, but not third, session of reversal learning in mice treated with 100 or 250 μg/kg SKF81297, as compared to vehicle (VEH)-treated controls. (B) Visual discrimination performance was not affected by SKF81297. $n = 7$–8/dose. $^{*}p < 0.05$ vs. VEH.

Further analysis of performance on the first reversal session revealed no significant differences in the number of correction trials ($F_{2,20} = 0.31$, $p = 0.73$), or test times ($F_{2,20} = 1.62$, $p = 0.22$) by dose. Both drug groups, however, did display marginally shorter latencies to retrieve the food reward in the pellet tray ($F_{2,19} = 2.77$, $p = 0.09$). Furthermore, partitioning the first reversal session into 3× 10-trial blocks revealed that mice receiving the highest dose (250 μg/kg) made a greater number of errors during the first 10-trial block of the first session ($F_{2,20} = 3.36$, $p = 0.05$), but not the second or third 10-trial block.

SKF81297 had no effect on sessions to criterion on visual discrimination ($F_{2,17} = 0.48$, $p = 0.63$) (Fig. 4B), nor was there an effect of drug on percent correct during any of the first three sessions of discrimination learning ($0.20 < F\text{'s} < 0.79$; $p\text{'s} > 0.47$). There was no effect of dose on auxiliary measures of behavioral performance during either discrimination or reversal learning (Table 2).

3.4. Effect of SKF81297 on the T-maze delayed alternation working memory task

Treatment with SKF81297 produced a significant impairment on the T-maze delayed alternation working memory task. During training, mice learned to alternate goal arms to an 85% criterion in an average of 17.8 sessions. Following the introduction of the delay between trials, mice dropped to a 71.3% performance average. There was a significant effect of the 100 μg/kg dose

Table 2
Auxiliary measures of reversal learning performance in mice treated with the D1-like agonist SKF81297

	Vehicle	100 μg/kg	250 μg/kg
Number of correction trials	59.5 ± 3.0	59.7 ± 1.4	58.2 ± 3.9
Test time (min)	21.0 ± 2.7	18.2 ± 1.8	20.7 ± 2.2
Reaction time to stimuli (s)	7.4 ± 0.8	8.7 ± 1.3	8.2 ± 1.0
Latency to retrieve pellet (s)	1.3 ± 0.2	1.6 ± 0.3	1.9 ± 0.4

Drug treatment did not significantly affect the number of correction trials received, test time, reaction time to respond to stimuli, or the latency to retrieve the pellet. $n = 7$–8/dose. Data are means ± S.E.M. averaged across all sessions taken to reach criterion

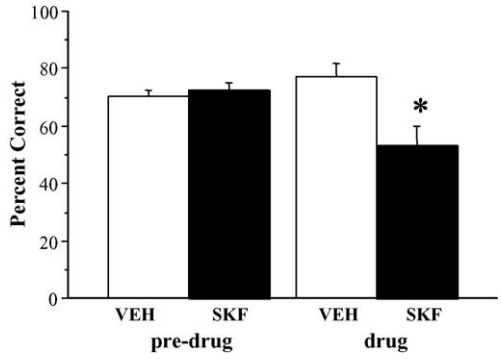

Fig. 5. Treatment with the D1-like agonist SKF81297 impairs delayed alternation working memory performance. A dose of SKF81297 (100 μg/kg) that impaired touchscreen-based reversal learning significantly impaired working memory on the T-maze delayed alternation task, as compared to vehicle (VEH)-treated controls. $n = 18$–20/dose. *$p < 0.05$ vs. VEH/drug.

of SKF81297 on delayed alternation performance ($F_{1,17} = 9.38$, $p < 0.01$). Drug-treated mice made significantly fewer correct responses than vehicle controls ($F_{1,17} = 8.66$, $p < 0.01$), and showed chance levels of performance (Fig. 5).

3.5. Effect of SKF81297 on open field locomotor activity and anxiety-like behavior

Treatment with SKF81297 did not significantly affect total distance traveled, velocity or center time in C57BL/6J mice in the open field test (Table 3).

Table 3
Open field locomotor exploration in C57BL/6J mice treated with the D1-like agonist SKF81297

	Vehicle	0.01 μg/kg	1 μg/kg	100 μg/kg
Total distance traveled (m)	6.7 ± 0.3	6.2 ± 0.3	6.3 ± 0.3	6.5 ± .4
Velocity (m/s)	3.7 ± 0.2	3.4 ± 0.2	3.5 ± 0.2	3.6 ± 0.2
Center time (% session)	13.4 ± 2.0	12.1 ± 2.2	9.5 ± 1.6	9.1 ± 1.3

Drug treatment did not significantly affect total distance traveled, velocity or center time. $n = 13$–15/dose. Data are means ± S.E.M.

4. Discussion

The present study provides an extension and further validation of a touchscreen-based operant procedure for measuring cognition in mice. Data confirm the utility of the paradigm for measuring visual discrimination and reversal learning and demonstrate the sensitivity of these behaviors to genetic factors and pharmacological challenge.

C57BL/6J mice readily learned a visual discrimination problem and its reversal on the touchscreen-based operant task. C57BL/6J mice progressed from chance-level performance to criterion within ∼10 sessions. When the reward contingencies were reversed, performance initially dropped to below chance, and then progressed to criterion within ∼20 sessions. The acquisition curves demonstrated by C57BL/6J mice are similar to those previously reported [5,8] and confirm good discrimination and reversal learning in this commonly-used mouse strain. These data provide a clear demonstration of the ability of C57BL/6J mice to accurately perceive and discriminate two-dimensional visual stimuli presented on a computer screen [50], to associate these stimuli with the occurrence of reward, and to switch responding when reward contingencies change. Thus, this strain provides an appropriate model system for future studies using this touchscreen-based paradigm, including those using genetically modified mice on the C57BL/6J background [14].

