Published in final edited form as:

Behav Brain Res. 2014 March 15; 261: 158–170. doi:10.1016/j.bbr.2013.12.015.

Performance of C57BL/6J and DBA/2J mice on a touchscreen-based attentional set-shifting task

Price E. Dickson¹, Michele A. Calton², and Guy Mittleman²

¹The Jackson Laboratory, 600 Main Street, Bar Harbor, ME 04609

²Department of Psychology, University of Memphis, Memphis, TN 38152

Abstract

Attentional set-shifting deficits are a feature of multiple psychiatric disorders. However, the neurogenetic mechanisms underlying these deficits are largely unknown. In the present study we assessed performance of C57BL/6J and DBA/2J mice on a touchscreen-based attentional set-shifting task similar to those used with humans and non-human primates. In experiment 1, mice discriminated simple white lines followed by compound stimuli composed of white lines superimposed on grey shapes. Although performance of the two strains was largely equivalent during early stages of the task, DBA/2J mice committed significantly more errors compared to C57BL/6J mice on the extra-dimensional shift. Additionally, performance of mice as a group declined across the three compound discrimination reversals. In experiment 2 we assessed salience of the shapes and lines dimensions and determined if dimensional salience, a variable previously shown to affect set-shifting abilities in humans and non-human primates, could be systematically manipulated. Findings from experiment 2 suggested that strain differences during the extra-dimensional shift in experiment 1 were most parsimoniously explained by a consistently impaired ability in DBA/2J mice to discriminate a subset of the compound stimuli. Additionally, unlike maze-based tasks, the relative salience of the two dimensions could be manipulated by systematically altering the width of lines exemplars while retaining other potentially-relevant attributes of the compound stimuli. These findings reveal unique and in some cases strain-dependent phenomena related to discriminations of simple and multidimensional visual stimuli which may facilitate future efforts to identify and fully characterize visual discrimination, reversal learning, and attentional set-shifting deficits in mice.

Keywords

attentional set-shifting; reversal learning; touchscreen; visual discrimination; C57BL/6J; DBA/2J; operant conditioning

© 2013 Elsevier B.V. All rights reserved.

Communicating author: Guy Mittleman, gmittlemn@memphis.edu, Telephone: 901.678.4213, Fax: 901.678.2579.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Conflicts of Interest: None

1. Introduction

Attentional set-shifting deficits are a feature of multiple disorders including schizophrenia [1, 2], autism spectrum disorder [3–6], Alzheimer's disease [7], and Parkinson's disease [8–12]. Studies in humans, non-human primates, and rodents have revealed that multiple brain regions and psychological processes are involved in the development and shifting of attentional sets [6, 11–18]. However, the neurogenetic mechanisms which underlie these deficits are unknown.

Mice are a powerful model system useful for dissecting the genetic and neurobiological mechanisms which underlie complex behaviors. The C57BL/6J (B6) and DBA/2J (D2) mouse strains are frequently compared [19–33] because they are genetically divergent [34] and because they are the progenitor strains of the BXD recombinant inbred lines [35]. Specifically, the existence of a phenotypic difference between the progenitor strains indicates that a systems genetics approach [36] using the BXD lines could be utilized to determine the genes and gene mechanisms underlying that phenotype. Indeed, the BXD lines have been used successfully for years to identify underlying genetic mechanisms of complex behavioral traits such as addiction and cognitive function [37–51]. Although simple visual discrimination and various executive function abilities of B6 and D2 strains have been assessed using several common operant conditioning tasks [20, 26, 32, 33, 52], multidimensional visual discrimination and attentional set-shifting performance of these strains has never been compared.

A frequently used task to assess attentional set-shifting abilities in humans is the intra-extra dimensional set-shifting (IED) task in the Cambridge Neuropsychological Test Automated Battery (CANTAB) [53]. Like other tests in the CANTAB battery, the IED task is performed using a touchscreen and is visually mediated. These two characteristics have enabled the development of similar versions for non-human primates [15, 17, 54, 55] and mice [56, 57], facilitating cross-species comparisons.

In the present study, performance of male B6 and D2 mice was compared using a mouse analog of the CANTAB intra-extra dimensional set-shifting task. In an initial experiment, mice sequentially learned to discriminate simple visual stimuli composed of white lines followed by compound stimuli composed of white lines superimposed on gray shapes. Mice then performed two intra-dimensional shifts, each followed by a reversal. During a final stage, mice performed an extra-dimensional shift during which the relevant dimension changed from white lines to gray shapes. In a second experiment, additional B6 and D2 mice were tested on the first three stages of the attentional set-shifting task. During this experiment, relevant dimension (lines or shapes) and line width (wide or narrow) were included as variables to determine if strain differences observed on the extra-dimensional shift in experiment 1 were unique to that stage or would also appear on shapes-relevant compound discriminations in earlier stages of the task. Additionally, we assessed relative salience of the lines and shapes dimensions used in the study and determined if it could be altered by systematically manipulating stimulus attributes. Dimensional salience may be an important factor in mouse attentional set-shifting studies because it has been shown to affect set-shifting abilities in humans and non-human primates [11, 17]. Finally, we assessed the

possibility that performance was dependent on the specific visual stimuli being discriminated and that this effect was dependent on strain and salience of the relevant dimension.

2. Materials and Methods

The following experiments were approved by the Institutional Animal Care and Use Committee at the University of Memphis and conducted in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. Efforts were made to reduce the number of animals used and to minimize animal pain and discomfort.

2.1. Subjects

Male and female C57BL/6J (B6; Stock Number: 000664) and DBA/2J (D2; Stock Number: 000671) mice were purchased from The Jackson Laboratory (Bar Harbor, ME). A single male and a single female of the same strain were housed together in standard cages in ventilated racks in the Animal Care Facility in the Department of Psychology at the University of Memphis. Adult male offspring from these breeder pairs were used as experimental subjects. After weaning at 4 weeks of age, experimental subjects were housed in groups of 3 – 5 until they entered the experiment at 12 weeks of age, at which point they were individually housed to facilitate food restriction. Mice had free access to food until they were individually housed, at which point they were food restricted to 90% of baseline weight. To avoid a litter effect, only one animal from each litter was assigned to an experimental group. Throughout the experiment, mice had free access to water and were maintained in a temperature controlled environment ($21 \pm 1^\circ\text{C}$) on a 12:12 light:dark cycle (lights on at 0800).

2.2. Apparatus

Training and testing was conducted in four operant conditioning chambers which have been described in detail previously [58]. Briefly, the front wall of each chamber consisted of an infrared touchscreen. The rear wall consisted of (1) a centrally mounted liquid dipper which provided access to 0.01 cc of Silk Vanilla Soymilk as a reward, (2) a trial initiation stimulus light located above the food receptacle, and (3) a house light centrally mounted at the top of the chamber. Operant conditioning chambers were controlled by a Lafayette Instruments control unit running ABET II and Whisker software. All operant conditioning schedules were written in-house using ABET II.

2.3. Visual Stimuli

During behavioral testing, mice discriminated pairs of visual stimuli presented on the touchscreen (Figure 1A). During the first stage of the task, mice discriminated exemplars from a single dimension of white lines (Figure 1B; experiments 1 and 2) or grey shapes (Figure 1C; experiment 2). In all subsequent stages, mice discriminated compound stimuli created by superimposing exemplars from the lines dimension onto exemplars from the shapes dimension. When compound stimuli were used, the location of the two exemplars from each dimension was randomized independently on each trial resulting in two possible stimulus configurations (Figure 1D and 1E). Lines were 6.5 cm long and either 0.2 cm wide

(experiments 1 and 2) or 0.04 cm wide (experiment 2). Figure 1F shows a comparison of stimuli containing wide and narrow lines. Length and width of exemplars from the shapes dimension varied. Simple and compound stimuli were always presented on a black background. All stimuli used in the experiment are shown in Figure 2 and were created using Adobe Photoshop. RGB values of the stimulus components were as follows: white lines (255, 255, 255), grey shapes (78, 78, 78), black background (0, 0, 0). The RGB values are relevant because they affect relative salience of the dimensions and the ability to dissociate the two exemplars in a compound stimulus (unpublished observations).

2.4. Operant Conditioning Task

2.4.1. Training—Mice were trained and tested in the same chamber and at the same time daily seven days per week until they completed the experiment. In order to control for idiosyncratic effects on performance caused by a specific chamber or testing time, we counterbalanced the number of mice from each strain that were tested in each chamber and in each testing group. Mice completed three training stages before testing began. Training stages lasted for 60 minutes or until mice reached criterion, whichever occurred first. In the first stage, the house light was always illuminated, the stimulus light was never illuminated, and stimuli were never shown on the touchscreen. The dipper arm was alternately raised for 20 seconds and then lowered for 20 seconds. The mouse reached criterion on this stage when he collected at least 20 rewards during a session.

In the second stage, a white square (6.5 cm × 6.5 cm) was randomly presented on the right or left of the screen. When the mouse nose-poked the square it disappeared from the screen and the dipper arm was raised for 10 seconds. The next trial began immediately following this. The mouse reached criterion on this stage when he collected at least 20 rewards in 60 minutes.

In the third and final training stage, an intertrial interval (ITI) and trial initiation requirement were added. The ITI, during which only the house light was illuminated, began immediately following the 10 second reward and lasted for 20 seconds. Following the ITI, the stimulus light located above the food receptacle was illuminated and the mouse was required to nose-poke the food receptacle to initiate the next trial. When a nose-poke occurred, the stimulus light was turned off and the white square appeared on the screen. The mouse reached criterion on the final training stage when he completed 80 trials in a single session.

2.4.2. Experiment 1: Attentional Set-shifting—B6 (n = 15) and D2 (n = 15) mice were tested on eight stages during which they discriminated pairs of simple or compound stimuli (Figure 2). The stages used in the experiment are shown in Figure 3. All testing stages were identical to the third training stage with the following exception. Immediately after a trial was initiated by the mouse, two visual stimuli were randomly presented on the right and left side of the screen. One of these stimuli was correct and the other was incorrect. A nose-poke to the correct stimulus resulted in access to the reward for 10 seconds. A nose-poke to the incorrect stimulus resulted in a 10 second timeout which was signaled by turning off the house light. Immediately following a nose-poke to either stimulus, the visual stimuli were removed from the screen. A 20 second ITI followed reward or timeout, at

which point the trial initiation light above the food receptacle was turned on signaling that the mouse could initiate another trial. Session duration was 60 minutes or until the mouse completed 80 trials, whichever occurred first. Once mice reached a criterion of 80% correct choices during a session, they advanced to the next stage of the experiment. This criterion was used for all experimental stages. All stimuli that were used in the study are shown in Figure 2. As shown in Table 1, we counterbalanced the order of presentation of these stimuli and the exemplar that was rewarded in each of these groups.

During the simple discrimination (SD) stage, mice discriminated exemplars from a single dimension of white lines presented randomly on the right and left of the touchscreen. Once mice reached criterion on the SD stage, they advanced to the compound discrimination (CD) stage. During the CD stage, a second dimension consisting of grey shapes was added to produce two compound stimuli. On each trial, the two exemplars from the lines dimension were superimposed on the two exemplars from the shapes dimension such that the four stimuli together formed two compound stimuli. Mice were rewarded when they nose-poked the compound stimulus containing the exemplar from the lines dimension which was correct on the SD stage. Stimuli were presented such that the correct exemplar from the lines dimension was superimposed on each of the two exemplars from the shapes dimension an equal number of times (e.g., Figure 1D and 1E). Therefore, exemplars from the shapes dimension had no predictive value with regard to reward. Exemplars from the lines dimension, however, perfectly predicted reward in that one of the two lines exemplars was always part of the correct compound stimulus and the other was never part of the correct compound stimulus. Thus, to learn the task, mice had to ignore exemplars from the non-relevant shapes dimension and selectively attend to exemplars from the relevant lines dimension.

Similar to our previously reported methods [58, 59], during each session of the SD stage and all subsequent stages, trials were considered either “correction” or “non-correction” trials depending on the correctness of the previous trial. Specifically, a correction trial followed an incorrect trial and a non-correction trial followed a correct trial. During correction trials, stimulus presentation was not randomized. Rather, the correct and incorrect stimuli were presented on the same side as in the previous trial. The purpose of this was to prevent the mouse from developing a strategy in which he ignored the visual stimuli, always chose the same side, and was therefore rewarded on 50% of the trials. A non-correction trial followed a correct trial, and stimulus presentation was randomized.

Once mice reached criterion on the CD stage, they advanced to the compound discrimination reversal stage (CDR). The CDR stage was identical to the CD stage with the exception that the previously correct exemplar from the lines dimension became the incorrect exemplar and vice versa. Once mice reached criterion on the CDR stage, they advanced to the first intra-dimensional shift stage (IDS1). The IDS1 stage was identical to the CD stage with the exception that novel exemplars from the lines and shapes dimensions were used. Importantly, because the IDS1 stage was an *intra*-dimensional shift, the lines dimension remained the relevant dimension. Once mice reached criterion on the IDS1 stage, they advanced to the first intra-dimensional shift reversal (IDS1R). The IDS1R stage was identical to the IDS1 stage with the exception that the previously correct exemplar from the

lines dimension became the incorrect exemplar and vice versa. Once mice reached criterion on the IDS1R stage, they advanced to the IDS2 stage and then to the IDS2R stage. These two stages were identical to the IDS1 and IDS1R stages with the exception that novel exemplars were used (Figure 3). Once mice reached criterion on the IDS2R stage, they advanced to the extra-dimensional shift (EDS) stage.

The EDS stage was identical to the IDS stages with two exceptions. First, novel stimuli from the lines and shapes dimensions were used. Second, and most importantly, *the relevance of the dimensions was reversed*. Specifically, exemplars from the previously-relevant lines dimension no longer had predictive value with respect to reward. Instead, the exemplars from the shapes dimension predicted reward. Thus, mice were required to stop attending to the previously-relevant lines dimension, which had been the relevant dimension during all previous stages, and begin selectively attending to the shapes dimension. Additionally, mice had to learn which of the two exemplars from the shapes dimension was correct. Mice were tested on the EDS stage until they reached criterion (80% correct on a session) or had completed 50 sessions (first cohort) or 80 sessions (second cohort), whichever occurred first.

2.4.3. Experiment 2: Dimensional Saliency—Although impaired performance at the EDS stage of the attentional set-shifting task could result from an impaired ability to shift an attentional set, it could also result from an impaired ability to discriminate grey shapes or greater saliency of white lines relative to grey shapes. To account for these possibilities and to assess if relative saliency of the lines and shapes dimensions could be systematically manipulated, we tested additional B6 ($n = 22$) and D2 ($n = 24$) mice on the SD, CD, and CDR stages. These stages were identical to those in experiment 1 with the following two exceptions. First, relevant dimension was added as a variable. Specifically, during the SD stage, mice discriminated exemplars from either the shapes dimension or the lines dimension. As in experiment 1, the relevant dimension during the SD stage (i.e., shapes or lines) remained the same during the CD and CDR stages. Second, line width was added as a variable. Specifically, during experiment 2, line width in simple and compound stimuli was either wide (0.2 cm in width) or narrow (0.04 cm in width). Line width in the wide lines conditions was the same as that used in experiment 1 (see Figure 1F for line width comparison). Thus, as shown in Table 2, experiment 2 was composed of four conditions: the *lines-relevant-wide* condition in which mice were required to attend to wide white lines and ignore grey shapes, the *shapes-relevant-wide* condition in which mice were required to attend to gray shapes and ignore wide white lines, the *lines-relevant-narrow* condition in which mice were required to attend to narrow white lines and ignore grey shapes, and the *shapes-relevant-narrow* condition in which mice were required to attend to gray shapes and ignore narrow white lines. Because conditions and stimuli in the *lines-relevant-wide* condition were identical to those from experiment 1, we used the B6 ($n = 15$) and D2 ($n = 15$) mice from experiment 1 for the *lines-relevant-wide* condition, and tested additional mice for the *shapes-relevant-wide* condition (B6: $n = 7$; D2: $n = 9$), the *lines-relevant-narrow* condition (B6: $n = 7$; D2: $n = 7$), and the *shapes-relevant-narrow* condition (B6: $n = 8$; D2: $n = 8$). In all conditions, mice were advanced from the SD and CD stages when they reached criterion (80% correct on a session) and completed the experiment once they reached

criterion (80% correct on a session) or had been tested for 50 sessions on the CDR stage, whichever occurred first.

2.5. Variables

2.5.1. Dependent Variables—The following dependent variables were collected at each stage of the attentional set-shifting task: errors to criterion (non-correction trials only), latency to stimulus choice, latency to collect a reward, and propensity to collect a reward. Latency to stimulus choice was defined as the time in seconds between stimulus onset and a nosepoke to one of the stimuli presented on the screen. Latency to collect a reward was defined as the time in seconds between a nosepoke to the correct stimulus on the screen and a head entry into the food receptacle. Propensity to collect a reward was defined as the percentage of correct trials on which a head entry occurred during the reward period following a nosepoke to the correct visual stimulus.

During the reversal stages, all errors during a session were defined as perseverative or learning errors depending on performance during that stage [58–61]. Specifically, errors committed during sessions on which performance was below chance levels (< 40% correct) were classified as perseverative errors, and errors committed during sessions on which performance did not differ from or was above chance (41% – 80% correct) were classified as learning errors.

2.5.2. Independent Variables—In addition to strain, stage, line width (experiment 2), and relevant dimension (experiment 2), exemplar group and stimulus counterbalancing-group were used as independent variables. Exemplar group (A – D) reflects the four groupings of 2 shapes and 2 lines that were presented together at a stage (Figure 2). Stimulus counterbalancing-group (1 – 4) reflects the order in which the four exemplar groups were presented across the SD - EDS stages in experiment 1 (Table 1). This order was counterbalanced to account for performance differences due to the order in which exemplar groups were presented or differences in discrimination difficulty of the exemplar groups themselves, as opposed to performance differences due to the unique requirements of each stage.

2.6. Statistical Methods

Analysis of Variance (ANOVA) was used to assess performance on the attentional set-shifting task. Prior to performing inferential statistical analysis, normality of all measures was assessed by inspecting normal probability plots. The assumption of homogeneity of variance across groups was assessed using Mauchly's test of sphericity. When this assumption was violated, the Huynh–Feldt correction was used. The criterion for statistical significance was $p < .05$. When performing multiple comparisons, Fisher's Least Significant Difference procedure was used.

3. Results

3.1. Experiment 1: Intra-Extra-dimensional Set-Shifting

3.1.1. Errors to Criterion—To examine performance of B6 and D2 mice on the attentional set-shifting task, we performed a $2 \times 4 \times 8$ mixed-model analysis of variance (ANOVA) using errors to criterion as the dependent variable, strain (B6 and D2) and stimulus counterbalancing-group (1 – 4) as between-subjects factors, and stage (SD - EDS) as a within-subjects factor. Repeated measures ANOVA revealed a significant stage $\times$ strain interaction [$F(7, 154) = 3.88, p < .05$], a significant stage $\times$ stimulus counterbalancing-group interaction [$F(21, 154) = 10.26, p < .001$], and a significant main effect of stage [$F(7, 154) = 42.16, p < .001$]. To determine the cause of the significant interactions, we examined performance of B6 and D2 mice on each stage of the attentional set-shifting task. As shown in Figure 4A, post hoc tests indicated that performance of B6 and D2 mice differed significantly only on the EDS stage. Specifically, the number of errors committed by D2 mice ($M = 734.60, SD = 539.25$) on the EDS stage was significantly greater ($p < .05$) than the number committed by B6 mice ($M = 444.47, SD = 295.72$). Additionally, as shown in Figure 4B, post hoc tests following up on the significant stage $\times$ stimulus counterbalancing-group interaction revealed that performance at most stages was significantly affected by the visual stimuli themselves, indicating that some exemplar groups were inherently more difficult to discriminate than others. The absence of a main effect of stimulus counterbalancing-group also indicates that differences in these subgroups across stages were due to differences in the discriminability of the stimuli themselves, not preexisting differences in discrimination abilities of mice in these subgroups.

The number of errors committed on the SD stage, IDS1 stage, and IDS2 stage did not differ significantly. This was true in both B6 and D2 mice and indicates that compound discriminations were not significantly more difficult than simple discriminations. However, the number of errors committed on the three reversal stages (i.e., CDR, IDS1R, IDS2R) increased significantly across reversals. Specifically, the number of errors committed on the CDR stage (B6: $M = 165.47, SD = 92.40$; D2: $M = 194.47, SD = 129.14$) was significantly less than the number committed on the IDS2R stage (B6: $M = 350.93, SD = 270.46$; D2: $M = 272.73, SD = 303.30$) in both strains. An analysis of perseverative errors (i.e., those committed when overall session performance was $\leq 40\%$ correct) and learning errors (i.e., those committed when overall session performance was $> 41\%$ correct) indicated that both strains committed significantly fewer learning errors on the CDR stage (B6: $M = 103.13, SD = 73.10$; D2: $M = 128.13, SD = 102.43$) relative to the IDS2R stage (B6: $M = 267.07, SD = 232.32$; D2: $M = 206.67, SD = 254.30$). The number of perseverative errors committed on the CDR stage (B6: $M = 62.33, SD = 33.52$; D2: $M = 66.33, SD = 39.30$) did not differ significantly from the number committed on the IDS2R stage (B6: $M = 83.87, SD = 45.84$; D2: $M = 66.07, SD = 63.19$) in either strain.