The present study also confirms the applicability of the touchscreen-based apparatus in another mouse strain frequently used in pharmacological and genetic studies of behavior, DBA/2J. In fact, DBA/2J mice displayed markedly superior learning to C57BL/6J on both the visual discrimination and reversal problems. Both strains began at the same level of performance in both learning conditions, indicating that DBA/2J mice did not show any initial visual or perceptual bias that could account for strain differences in subsequent learning. Nonetheless, DBA/2J mice took half as many trials to reach criterion on the discrimination problem as C57BL/6J mice. Strain differences were even more pronounced on reversal. High rates of responding according to the previous stimulus-reward contingency is expected early in reversal learning [27], and this is the pattern demonstrated by C57BL/6J mice, which took twice as many sessions to reach criterion on the reversal as compared to the initial discrimination problem. In contrast, while DBA/2J showed below-chance performance on the first session of reversal equivalent to that seen in C57BL/6J, the rate of reversal learning over subsequent sessions was remarkably rapid in the DBA/2J strain. Indeed, DBA/2J mice required the same number of sessions to achieve criterion on reversal learning as the initial visual discrimination.

Taken together, the pattern of performance in the DBA/2J strain can be construed as superior learning of the stimulus-reward association in visual discrimination learning as well as more flexible behavior and/or rule learning in their rapid behavioral shifting in response to reversal in reward contingency. Previous studies have shown that C57BL/6J and DBA/2J differ in learning and memory performance on various tasks, including the Morris water maze [25,36,49], Pavlovian fear conditioning [25,36,38], conditional spatial alternation [37], and an operant

delayed non-matching to position task [20,21]. Interestingly, some, but not all, studies found relatively better learning in C57BL/6J than DBA/2J [25,36,38,49], rather than vice versa as presently found. This suggests that cognitive differences between these two strains appear to be highly task-related; possibly due to the fact that tasks differentially recruit specific neural systems that are functionally diverse between the strains. Interestingly in this context, recent studies have shown that C57BL/6J and DBA/2J exhibit marked differences in baseline expression of multiple genes in brain regions thought to mediate reversal learning, such as the prefrontal cortex and hippocampus [17,28]. This raises the possibility of using these strains in this paradigm as a model to identify neural and genetic factors subserving reversal learning. As noted in the Introduction, DBA/2J and C57BL/6J strains are ideally suited to this type of approach because of the availability of recombinant inbred crosses between the strains (BXD RI). BXD RI strains have proven valuable in elucidating the mechanistic basis of various behavioral phenotypes in mice [13].

Present data also demonstrated that the touchscreen paradigm is sensitive to pharmacological as well as genetic factors. Systemic treatment with the selective D1-like receptor agonist SKF81297 selectively impaired reversal learning, not visual discrimination, in C57BL/6J mice. This dissociation is consistent with previous evidence that reversal learning is impaired by experimental dopaminergic manipulations in rats and non-human primates [12,19,29,40,45–47] (but see [15]) and is the first demonstration of such an effect in mice. Importantly, this effect was not explained by alterations in motivation levels (as indicated by normal reaction times to stimuli and latencies to retrieve pellet rewards after nosepoking stimuli), or disturbances to locomotor activity (as evidenced by normal open field behavior). It is also unlikely that impaired reversal learning was the result of attentional deficits, as doses as high as 300 μg/kg administered directly into the rat prefrontal cortex have been shown to improve rather than impair visual attention [10]. Moreover, we demonstrated that the same dose (100 μg/kg) of SKF81297 that impaired reversal learning produced a significant impairment on the T-maze delayed alternation test for spatial working memory in separate cohort of mice. This finding provides an important replication of only one previous study showing the same effect in mice [30]. Moreover, because performance on this task is suggested to recruit at least partially overlapping neural circuits as reversal learning [1,22], present data further suggest that the effects of SKF81297 on reversal learning are indicative of a true positive effect on a higher order cognitive function common to both tasks, such as cognitive flexibility.

It should be noted that the impairing effect of SKF81297 was limited to the early phase of reversal learning, suggesting a significant but modest and highly selective effect overall. This behavioral profile might be indicative of the relative sensitivity of the early phases of reversal learning to dopaminergic perturbation, when demands upon flexible responding and behavioral inhibition are greatest. It is also possible that greater impairing effects of a D1-like agonist on reversal learning would be uncovered by direct (rather than systemic) administration of the drug into specific brain regions mediating this behavior, and this will be an interesting avenue for future research using this paradigm.

In sum, present data provide further evidence in support of the utility and applicability of a touchscreen-based operant procedure for measuring complex cognitive processes in mice. The commonly used C57BL/6J and DBA/2J strains were found to show good visual discrimination and reversal learning performance, with clear cognitive differences between the two. In addition, systemic treatment with the D1-like receptor agonist, SKF81297, produced a selective impairment in reversal learning in the C57BL/6J strain.

While there has been an explosion in the use of mice in behavioral neuroscience and drug discovery in the past decade, there has arguably been a lag in the use of this species on more sophisticated behavioral paradigms [16]. The touchscreen-based operant system provides a novel example of the capacity for measuring complex behavioral processes in the mouse. When combined with emerging molecular techniques uniquely suited to this species such as genetic engineering and RNA modification [16,48], this paradigm could provide a powerful new tool for behavioral neuroscience.

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