As shown in Figure 4C, worsening performance across reversal stages was most evident in mice that were discriminating stimuli from exemplar group B. These mice were in stimulus counterbalancing-groups 2, 1, and 3 on the CDR, IDS1R, and IDS2R stages, respectively. However, even when excluding these mice, errors consistently increased across the CDR (M

= 141.91, SD = 74.93), IDS1R (M = 158.36, SD = 63.04), and IDS2R stages (M = 189.61, SD = 112.20). Similar to the overall group, this pattern was due to consistent increases in learning errors (CDR: M = 86.45, SD = 61.83; IDS1R: M = 109.45, SD = 52.95; IDS2R: M = 135.91, SD = 98.12; CDR vs IDS2R: $F(1, 43) = 4.04$, $p = .05$). Perseverative errors remained stable across reversal stages (CDR: M = 55.45, SD = 28.39; IDS1R: M = 48.91, SD = 29.42; IDS2R: M = 53.70, SD = 27.58).

3.1.2. EDS Stage Failure Rate—Mice from experiment 1 were tested in two cohorts. In the first cohort, mice that failed to reach criterion following 50 sessions on the EDS stage were not tested further. This is similar to the 50-trial cut-off rule used with human subjects in the touchscreen attentional set-shifting task [9, 11]; this rule has also been used in the mouse U-maze version of the task [62]. In the first cohort, four out of seven D2 mice failed to reach criterion by session 50. In contrast, all seven B6 mice in the first cohort reached criterion by session 50. All mice reached criterion at all stages prior to the extra-dimensional shift in experiment 1.

To reduce the impact of this ceiling effect, in the second cohort we adjusted the cut-off to 80 sessions. In this cohort, one out of eight D2 mice failed to reach criterion by session 80 (including this mouse, 3 out of 8 D2 mice in cohort 2 failed to reach criterion by session 50). In contrast, all 8 B6 mice in the second cohort reached criterion before completing 50 sessions.

Of the seven D2 mice in experiment 1 that had not reached criterion on or before session 50 of the EDS stage, three mice were discriminating exemplar group D, one mouse was discriminating exemplar group A, and three mice were discriminating exemplar group B (stimulus counterbalancing-groups 2, 3, and 4, respectively). Out of the six D2 mice discriminating stimuli from exemplar groups B and D, some were rewarded for responding to exemplar 1 (left column Figure 2) in the B ($n = 1$) and D ($n = 1$) exemplar groups, and others were rewarded for responding to exemplar 2 (right column Figure 2) in the B ($n = 2$) and D ($n = 2$) exemplar groups. Because EDS stage failure rates indicated that performance of D2 mice seemed to be particularly impaired when discriminating these two exemplar groups, we compared performance of each strain within each of the four stimulus counterbalancing-groups. As shown in Figure 4C, the strain difference at the EDS stage was driven almost completely by performance differences between B6 and D2 mice in stimulus counterbalancing-groups 2 and 4 (discriminating exemplar-groups D and B, respectively). Notably, it is likely that the EDS strain differences illustrated in Figure 4A would have been substantially greater if the D2 mice that failed to reach criterion (four in cohort 1 and one in cohort 2) were not terminated at the 50 and 80 session cut-offs, respectively.

3.1.3. Latency to Stimulus Choice—We performed a $2 \times 4 \times 8$ mixed-model ANOVA to examine the effects of strain and stage on the latency to stimulus choice following stimulus onset. We used latency to stimulus choice as the dependent variable, strain and stimulus counterbalancing-group as between-subjects factors, and stage as a within-subjects factor. Repeated measures ANOVA revealed a significant main effect of stage [$F(7, 154) = 16.44$, $p < .001$]. Post hoc comparisons revealed that latency to stimulus choice became shorter across stages in both B6 (SD: M = 4.38, SD = 1.76; CD: M = 3.72, SD = 1.82; CDR:

M = 3.82, SD = 1.16; IDS1: M = 2.93, SD = 0.68; IDS1R: M = 3.02, SD = 0.83; IDS2: M = 2.80, SD = 0.84; IDS2R: M = 2.84, SD = 0.72; EDS: M = 2.64, SD = 0.62) and D2 mice (SD: M = 5.27, SD = 2.41; CD: M = 3.26, SD = 1.37; CDR: M = 3.82, SD = 1.56; IDS1: M = 2.93, SD = 0.71; IDS1R: M = 3.02, SD = 0.97; IDS2: M = 2.99, SD = 1.25; IDS2R: M = 3.52, SD = 1.42; EDS: M = 3.05, SD = 0.75).

3.1.4. Latency and Propensity to Collect Reward—We performed a $2 \times 4 \times 8$ mixed-model ANOVA to examine the effects of strain and stage on the latency and propensity to collect a reward following a nosepoke to the correct stimulus. We used strain and stimulus counterbalancing-group as between-subjects factors and stage as a within-subjects factor.

Using latency to collect a reward as the dependent variable, repeated measures ANOVA revealed a significant main effect of strain [$F(1, 22) = 14.74, p < .01$] and main effect of stage [$F(7, 154) = 6.30, p < .01$]. Post hoc comparisons revealed that reward collection latency (s) of B6 mice (SD: M = 1.58, SD = 0.22; CD: M = 1.49, SD = 0.29; CDR: M = 1.48, SD = 0.24; IDS1: M = 1.38, SD = 0.16; IDS1R: M = 1.38, SD = 0.18; IDS2: M = 1.35, SD = 0.22; IDS2R: M = 1.35, SD = 0.17; EDS: M = 1.33, SD = 0.15) was significantly shorter than reward collection latency of D2 mice (SD: M = 1.76, SD = 0.30; CD: M = 1.61, SD = 0.28; CDR: M = 1.72, SD = 0.25; IDS1: M = 1.61, SD = 0.15; IDS1R: M = 1.61, SD = 0.16; IDS2: M = 1.62, SD = 0.12; IDS2R: M = 1.66, SD = 0.15; EDS: M = 1.63, SD = 0.07) on the CDR – EDS stages. Additionally, reward collection latency decreased significantly in both strains across stages.

Using percentage of trials on which a reward collection occurred as the dependent variable, repeated measures ANOVA revealed no significant main effects or interactions. Examination of group means indicated that a reward was collected on almost all rewarded trials by B6 (M = 97.28%; SD = 2.10%) and D2 mice (M = 98.05%; SD = 1.50%).

3.1.5. Trials Completed Per Session—We performed a $2 \times 4 \times 8$ mixed-model ANOVA to examine the effects of strain and stage on the number of trials completed during a session. We used the average number of trials completed during a session at each stage as the dependent variable, strain and stimulus counterbalancing-group as between-subjects factors, and stage as a within-subjects factor. Repeated measures ANOVA revealed a significant main effect of stage [$F(7, 154) = 8.97, p < .001$] but no significant effects of strain or stimulus counterbalancing-group. The significant main effect of stage resulted from mice completing more trials per session over time (SD: M = 71.80, SD = 10.27; CD: M = 76.72, SD = 6.92; CDR: M = 75.78, SD = 5.38; IDS1: M = 78.60, SD = 5.01; IDS1R: M = 77.91, SD = 5.02; IDS2: M = 78.24, SD = 3.78; IDS2R: M = 79.48, SD = 1.15; EDS: M = 79.06, SD = 1.63).

3.2. Experiment 2: Effects of Line Width, Dimension, and Exemplar Group on Performance of B6 and D2 Mice

In experiment 2, we examined effects of relevant dimension and line width to determine how discrimination performance and dimensional salience were affected by these variables and to determine if any of these effects varied as a function of strain. Additionally, we

included exemplar group as a variable to account for the possibility that performance on any of these factors was affected by stimulus attributes unique to an exemplar group. Additionally, with respect to experiment 1, we wanted to determine if strain differences observed on the extra-dimensional shift in experiment 1 were unique to that stage or would also appear on shapes-relevant compound discriminations in earlier stages of the task.

We performed a $3 \times 2 \times 2 \times 2 \times 4$ mixed-model ANOVA using errors to criterion as the dependent variable, stage (SD - CDR) as a within-subjects factor, and strain, relevant dimension (white lines or grey shapes), line width (wide or narrow), and exemplar group (A - D) as between-subjects factors. Repeated measures ANOVA revealed multiple significant interactions including a significant 5-way interaction of all independent variables [$F(6, 88) = 2.99, p < .05$], a significant 4-way stage $\times$ relevant dimension $\times$ line width $\times$ exemplar group interaction [$F(6, 88) = 3.26, p < .01$], a significant 3-way stage $\times$ line width $\times$ exemplar group interaction [$F(6, 88) = 3.58, p < .01$], a significant 3-way stage $\times$ relevant dimension $\times$ exemplar group interaction [$F(6, 88) = 5.58, p < .0001$], a significant 3-way stage $\times$ relevant dimension $\times$ line width interaction [$F(2, 88) = 93.22, p < .0001$], a significant stage $\times$ exemplar group interaction [$F(6, 88) = 4.71, p < .001$], a significant strain $\times$ exemplar group interaction [$F(3, 44) = .6.69, p < .01$], and a significant relevant dimension $\times$ line width interaction [$F(1, 44) = 87.52, p < .0001$]. There were also main effects of stage [$F(2, 88) = 206.80, p < .0001$] and exemplar group [$F(3, 44) = 7.64, p < .01$]. To determine the cause of the multiple interactions in experiment 2, we examined post hoc tests as detailed below.

3.2.1. Effects of Line Width Manipulation on Dimensional Salience—To examine the effects of relevant dimension and line width on dimensional salience and the possibility that this relationship varied as a function of strain, we compared performance of each strain at each stage in each of the relevant dimension $\times$ line width subgroups (i.e., lines-relevant-wide, shapes-relevant-wide, lines-relevant-narrow, and shapes-relevant-narrow). In this analysis, we collapsed across exemplar group to examine the overall effect of relevant dimension and line width on dimensional salience. As shown in Figure 5A and 5B, post hoc comparisons indicated that performance of B6 and D2 mice did not vary as a function of relevant dimension or line width during the SD stage. This indicates that mice were able to discriminate grey shapes, narrow white lines, and wide white lines equally well when they were presented as simple, non-compound stimuli. During the CD and CDR stages when exemplars from the non-relevant dimension were combined with exemplars from the relevant dimension to make compound stimuli, two relationships were observed.

First, as shown in Figure 5A and 5B, when compound stimuli were composed of wide white lines and grey shapes (i.e., lines-relevant-wide and shapes-relevant-wide conditions), mice committed more errors to criterion when the relevant dimension was shapes. This indicates that when lines were wide, the lines dimension was significantly *more salient* than the shapes dimension. D2 mice in the shapes-relevant-wide condition committed significantly ($p < .05$) more errors at the CD ($M = 254.22, SD = 293.50$) and CDR stages ($M = 679.22, SD = 348.80$) than D2 mice in the lines-relevant-wide condition (CD: $M = 61.80, SD = 59.54$; CDR: $M = 194.47, SD = 129.14$). B6 mice in the shapes-relevant-wide condition also committed more errors at the CD ($M = 144.14, SD = 104.99$) and CDR stage ($M = 646.43$,

SD = 249.41) than B6 mice in the lines-relevant-wide condition (CD: M = 32.40, SD = 33.42; CDR: M = 165.47, SD = 92.40). In B6 mice, this difference reached statistical significance ($p < .05$) on the CDR but not CD stage.

Second, when compound stimuli were composed of narrow white lines and grey shapes (i.e., lines-relevant-narrow and shapes-relevant-narrow conditions), mice committed significantly more errors to criterion when the relevant dimension was lines. This indicates that when lines were narrow, the lines dimension was significantly *less salient* than the shapes dimension. D2 mice in the shapes-relevant-narrow condition committed significantly fewer ($p < .05$) errors at the CD (M = 92.88, SD = 176.77) and CDR stage (M = 212.00, SD = 121.70) than D2 mice in the lines-relevant-narrow condition (CD: M = 300.71, SD = 305.73; CDR: M = 609.29, SD = 348.89). B6 mice in the shapes-relevant-narrow condition committed fewer errors at the CD (M = 27.63, SD = 22.72) and CDR stage (M = 183.75, SD = 96.75) than B6 mice in the lines-relevant-narrow condition (CD: M = 188.71, SD = 161.54; CDR: M = 556.14, SD = 223.38). In B6 mice, this difference reached statistical significance ($p < .05$) on the CDR stage and approached statistical significance ($p < .06$) on the CD stage.

3.2.2. Effects of Exemplar Group on Performance—The strain difference at the EDS stage in experiment 1 was primarily driven by mice that were discriminating stimuli from exemplar groups B and D (Figure 4C). While this could indicate a strain difference in set shifting abilities mediated by specific exemplar attributes, it is also possible that this difference can be more parsimoniously explained by a strain difference in the overall ability to discriminate those specific exemplar groups or a strain difference in the ability to discriminate those exemplar groups under the specific salience conditions present during the EDS stage (i.e., a non-salient relevant dimension). To assess these possibilities, we examined performance of B6 and D2 mice in all exemplar groups at all stages (SD - CDR) under conditions in which the relevant dimension was salient (i.e., lines-relevant-wide and shapes-relevant-narrow) and conditions in which the relevant dimension was non-salient (i.e., shapes-relevant-wide and lines-relevant-narrow). As shown in Figure 6 panels A and B, performance of B6 and D2 mice differed on multiple exemplar groups under multiple conditions during experiment 2. Two patterns are notable in these data.

First, although several significant strain differences were observed on simple discriminations of both lines and shapes stimuli during the SD stage, the most robust strain differences occurred on compound discriminations when the relevant dimension was non-salient (i.e., shapes-relevant-wide and lines-relevant-narrow conditions). This was particularly true on compound discrimination reversals. This is underscored by the failure of multiple D2 mice to reach criterion during the CDR stage when the relevant dimension was non-salient. These D2 mice were in the (1) shapes-relevant-wide condition discriminating exemplar groups B ($n = 2$) and D ($n = 3$) and (2) lines-relevant-narrow condition discriminating exemplar group B ($n = 1$). Out of these six D2 mice, some were rewarded for responding to exemplar 1 (left column Figure 2) in the B ($n = 2$) and D ($n = 2$) exemplar groups, and others were rewarded for responding to exemplar 2 (right column Figure 2) in the B ($n = 1$) and D ($n = 1$) exemplar groups. Three B6 mice also failed to reach criterion on the CDR stage under these salience conditions. These mice were in the (1) shapes-relevant-wide condition

discriminating exemplar groups A ($n = 1$) and B ($n = 1$), and (2) lines-relevant-narrow condition discriminating exemplar group C ($n = 1$). All B6 and D2 mice in these conditions reached criterion during the SD and CD stages. With respect to D2 mice, these data mirror experiment 1 (Figure 4C) in that performance of D2 mice relative to B6 mice was significantly impaired when discriminating the B and D exemplars groups on the EDS stage during which shapes were relevant but non-salient. However, the significant impairments of B6 mice discriminating exemplar group A in experiment 2 were not observed at the EDS stage in experiment 1 (Figure 4C, stimulus counterbalancing-group 3).

Second, with one exception, significant strain differences were not observed on compound discriminations when the relevant dimension was salient (i.e., lines-relevant-wide and shapes-relevant-narrow conditions). Additionally, all mice in these conditions reached criterion at all stages. These data mirror performance in experiment 1 (Figure 4C) during which strain differences were not observed on the lines-relevant IDS1, IDS1R, IDS2, and IDS2R stages (when lines were salient) which preceded the EDS stage.

4. Discussion

In the present study, performance of B6 and D2 mice was assessed using an attentional set-shifting task, a touchscreen-based task similar to those used with humans and non-human primates. In experiment 1, mice discriminated simple white lines followed by compound stimuli composed of white lines (relevant dimension) superimposed on grey shapes (non-relevant dimension). On the extra-dimensional shift when the relevant dimension changed from white lines to grey shapes, D2 mice committed significantly more errors compared to B6 mice (Figure 4A). However, this overall effect was the result of strain differences which were limited to exemplar groups B and D (Figure 4C); significant strain differences did not occur in exemplar groups A and C. Indeed, performance of D2 mice discriminating stimuli from exemplar groups B and D was impaired to such a degree on the EDS stage that multiple mice did not reach criterion. With the exception of a strain difference in mice discriminating exemplar group B during the SD and CDR stage, no significant strain differences were observed between B6 and D2 mice in any of the four exemplar subgroups during any stage other than the EDS stage. However, performance of mice as a group declined across the three compound discrimination reversals.

In a second experiment, we examined the effects of relevant dimension (lines or shapes) and line width (wide or narrow) on performance during the SD, CD, and CDR stages to determine (1) if strain differences observed on the extra-dimensional shift in experiment 1 were unique to that stage or would also appear on shapes-relevant compound discriminations in earlier stages of the task, and (2) if salience of the lines and shapes dimensions differed and if relative salience of the two dimensions could be altered by systematically manipulating line width. During the SD stage, mice were able to discriminate grey shapes, narrow white lines, and wide white lines equally well when they were presented as simple, non-compound stimuli (Figure 5). However, during the CD and CDR stages when mice discriminated compound stimuli composed of exemplars from the relevant and non-relevant dimensions, the pattern of errors indicated that white lines were more salient than grey shapes when lines were wide, but less salient than grey shapes when lines

were narrow. Analysis of strain differences during experiment 2 indicated that performance of D2 mice was impaired when discriminating exemplar groups B and D and performance of B6 mice was impaired when discriminating exemplar group A. This effect was most robust during compound discriminations when the relevant dimension was non-salient, an effect which likely contributed to observed strain differences during the EDS stage of experiment 1.

4.1. Performance Differences between C57BL/6J and DBA/2J Mice on the Attentional Set-shifting Task

During the EDS stage of experiment 1, overall performance of D2 relative to B6 mice was significantly impaired. Several factors could account for this impairment including strain differences in relative salience of the shapes and lines dimensions, strain differences in fundamental simple or compound discrimination abilities, or strain differences in set shifting abilities. Although strain differences were not observed in overall discrimination abilities or relative salience of the lines and shapes dimensions in experiment 2, a more complex pattern emerged when examining performance of the two strains within each of the four exemplar groups. Specifically, performance of D2 mice during experiments 1 and 2 was consistently impaired relative to B6 mice, but only when discriminating stimuli from exemplar groups B and D; these impairments were most evident when performing compound discriminations when the relevant dimension was non-salient. Impairments in D2 mice were not observed when discriminating stimuli from exemplar groups A and C, regardless of the relevant dimension. Considering this, the most parsimonious explanation for the strain difference observed during the EDS stage of experiment 1 may be a robust impairment in the ability of D2 mice to discriminate shapes-relevant compound stimuli from exemplar groups B and D when the relevant dimension is non-salient.

One question, however, is what properties of certain exemplar groups would result in strain-dependent differences in simple and compound discrimination performance. One possible explanation is that D2 and B6 mice tend to observe different regions of the screen when making a response. Because stimuli are quite complex and relatively large (6.5 cm × 6.5 cm), this tendency would result in B6 and D2 mice consistently viewing different regions of each stimulus when making a response, and these regions could differ in their discriminability. Strain differences in the probability of sampling a particular stimulus region could have resulted from strain-dependent differences in response patterns (e.g., rearing when responding or responding preferentially to edges of the stimuli) or subtle strain differences in body or head position when making a response. It is also possible that strain differences in the probability of attending to a certain region of the visual field could result in preferential sampling of different stimulus regions. We should note, however, that although we have observed varying response patterns in individual mice (e.g., rearing to respond), we have not systematically characterized these behaviors in individual mouse strains. Thus, it is possible that mechanisms unrelated to strain-dependent differences in stimulus sampling could contribute to or fully explain the observed strain differences in discrimination performance of the B and D exemplar groups.

A second question is why strain-dependent differences in simple and compound discrimination performance would be more robust when the relevant dimension is non-salient. One possible explanation is that under these conditions, the non-relevant but salient exemplars provide highly effective distractor stimuli which increase attentional load and therefore the difficulty of the underlying discrimination. Additionally, because many of the strain differences occurred during the reversal stage of experiment 2 and the extra dimensional shift of experiment 1, it is possible that increased behavioral flexibility requirements during these stages interacted with increased attentional load to increase the overall difficulty of these discriminations [58]. Regardless of the exact mechanisms involved, these results indicate that visual discrimination abilities of differing mouse strains do not exist in isolation, but rather can depend to a large degree on the visual stimuli being discriminated. This may be an important consideration when comparing the effects of mutations on different genetic backgrounds or employing a systems genetics approach to discover genes and gene mechanisms underlying visual discrimination, reversal learning, or attentional set-shifting abilities.

Although collectively the data from experiment 1 and 2 reveal underlying strain-differences in discrimination abilities of some exemplar groups, it remains possible that performance during the EDS stage of experiment 1 was also influenced in a strain-dependent manner by abilities related to set shifting or development of an attentional set. Regarding this, it is interesting to note that the impaired ability of B6 mice to discriminate compound stimuli from the A exemplar group which was observed in experiment 2 (Figure 6 panel A and B) was not present during the EDS stage of experiment 1 (Figure 4C, stimulus counterbalancing-group 3). However, when considering this, it is important to note that although B6 and D2 mice as a group committed significantly more errors during the EDS stage relative to the previous IDS2 stage, this by itself does not indicate the development of an attentional set. Specifically, because relevant exemplars on the EDS stage were relatively less salient than non-relevant exemplars, it is possible that the overall increase in errors committed during the EDS stage relative to the IDS2 stage simply reflects a greater difficulty attending to a relevant but non-salient dimension in the presence of non-relevant but highly-salient distractor stimuli. Thus, it is possible that neither B6 nor D2 mice developed an attentional set in the present study. In this regard, many but not all previous studies have concluded that mice have the ability to develop an attentional set [14, 56, 57, 62–73], although the majority of those studies used the U-maze version of the task and all reported using equally salient dimensions. Future studies will be needed to clearly dissociate strain differences in discrimination abilities related to unique sets of stimuli from those related to the development and shifting of an attentional set.

One strategy for establishing the development of an attentional set is the use of an experimental design in which mice perform both a line-to-shape shift and a shape-to-line shift. In such a scenario, it is necessary for the lines and shapes dimensions to be equally salient in order to determine if increased errors during the EDS stage are due to the development of an attentional set or greater difficulty attending to a non-salient dimension. As we've shown in the present study, this can be accomplished in the touchscreen version of the attentional set-shifting task by systematically increasing or decreasing the width of lines exemplars in order to create equally salient dimensions. Notably, while this manipulation

has a large effect on the relative salience of the two dimensions, other potentially-relevant attributes of the stimuli such as the relative position of exemplar components in a compound stimulus are retained (Figure 1F). This may be particularly important when considering that strain differences in discrimination abilities varied across exemplar groups in the present study. Although this dual-shift design removes the potentially confounding effect of differential dimensional salience on performance during the EDS stage, it leaves unassessed the possibility that stimulus salience itself may interact with set-shifting abilities.

4.2. The importance of including a salience component in mouse attentional set-shifting tasks

Although attentional set-shifting abilities of humans and non-human primates have typically been assessed using a touchscreen-based paradigm similar to the one used in the present study, attentional set-shifting abilities of mice have most frequently been assessed using a maze-based paradigm (i.e., the U-maze) in which mice dig for a food reward in bowls filled with scented digging media [e.g., 14, 63, 65, 73]. An important characteristic of the U-maze task is that the odor and media dimensions have consistently been reported to be equally salient. One reason for this may be that salience of the media-texture and media-odor dimensions typically used in the U-maze task are difficult to precisely control, or that attending to dimensions from separate sensory modalities in the U-maze is less demanding on attentional control in comparison to attending to dimensions from the same sensory modality in the touchscreen task. In any case, consistent findings of equally salient dimensions in the mouse U-maze task contrast with findings from many [11, 17, 74, 75], but not all [76, 77] touchscreen-based attentional set-shifting tasks used in humans and non-human primates in which the two dimensions have been found to be differentially salient.

In some human and non-human primate studies, performance on the attentional set-shifting task has been shown to depend not only on the ability to develop and shift an attentional set, but also on the ability to direct attention and respond based on task-dependent (top-down) as opposed to sensory-driven (bottom-up) factors [11, 17]. Therefore, the ability to manipulate dimensional salience in a controlled, systematic way in mouse attentional set-shifting studies may prove useful. Indeed, this ability may be crucial when using mouse models to probe the neurobiological mechanisms of executive dysfunction in some human diseases. For example, using a touchscreen-based attentional set-shifting task in combination with exemplars from differentially salient dimensions, Cools et al. (2010) [11] found that responding by individuals with Parkinson's disease was driven to a relatively greater degree by bottom-up relative to top-down processing, resulting in apparently facilitated extra-dimensional set-shifting performance when the relevant dimension was salient, and impaired performance when the relevant dimension was non-salient. These findings in humans are consistent with a similar study in which extra-dimensional set-shifting performance of marmosets with 6-hydroxydopamine lesions in the prefrontal cortex was facilitated when the relevant dimension was salient and impaired when the relevant dimension was non-salient [17].

Considering these findings, when assessing cognitive function of a mouse model in which a set-shifting impairment is suspected to involve a deficit in relative top-down and bottom-up

control, an ideal set-shifting task would include a shift between equally salient dimensions, a shift from a salient to a non-salient dimension, and a shift from a non-salient to a salient dimension. The shift between equally salient dimensions would establish whether and to what degree mice had developed an attentional set, and the shift between dimensions of unequal salience would examine the possibility that set-shifting ability itself was dependent on salience of the relevant dimension. During shifts between dimensions of unequal salience, the salience component could be manipulated at, but not before the EDS stage to eliminate the confounding effects of increased trials to criterion on IDS stages during which dimensions were not equally salient. We have shown in the present study that this design is possible with a touchscreen-based set-shifting task by systematically manipulating line width to control relative dimensional salience while retaining other potentially-relevant stimulus attributes.

4.3. Visual Discrimination and Reversal Learning

Visual discrimination and reversal learning abilities of B6 and D2 mice have previously been assessed by Izquierdo et al. [52] using a touchscreen-based reversal learning task which was similar in some ways to the attentional set-shifting task used in the present study. In Izquierdo et al., B6 and D2 mice performed a simple discrimination of white shapes presented on a black background followed by a single reversal stage. In contrast to the present study in which visual discrimination and reversal learning performance of B6 and D2 mice did not differ, these authors found that performance of D2 mice was superior to performance of B6 mice on the simple discrimination and reversal stage. A possible explanation for these differential findings is that multiple characteristics of the operant conditioning schedules used in the present study and Izquierdo et al. differed including length of session time, length of intertrial interval, number of trials per session, criterion, size of visual stimuli, and type of stimuli used during the reversal (i.e., simple or compound). These differences are potentially relevant because Bussey et al. [78] have recently shown that many of these schedule characteristics affect rate of learning in rats. Thus, it seems plausible that strain-dependent effects of any of these variables on rate of learning could explain the differential findings. Indeed, in the present study although overall discrimination performance of the two strains was equivalent, performance of D2 mice was observed to be significantly better than B6 mice when discriminating some stimuli and worse when discriminating others. In addition to these factors, it is also possible that housing differences, unreported handling differences, or environmental stress could have contributed to reported differences between the two studies. It should be noted that there is evidence of strain-dependent effects of light-phase on some behavioral tasks such as the elevated plus maze and open field [79, 80]. However, in both the present study and Izquierdo et al., mice were tested during the light phase of a standard 12:12 light:dark cycle and an incorrect response was signaled by extinguishing the house light. Thus, it is unlikely that differences in these two variables could account for differences between Izquierdo et al. and the present study.

4.4 Serial Reversal Learning

Although the present study did not explicitly assess serial reversal learning (SRL), three reversal stages were components of the attentional set-shifting task. Because the SRL effect

(i.e., improved performance across reversals) has been well established [e.g., 81, 82, 83], it may seem surprising that this effect was not observed in the present study. However, performance improvements across reversal stages have not typically been observed in rodent SRL touchscreen studies. Specifically, animals in these tasks frequently fail to show significant improvement across serial reversals [60, 61, 84]. Why this occurs is unclear, although one reason may be that it is common to use relatively few reversal stages in rodent touchscreen SRL studies, and the SRL effect may be most robust, and therefore more consistently observed, when many reversals are used [85]. However, this phenomenon may be specific to touchscreen SRL tasks involving non-spatial discriminations of complex visual stimuli because robust performance improvements have been routinely observed in spatial SRL tasks after only a few reversals [83, 86, 87]. Additionally, improvements in learning-phase SRL performance have sometimes [88] though not always [59] been observed after four reversals of a conditional visual discrimination in which stimulus lights were used as the discriminanda.

In the present study, it is notable that reversal learning performance not only failed to improve, but in fact declined across reversals. A possible explanation for this is that proactive interference from prior stages impaired performance at subsequent stages. Specifically, as shown in Figure 2, many components of the lines exemplars in the four exemplar groups were identical. For example, a vertical line of the same length was a component of exemplar group A and B, and diagonal lines were components of group B and C. While these similarities between exemplar groups would not have affected performance during the first three stages of the IED task, discriminations between exemplars in the relevant dimension may have become more difficult during later stages of the task because correct exemplars would be increasingly likely to contain stimulus components which were both rewarded and not rewarded during prior stages; the same would apply to incorrect exemplars. This putative effect would not have occurred in past rodent touchscreen studies which explicitly examined SRL performance [60, 61, 84] because in those studies identical stimuli were used at each stage. Although proactive interference has been proposed to account for *improvements* across serial reversals [e.g., 89, 90], those studies have typically involved discriminations of the same two stimuli or locations across many back-to-back reversal stages, while the present study involved a series of novel-stimulus discriminations with each followed by a single discrimination-reversal of those newly-learned compound stimuli. Thus, the mechanisms of these two effects likely differ. Regarding the lack of a significant increase in errors across intra-dimensional shifts in the present study, the putative effects of proactive interference may have been intensified on reversal stages because of added interference from the previously-correct exemplar from the preceding compound discrimination. However, although not significant, errors did increase by ~11% from IDS1 ($M = 122.83$, $SD = 91.41$) to IDS2 ($M = 135.97$, $SD = 125.12$). Finally, it should be noted that the ability to discriminate between exemplars within the relevant dimension and the ability to selectively attend to the relevant dimension may independently affect performance on set-shifting tasks.

In the present study, we chose to include multiple reversal stages with the intention of further locking in the attentional set. An additional advantage of including reversal stages

was that it provided a measure of reversal learning ability. However, it is possible that reversal stages in the present study could have been replaced with intra-dimensional shifts to reduce the total number of testing sessions per mouse without altering performance on the EDS stage.

5. Conclusion

In the present study, we compared attentional set-shifting abilities of C57BL/6J and DBA/2J mice using a touchscreen-based attentional set-shifting task similar to the type used in human and non-human primate studies. We reported three principal findings. First, during experiment 1, performance of C57BL/6J mice was superior to that of DBA/2J mice on the EDS stage. However, findings from experiment 2 suggested that these strain differences were due to a relatively impaired ability in DBA/2J mice to discriminate stimuli in two of the four exemplar groups, particularly on compound discriminations when the relevant dimension was non-salient. Second, although significant strain differences were not observed on reversal learning stages, mice as a group committed more errors across reversals, possibly as a result of proactive interference from previous exemplar groups. Third, the lines and shapes dimensions were observed to be differentially salient, and relative salience of the dimensions could be controlled by manipulating line width while retaining other potentially-relevant attributes of the compound stimuli. These findings reveal unique and in some cases strain-dependent phenomena related to discriminations of simple and multidimensional visual stimuli which may facilitate future efforts to identify and fully characterize visual discrimination, reversal learning, and attentional set-shifting deficits in mice.

Acknowledgments

Funding and Acknowledgements: This project was made possible by NINDS grant 1R01NS063009. The authors gratefully acknowledge Erin Clardy for assistance with mouse breeding and data collection.

References

1. Jazbec S, Pantelis C, Robbins T, Weickert T, Weinberger DR, Goldberg TE. Intra-dimensional/extra-dimensional set-shifting performance in schizophrenia: impact of distractors. *Schizophr Res.* 2007; 89:339–349. [PubMed: 17055703]
2. Tyson PJ, Laws KR, Roberts KH, Mortimer AM. Stability of set-shifting and planning abilities in patients with schizophrenia. *Psychiatry Res.* 2004; 129:229–239. [PubMed: 15661316]
3. Hughes C, Russell J, Robbins TW. Evidence for executive dysfunction in autism. *Neuropsychologia.* 1994; 32:477–492. [PubMed: 8047253]
4. Yerys BE, Wallace GL, Harrison B, Celano MJ, Giedd JN, Kenworthy LE. Set-shifting in children with autism spectrum disorders: reversal shifting deficits on the Intradimensional/Extradimensional Shift Test correlate with repetitive behaviors. *Autism.* 2009; 13:523–538. [PubMed: 19759065]
5. Ozonoff S, Cook I, Coon H, Dawson G, Joseph RM, Klin A, et al. Performance on Cambridge Neuropsychological Test Automated Battery subtests sensitive to frontal lobe function in people with autistic disorder: evidence from the Collaborative Programs of Excellence in Autism network. *J Autism Dev Disord.* 2004; 34:139–150. [PubMed: 15162933]
6. Maes JH, Eling PA, Wezenberg E, Vissers CT, Kan CC. Attentional set shifting in autism spectrum disorder: differentiating between the role of perseveration, learned irrelevance, and novelty processing. *J Clin Exp Neuropsychol.* 2011; 33:210–217. [PubMed: 20694871]

7. Perry RJ, Hodges JR. Attention and executive deficits in Alzheimer's disease. A critical review. *Brain*. 1999; 122(Pt 3):383–404. [PubMed: 10094249]
8. Sawada Y, Nishio Y, Suzuki K, Hirayama K, Takeda A, Hosokai Y, et al. Attentional set-shifting deficit in Parkinson's disease is associated with prefrontal dysfunction: an FDG-PET study. *PLoS One*. 2012; 7:e38498. [PubMed: 22685575]
9. Owen AM, Roberts AC, Hodges JR, Summers BA, Polkey CE, Robbins TW. Contrasting mechanisms of impaired attentional set-shifting in patients with frontal lobe damage or Parkinson's disease. *Brain*. 1993; 116(Pt 5):1159–1175. [PubMed: 8221053]
10. Owen AM, James M, Leigh PN, Summers BA, Marsden CD, Quinn NP, et al. Fronto-striatal cognitive deficits at different stages of Parkinson's disease. *Brain*. 1992; 115(Pt 6):1727–1751. [PubMed: 1486458]
11. Cools R, Rogers R, Barker RA, Robbins TW. Top-down attentional control in Parkinson's disease: salient considerations. *J Cogn Neurosci*. 2010; 22:848–859. [PubMed: 19320545]
12. Slabosz A, Lewis SJ, Smigajewicz K, Szymura B, Barker RA, Owen AM. The role of learned irrelevance in attentional set-shifting impairments in Parkinson's disease. *Neuropsychology*. 2006; 20:578–88. [PubMed: 16938020]
13. Owen AM, Roberts AC, Polkey CE, Sahakian BJ, Robbins TW. Extra-dimensional versus intra-dimensional set shifting performance following frontal lobe excisions, temporal lobe excisions or amygdalo-hippocampectomy in man. *Neuropsychologia*. 1991; 29:993–1006. [PubMed: 1762678]
14. Bissonette GB, Martins GJ, Franz TM, Harper ES, Schoenbaum G, Powell EM. Double dissociation of the effects of medial and orbital prefrontal cortical lesions on attentional and affective shifts in mice. *J Neurosci*. 2008; 28:11124–11130. [PubMed: 18971455]
15. Dias R, Robbins TW, Roberts AC. Dissociation in prefrontal cortex of affective and attentional shifts. *Nature*. 1996; 380:69–72. [PubMed: 8598908]
16. Gotham AM, Brown RG, Marsden CD. 'Frontal' cognitive function in patients with Parkinson's disease 'on' and 'off' levodopa. *Brain*. 1988; 111(Pt 2):299–321. [PubMed: 3378138]
17. Crofts HS, Dalley JW, Collins P, Van Denderen JC, Everitt BJ, Robbins TW, et al. Differential effects of 6-OHDA lesions of the frontal cortex and caudate nucleus on the ability to acquire an attentional set. *Cereb Cortex*. 2001; 11:1015–1026. [PubMed: 11590111]
18. Courchesne E, Townsend J, Akshoomoff NA, Saitoh O, Yeung-Courchesne R, Lincoln AJ, et al. Impairment in shifting attention in autistic and cerebellar patients. *Behav Neurosci*. 1994; 108:848–865. [PubMed: 7826509]
19. Dickson PE, Rogers TD, Lester DB, Miller MM, Matta SG, Chesler EJ, et al. Genotype-dependent effects of adolescent nicotine exposure on dopamine functional dynamics in the nucleus accumbens shell in male and female mice: a potential mechanism underlying the gateway effect of nicotine. *Psychopharmacology*. 2011; 215:631–642. [PubMed: 21212937]
20. Patel S, Stolerman IP, Asherson P, Sluyter F. Attentional performance of C57BL/6 and DBA/2 mice in the 5-choice serial reaction time task. *Behav Brain Res*. 2006; 170:197–203. [PubMed: 16616787]
21. Voikar V, Polus A, Vasar E, Rauvala H. Long-term individual housing in C57BL/6J and DBA/2 mice: assessment of behavioral consequences. *Genes Brain Behav*. 2005; 4:240–252. [PubMed: 15924556]
22. Kerns RT, Ravindranathan A, Hassan S, Cage MP, York T, Sikela JM, et al. Ethanol-responsive brain region expression networks: implications for behavioral responses to acute ethanol in DBA/2J versus C57BL/6J mice. *J Neurosci*. 2005; 25:2255–2266. [PubMed: 15745951]
23. van der Veen R, Piazza PV, Deroche-Gamonet V. Gene-environment interactions in vulnerability to cocaine intravenous self-administration: a brief social experience affects intake in DBA/2J but not in C57BL/6J mice. *Psychopharmacology*. 2007; 193:179–186. [PubMed: 17396246]
24. Flanigan TJ, Cook MN. Effects of an early handling-like procedure and individual housing on anxiety-like behavior in adult C57BL/6J and DBA/2J mice. *PLoS One*. 2011; 6:e19058. [PubMed: 21533042]
25. Orsini C, Bonito-Oliva A, Conversi D, Cabib S. Susceptibility to conditioned place preference induced by addictive drugs in mice of the C57BL/6 and DBA/2 inbred strains. *Psychopharmacology*. 2005; 181:327–336. [PubMed: 15864555]

26. Escher T, Mittleman G. Effects of ethanol and GABAB drugs on working memory in C57BL/6J and DBA/2J mice. *Psychopharmacology*. 2004; 176:166–174. [PubMed: 15064920]
27. Kafkafi N, Lipkind D, Benjamini Y, Mayo CL, Elmer GI, Golani I. SEE locomotor behavior test discriminates C57BL/6J and DBA/2J mouse inbred strains across laboratories and protocol conditions. *Behav Neurosci*. 2003; 117:464–477. [PubMed: 12802875]
28. Podhorna J, Brown RE. Strain differences in activity and emotionality do not account for differences in learning and memory performance between C57BL/6 and DBA/2 mice. *Genes Brain Behav*. 2002; 1:96–110. [PubMed: 12884980]
29. Rocha BA, Odom LA, Barron BA, Ator R, Wild SA, Forster MJ. Differential responsiveness to cocaine in C57BL/6J and DBA/2J mice. *Psychopharmacology*. 1998; 138:82–88. [PubMed: 9694530]
30. Peirce JL, Derr R, Shendure J, Kolata T, Silver LM. A major influence of sex-specific loci on alcohol preference in C57BL/6 and DBA/2 inbred mice. *Mamm Genome*. 1998; 9:942–948. [PubMed: 9880657]
31. Grahame NJ, Cunningham CL. Genetic differences in intravenous cocaine self-administration between C57BL/6J and DBA/2J mice. *Psychopharmacology*. 1995; 122:281–291. [PubMed: 8748397]
32. Estape N, Steckler T. Effects of cholinergic manipulation on operant delayed non-matching to position performance in two inbred strains of mice. *Behav Brain Res*. 2001; 121:39–55. [PubMed: 11275283]
33. Estape N, Steckler T. Cholinergic blockade impairs performance in operant DNMTPT in two inbred strains of mice. *Pharmacol Biochem Behav*. 2002; 72:319–334. [PubMed: 11900803]
34. Taylor BA. Genetic relationships between inbred strains of mice. *J Hered*. 1972; 63:83–86. [PubMed: 5031317]
35. Peirce JL, Lu L, Gu J, Silver LM, Williams RW. A new set of BXD recombinant inbred lines from advanced intercross populations in mice. *BMC Genet*. 2004; 5:7. [PubMed: 15117419]
36. Bubier JA, Chesler EJ. Accelerating discovery for complex neurological and behavioral disorders through systems genetics and integrative genomics in the laboratory mouse. *Neurotherapeutics*. 2012; 9:338–348. [PubMed: 22422471]
37. Philip VM, Duvvuru S, Gomero B, Ansah TA, Blaha CD, Cook MN, et al. High-throughput behavioral phenotyping in the expanded panel of BXD recombinant inbred strains. *Genes Brain Behav*. 2010; 9:129–159. [PubMed: 19958391]
38. Phillips TJ, Huson MG, McKinnon CS. Localization of genes mediating acute and sensitized locomotor responses to cocaine in BXD/Ty recombinant inbred mice. *J Neurosci*. 1998; 18:3023–3034. [PubMed: 9526019]
39. Grisell JE, Belknap JK, O'Toole LA, Helms ML, Wenger CD, Crabbe JC. Quantitative trait loci affecting methamphetamine responses in BXD recombinant inbred mouse strains. *J Neurosci*. 1997; 17:745–754. [PubMed: 8987796]
40. Kest B, Palmese CA, Juni A, Chesler EJ, Mogil JS. Mapping of a quantitative trait locus for morphine withdrawal severity. *Mamm Genome*. 2004; 15:610–617. [PubMed: 15457340]
41. Tarantino LM, McClearn GE, Rodriguez LA, Plomin R. Confirmation of quantitative trait loci for alcohol preference in mice. *Alcohol Clin Exp Res*. 1998; 22:1099–1105. [PubMed: 9726281]
42. Roberts AJ, Phillips TJ, Belknap JK, Finn DA, Keith LD. Genetic analysis of the corticosterone response to ethanol in BXD recombinant inbred mice. *Behav Neurosci*. 1995; 109:1199–1208. [PubMed: 8748968]
43. Gallaher EJ, Jones GE, Belknap JK, Crabbe JC. Identification of genetic markers for initial sensitivity and rapid tolerance to ethanol-induced ataxia using quantitative trait locus analysis in BXD recombinant inbred mice. *J Pharmacol Exp Ther*. 1996; 277:604–612. [PubMed: 8627537]
44. Crabbe JC, Belknap JK, Mitchell SR, Crawshaw LI. Quantitative trait loci mapping of genes that influence the sensitivity and tolerance to ethanol-induced hypothermia in BXD recombinant inbred mice. *J Pharmacol Exp Ther*. 1994; 269:184–192. [PubMed: 8169823]
45. Belknap JK, Metten P, Helms ML, O'Toole LA, Angeli-Gade S, Crabbe JC, et al. Quantitative trait loci (QTL) applications to substances of abuse: physical dependence studies with nitrous oxide and ethanol in BXD mice. *Behav Genet*. 1993; 23:213–222. [PubMed: 8512534]

46. Belknap JK, Crabbe JC. Chromosome mapping of gene loci affecting morphine and amphetamine responses in BXD recombinant inbred mice. *Ann N Y Acad Sci.* 1992; 654:311–323. [PubMed: 1632590]
47. Loos M, Staal J, Pattij T, Neuro BMPC, Smit AB, Spijker S. Independent genetic loci for sensorimotor gating and attentional performance in BXD recombinant inbred strains. *Genes Brain Behav.* 2012; 11:147–156. [PubMed: 22098762]
48. Beatty J, Laughlin RE. Genomic regulation of natural variation in cortical and noncortical brain volume. *BMC Neurosci.* 2006; 7:16. [PubMed: 16503985]
49. Kempermann G, Gage FH. Genetic determinants of adult hippocampal neurogenesis correlate with acquisition, but not probe trial performance, in the water maze task. *Eur J Neurosci.* 2002; 16:129–136. [PubMed: 12153537]
50. Laughlin RE, Grant TL, Williams RW, Jentsch JD. Genetic dissection of behavioral flexibility: reversal learning in mice. *Biol Psychiatry.* 2011; 69:1109–1116. [PubMed: 21392734]
51. Cervantes MC, Laughlin RE, Jentsch JD. Cocaine self-administration behavior in inbred mouse lines segregating different capacities for inhibitory control. *Psychopharmacology.* 2013; 229:515–525. [PubMed: 23681162]
52. Izquierdo A, Wiedholz LM, Millstein RA, Yang RJ, Bussey TJ, Saksida LM, et al. Genetic and dopaminergic modulation of reversal learning in a touchscreen-based operant procedure for mice. *Behav Brain Res.* 2006; 171:181–188. [PubMed: 16713639]
53. Sahakian BJ, Owen AM. Computerized assessment in neuropsychiatry using CANTAB: discussion paper. *J R Soc Med.* 1992; 85:399–402. [PubMed: 1629849]
54. Dias R, Robbins TW, Roberts AC. Dissociable forms of inhibitory control within prefrontal cortex with an analog of the Wisconsin Card Sort Test: restriction to novel situations and independence from "on-line" processing. *J Neurosci.* 1997; 17:9285–9297. [PubMed: 9364074]
55. Baxter MG, Gaffan D. Asymmetry of attentional set in rhesus monkeys learning colour and shape discriminations. *Q J Exp Psychol.* 2007; 60:1–8.
56. Brigman JL, Bussey TJ, Saksida LM, Rothblat LA. Discrimination of multidimensional visual stimuli by mice: intra- and extradimensional shifts. *Behav Neurosci.* 2005; 119:839–842. [PubMed: 15998206]
57. Brigman JL, Padukiewicz KE, Sutherland ML, Rothblat LA. Executive functions in the heterozygous reeler mouse model of schizophrenia. *Behav Neurosci.* 2006; 120:984–988. [PubMed: 16893304]
58. Dickson PE, Corkill B, McKimm E, Miller MM, Calton MA, Goldowitz D, et al. Effects of stimulus salience on touchscreen serial reversal learning in a mouse model of fragile X syndrome. *Behav Brain Res.* 2013
59. Dickson PE, Rogers TD, Del Mar N, Martin LA, Heck D, Blaha CD, et al. Behavioral flexibility in a mouse model of developmental cerebellar Purkinje cell loss. *Neurobiol Learn Mem.* 2010; 94:220–228. [PubMed: 20566377]
60. Chudasama Y, Robbins TW. Dissociable contributions of the orbitofrontal and infralimbic cortex to pavlovian autoshaping and discrimination reversal learning: further evidence for the functional heterogeneity of the rodent frontal cortex. *J Neurosci.* 2003; 23:8771–8780. [PubMed: 14507977]
61. Bussey TJ, Muir JL, Everitt BJ, Robbins TW. Triple dissociation of anterior cingulate, posterior cingulate, and medial frontal cortices on visual discrimination tasks using a touchscreen testing procedure for the rat. *Behav Neurosci.* 1997; 111:920–936. [PubMed: 9383514]
62. Laurent V, Podhorna J. Subchronic phencyclidine treatment impairs performance of C57BL/6 mice in the attentional set-shifting task. *Behav Pharmacol.* 2004; 15:141–148. [PubMed: 15096914]
63. Colacicco G, Welzl H, Lipp HP, Wurbel H. Attentional set-shifting in mice: modification of a rat paradigm, and evidence for strain-dependent variation. *Behav Brain Res.* 2002; 132:95–102. [PubMed: 11853862]
64. Glickstein SB, Desteno DA, Hof PR, Schmauss C. Mice lacking dopamine D2 and D3 receptors exhibit differential activation of prefrontal cortical neurons during tasks requiring attention. *Cereb Cortex.* 2005; 15:1016–1024. [PubMed: 15537671]

65. Garner JP, Thogerson CM, Wurbel H, Murray JD, Mench JA. Animal neuropsychology: validation of the Intra-Dimensional Extra-Dimensional set shifting task for mice. *Behav Brain Res.* 2006; 173:53–61. [PubMed: 16842867]
66. Zhuo JM, Prescott SL, Murray ME, Zhang HY, Baxter MG, Nicolle MM. Early discrimination reversal learning impairment and preserved spatial learning in a longitudinal study of Tg2576 APPsw mice. *Neurobiol Aging.* 2007; 28:1248–1257. [PubMed: 16828204]
67. DeSteno DA, Schmauss C. Induction of early growth response gene 2 expression in the forebrain of mice performing an attention-set-shifting task. *Neuroscience.* 2008; 152:417–428. [PubMed: 18280047]
68. Levi Y, Kofman O, Schwebel M, Shaldubina A. Discrimination and avoidance learning in adult mice following developmental exposure to diisopropylfluorophosphate. *Pharmacol Biochem Behav.* 2008; 88:438–445. [PubMed: 17963829]
69. Papaleo F, Crawley JN, Song J, Lipska BK, Pickel J, Weinberger DR, et al. Genetic dissection of the role of catechol-O-methyltransferase in cognition and stress reactivity in mice. *J Neurosci.* 2008; 28:8709–8723. [PubMed: 18753372]
70. De Steno DA, Schmauss C. A role for dopamine D2 receptors in reversal learning. *Neuroscience.* 2009; 162:118–127. [PubMed: 19401217]
71. Macri S, Granstrem O, Shumilina M, Antunes Gomes dos Santos FJ, Berry A, Saso L, et al. Resilience and vulnerability are dose-dependently related to neonatal stressors in mice. *Horm Behav.* 2009; 56:391–408. [PubMed: 19632235]
72. Macri S, Biamonte F, Romano E, Marino R, Keller F, Laviola G. Perseverative responding and neuroanatomical alterations in adult heterozygous reeler mice are mitigated by neonatal estrogen administration. *Psychoneuroendocrinology.* 2010; 35:1374–1387. [PubMed: 20452127]
73. Young JW, Powell SB, Geyer MA, Jeste DV, Risbrough VB. The mouse attentional-set-shifting task: a method for assaying successful cognitive aging? *Cogn Affect Behav Neurosci.* 2010; 10:243–251. [PubMed: 20498348]
74. Lawrence AD, Sahakian BJ, Rogers RD, Hodge JR, Robbins TW. Discrimination, reversal, and shift learning in Huntington's disease: mechanisms of impaired response selection. *Neuropsychologia.* 1999; 37:1359–1374. [PubMed: 10606011]
75. Clarke HF, Walker SC, Crofts HS, Dalley JW, Robbins TW, Roberts AC. Prefrontal serotonin depletion affects reversal learning but not attentional set shifting. *J Neurosci.* 2005; 25:532–538. [PubMed: 15647499]
76. Downes JJ, Roberts AC, Sahakian BJ, Evenden JL, Morris RG, Robbins TW. Impaired extra-dimensional shift performance in medicated and unmedicated Parkinson's disease: evidence for a specific attentional dysfunction. *Neuropsychologia.* 1989; 27:1329–1343. [PubMed: 2615934]
77. Roberts AC, Robbins TW, Everitt BJ. The effects of intradimensional and extradimensional shifts on visual discrimination learning in humans and non-human primates. *Q J Exp Psychol B.* 1988; 40:321–341. [PubMed: 3145534]
78. Bussey TJ, Padain TL, Skillings EA, Winters BD, Morton AJ, Saksida LM. The touchscreen cognitive testing method for rodents: how to get the best out of your rat. *Learn Mem.* 2008; 15:516–523. [PubMed: 18612068]
79. Hossain SM, Wong BK, Simpson EM. The dark phase improves genetic discrimination for some high throughput mouse behavioral phenotyping. *Genes Brain Behav.* 2004; 3:167–177. [PubMed: 15140012]
80. Post AM, Weyers P, Holzer P, Painsipp E, Pauli P, Wulsch T, et al. Gene-environment interaction influences anxiety-like behavior in ethologically based mouse models. *Behav Brain Res.* 2011; 218:99–105. [PubMed: 21111005]
81. Mackintosh NJ, McGonigle B, Holgate V, Vanderver V. Factors underlying improvement in serial reversal learning. *Can J Psychol.* 1968; 22:85–95. [PubMed: 5649040]
82. Jones B, Mishkin M. Limbic lesions and the problem of stimulus--reinforcement associations. *Exp Neurol.* 1972; 36:362–377. [PubMed: 4626489]
83. Boulougouris V, Glennon JC, Robbins TW. Dissociable effects of selective 5-HT_{2A} and 5-HT_{2C} receptor antagonists on serial spatial reversal learning in rats. *Neuropsychopharmacology.* 2008; 33:2007–2019. [PubMed: 17957219]

84. Chudasama Y, Bussey TJ, Muir JL. Effects of selective thalamic and prelimbic cortex lesions on two types of visual discrimination and reversal learning. *Eur J Neurosci.* 2001; 14:1009–1020. [PubMed: 11595039]
85. Rygula R, Walker SC, Clarke HF, Robbins TW, Roberts AC. Differential contributions of the primate ventrolateral prefrontal and orbitofrontal cortex to serial reversal learning. *J Neurosci.* 2010; 30:14552–14559. [PubMed: 20980613]
86. Castane A, Theobald DE, Robbins TW. Selective lesions of the dorsomedial striatum impair serial spatial reversal learning in rats. *Behav Brain Res.* 2010; 210:74–83. [PubMed: 20153781]
87. Boulougouris V, Dalley JW, Robbins TW. Effects of orbitofrontal, infralimbic and prelimbic cortical lesions on serial spatial reversal learning in the rat. *Behav Brain Res.* 2007; 179:219–228. [PubMed: 17337305]
88. Jones GH, Marsden CA, Robbins TW. Behavioural rigidity and rule-learning deficits following isolation-rearing in the rat: neurochemical correlates. *Behav Brain Res.* 1991; 43:35–50. [PubMed: 1677579]
89. Beale IL. The effects of amount of training per reversal on successive reversals of a color discrimination. *J Exp Anal Behav.* 1970; 14:345–352. [PubMed: 5491010]
90. Woodward WT, Schoel WM, Bitterman ME. Reversal learning with singly presented stimuli in pigeons and goldfish. *J Comp Physiol Psychol.* 1971; 76:460–467. [PubMed: 5127951]

Highlights

- We compared attentional set-shifting abilities of C57BL/6J and DBA/2J mice in a touchscreen-based task.
- Salience of the relevant dimension was manipulated to assess effects on discrimination performance
- Performance of C57BL/6J mice was superior to that of DBA/2J mice on the extra-dimensional shift
- Task performance was dependent on interactions between strain, exemplar group, and salience of the relevant dimension
- Reversal learning performance of all mice decreased across reversals, likely due to proactive interference

Figure 1.

Simple and compound visual stimuli used in the attentional set-shifting task. (A) An operant conditioning chamber used in the study. (B and C) During the simple discrimination stage, mice performed a pairwise discrimination of simple stimuli from the lines dimension (experiment 1 and 2) or shapes dimension (experiment 2). (D and E) Beginning with the compound discrimination stage, mice performed pairwise discriminations of compound stimuli consisting of exemplars from both the shapes and lines dimensions. With the exception of correction trials, the positions of the lines and shapes exemplars were independently randomized on each trial resulting in two possible stimulus configurations. (F) During experiment 2, we manipulated relative salience of the lines and shapes dimensions by systematically altering width of all lines exemplars. Thus, in experiment 2, compound stimuli were composed of shapes and either wide or narrow lines.

Figure 2.

The four exemplar groups used in the experiment. The exemplar groups have been termed A, B, C, and D, and exemplars from the left and right columns have been termed exemplars 1 and 2, respectively.

<u>Stage</u>	<u>Abbr.</u>	<u>Discrimination</u>	<u>Correct</u>
1. Simple Discrimination	SD
2. Compound Discrimination	CD
3. Compound Discrimination Reversal	CDR
4. Intra-dimensional Shift 1	IDS1
5. Intra-dimensional Shift 1 Reversal	IDS1R
6. Intra-dimensional Shift 2	IDS2
7. Intra-dimensional Shift 2 Reversal	IDS2R
8. Extra-dimensional Shift	EDS

Figure 3.

The sequence of stages in the attentional set-shifting task. The exemplar group used at each stage and the rewarded exemplar from the relevant dimension were counterbalanced as detailed in Table 1.

Figure 4.

Performance of B6 and D2 mice on a touchscreen-based attentional set-shifting task. (A) Relative to B6 mice, D2 mice committed significantly more errors on the EDS stage of experiment 1 when the relevant dimension changed from lines to shapes. Additionally, errors of mice as a group consistently increased across reversal stages, and the number of errors committed on the IDS2R stage was significantly greater than the number committed on the CDR stage. (B) A significant ($p < .001$) stage $\times$ stimulus counterbalancing-group interaction revealed that performance at most stages was significantly affected by the visual stimuli themselves, indicating that some exemplar groups were inherently more difficult to discriminate than others. (C) Significant strain differences in lines-relevant compound discriminations were not observed on the two intra-dimensional shifts and their reversals which preceded the EDS stage; this was true even when examining performance within each stimulus counterbalancing-group. However, the overall strain difference in EDS stage performance was predominantly driven by mice in stimulus counterbalancing-groups 2 and 4 (discriminating exemplar groups D and B, respectively). Additionally, while all B6 mice reached criterion on the EDS stage, several D2 mice discriminating exemplar groups D and B failed to reach criterion. Together, this indicates that visual stimulus characteristics of

some exemplar groups affected the ability to discriminate compound stimuli at the EDS stage in a strain-dependent manner.

* = strain difference ($p < .05$)

† = CDR differs from IDS2R in all mice as a group ($p < .05$)

= strain difference ($p < .06$)

¹ = In figure 4B, group differences at each stage are represented by displaying the group number above the bar from which that group differed significantly ($p < .05$).

Figure 5.

Performance of B6 and D2 mice on experiment 2. During the SD stage, mice were able to discriminate three types of stimuli (shapes, narrow lines, and wide lines) equally well when they were presented as simple, non-compound stimuli. During the CD and CDR stages when mice discriminated compound stimuli composed of shapes and wide or narrow lines, two patterns emerged. First, when the shapes dimension was relevant, B6 and D2 mice committed more errors to criterion when lines were *wide* and fewer errors to criterion when lines were *narrow*. Second, when the lines dimension was relevant, B6 and D2 mice

committed more errors to criterion when lines were *narrow* and fewer errors to criterion when lines were *wide*. This overall pattern was evident in both strains and indicates that when lines were wide, they were *more* salient than shapes, but when lines were narrow they were *less* salient than shapes.

* = $p < .05$

= $p < .06$

Figure 6.

Effects of exemplar group on shapes- and lines-relevant simple and compound discriminations under differing salience conditions in experiment 2. Several significant strain differences were observed on simple discriminations of both lines and shapes stimuli during the SD stage. However, the most robust strain differences were observed on compound discriminations when the relevant dimension was non-salient. This was especially true during the reversal stage, and six D2 mice discriminating the B ($n = 3$) and D ($n = 3$) exemplar groups and three B6 mice discriminating the A ($n = 1$), B ($n = 1$), and C ($n = 1$) exemplar groups failed to reach criterion under these conditions. With one exception, significant strain differences were not observed on compound discriminations when the relevant dimension was salient. Additionally, all mice in these conditions reached criterion at all stages.

* = $p < .05$

= $p = .06$

Table 1

Order of exemplar group presentation in Experiment 1

Stimulus-Counterbalancing Group	Strain		Stage			
	C57BL/6J	DBA/2J	SD, CD, and CDR	IDS1 and IDS1R	IDS2 and IDS2R	EDS
1	4 (2/2)	4 (2/2)	A	B	D	C
2	4 (2/2)	4 (2/2)	B	C	A	D
3	4 (2/2)	3 (2/1)	C	D	B	A
4	3 (2/1)	4 (2/2)	D	A	C	B

Notes. The first number in each cell in the two Strain columns represents the subgroup sample size. The two numbers in parentheses following the subgroup sample size represent the number of mice in that subgroup rewarded for responding to exemplar 1 and exemplar 2, respectively. The letters A – D in the Stage columns represent the exemplar groups shown in Figure 2.

Table 2

Counterbalancing of exemplar groups in Experiment 2

Wide Lines		Narrow Lines							
Lines Relevant		Shapes Relevant		Lines Relevant		Shapes Relevant			
Exemplar Group	C57BL/6J	DBA/2J	C57BL/6J	DBA/2J	C57BL/6J	DBA/2J	C57BL/6J	DBA/2J	
A	4 (2/2)	4 (2/2)	2 (1/1)	2 (1/1)	2 (1/1)	2 (1/1)	2 (1/1)	2 (1/1)	
B	4 (2/2)	4 (2/2)	2 (1/1)	2 (1/1)	1 (0/1)	2 (1/1)	2 (1/1)	2 (1/1)	
C	4 (2/2)	3 (2/1)	2 (1/1)	2 (1/1)	2 (1/1)	1 (1/0)	2 (1/1)	2 (1/1)	
D	3 (2/1)	4 (2/2)	1 (1/0)	3 (2/1)	2 (1/1)	2 (1/1)	2 (1/1)	2 (1/1)	
Total	15	15	7	9	7	7	8	8	

Notes. The first number in each cell represents the subgroup sample size. The two numbers in parentheses following the subgroup sample size represent the number of mice in that subgroup rewarded for responding to exemplar 1 and exemplar 2, respectively. The C57BL/6J (n = 15) and DBA/2J (n = 15) mice listed in the wide lines / lines relevant category are from experiment 1.