Cerebral Cortex Advance Access published November 28, 2013

Cerebral Cortex
doi:10.1093/cercor/bht293

Cognitive Abilities on Transitive Inference Using a Novel Touchscreen Technology for Mice

J.L. Silverman[1,2], P.T. Gastrell[2], M.N. Karras[2], M. Solomon[1,3] and J.N. Crawley[1,2]

[1]MIND Institute, Department of Psychiatry and Behavioral Science, University of California Davis School of Medicine, Sacramento, CA 95817, USA, [2]Laboratory of Behavioral Neuroscience, National Institute of Mental Health Intramural Research Program, Bethesda, MD 20892-3730, USA and [3]Imaging Research Center, University of California Davis, Sacramento, CA 95817, USA

Address correspondence to Dr Jill L. Silverman, MIND Institute, Department of Psychiatry and Behavioral Sciences, University of California Davis School of Medicine, Sacramento, CA 95817, USA. Email: jill.silverman@ucdmc.ucdavis.edu

Cognitive abilities are impaired in neurodevelopmental disorders, including autism spectrum disorder (ASD) and schizophrenia. Preclinical models with strong endophenotypes relevant to cognitive dysfunctions offer a valuable resource for therapeutic development. However, improved assays to test higher order cognition are needed. We employed touchscreen technology to design a complex transitive inference (TI) assay that requires cognitive flexibility and relational learning. C57BL/6J (B6) mice with good cognitive skills and BTBR T +tf/J (BTBR), a model of ASD with cognitive deficits, were evaluated in simple and complex touchscreen assays. Both B6 and BTBR acquired visual discrimination and reversal. BTBR displayed deficits on components of TI, when 4 stimuli pairs were interspersed, which required flexible integrated knowledge. BTBR displayed impairment on the A > E inference, analogous to the A > E deficit in ASD. B6 and BTBR mice both reached criterion on the B > D comparison, unlike the B > D impairment in schizophrenia. These results demonstrate that mice are capable of complex discriminations and higher order tasks using methods and equipment paralleling those used in humans. Our discovery that a mouse model of ASD displays a TI deficit similar to humans with ASD supports the use of the touchscreen technology for complex cognitive tasks in mouse models of neurodevelopmental disorders.

Keywords: autism, learning, mouse model, schizophrenia, translational

Introduction

Mouse models of autism spectrum disorder (ASDs) and schizophrenia have increased our understanding of mechanisms that may underlie these disease states. One roadblock for using rodent models in preclinical therapeutic development is that many cognitive and behavioral characteristics observed in the clinic are difficult to compare to phenotypes observed in rodents. Tasks that accurately assess complex cognitive abilities in mice are in high demand, yet a consensus around assays with high relevance to the clinical symptoms has yet to be reached (Koenig 2006; Buchanan et al. 2007; Carter and Barch 2007; Carpenter and Koenig 2008; Silverman, Yang et al. 2010; Young et al. 2010, 2012; Bussey et al. 2012; Dudchenko et al. 2012; Watson and Platt 2012). The present study introduces an innovative cognitive assay using forefront touch screen technology, to enhance the translation of preclinical rodent research into human clinical trials of treatments to prevent or reverse disease-associated cognitive abnormalities in ASD and schizophrenia.

One measureable form of cognitive advancement is the relational learning and memory task of transitive inference (TI). TI involves the acquisition of multiple overlapping discriminations and integration of these learned pairs into relational networks (Eichenbaum and Fortin 2009). Tasks assessing TI offer a means of measuring the organization of an underlying learning structure, which may support cognitive flexibility and the development of more elaborate memory networks (Dusek and Eichenbaum 1997). In TI tasks, subjects learn a hierarchical series of overlapping choice judgments, in which 1 of 2 stimuli is rewarded, across pairwise sequential presentations (e.g., A is rewarded over B, B is rewarded over C, C is rewarded over D, and D is rewarded over E). After the sequence is acquired, a probe is administered to evaluate the understanding of the relationship between indirectly related items. B > D represents the cardinal transitive inference, an indirect logical deduction since B was valued earlier in the sequence (A > B), and D was valued lower in the sequence (D > E), and neither B nor D was directly paired. A > E represents the end pair inference, thought to be trivial, since A was solely valued while E was never rewarded, and neither A nor E was directly paired. Robust TI impairments in complex B > D and end pair A > E inferences have been reported in neurodevelopmental disorders, including schizophrenia and ASD (Titone et al. 2004; Coleman et al. 2010; Solomon et al. 2011). Moreover, TI makes for an excellent translational assay since it has been well characterized across species including humans, nonhuman primates, pigeons, crows, fish, rats, and mice (Bryant and Trabasso 1971; Davis 1992; McGonigle 1992; Rapp et al. 1996; Dusek and Eichenbaum 1997; Lazareva et al. 2000; Frank et al. 2003, 2005; Van Elzakker et al. 2003; Buckmaster et al. 2004; Grosenick et al. 2007; Devito, Kanter et al. 2010; Devito, Lykken et al. 2010; Fijal and Popik 2011; Solomon et al. 2011; Andre et al. 2012; Gazes et al. 2012, 2013; Lazareva and Wasserman 2012). Hippocampus (HPC), fornix, perirhinal cortex, entorhinal cortex, and medial prefrontal cortex (PFC) have been implicated in the acquisition and expression of TI in rodents and nonhuman primates (Dusek and Eichenbaum 1997; Buckmaster et al. 2004; DeVito, Kanter et al. 2010; Devito, Lykken et al. 2010; Fijal and Popik 2011).

Olfactory-based TI tasks were developed and optimized for rodents by Eichenbaum and coworkers (Davis 1992; Dusek and Eichenbaum 1997; Devito, Kanter et al. 2010; Devito, Lykken et al. 2010; Andre et al. 2012). We considered the possibility that conversion to the visual modality might be feasible with the advent of new operant touchscreen technology, pioneered in the Bussey, Holmes, and Rothblat laboratories (Bussey et al. 2001, 2008; Brigman et al. 2005; Izquierdo et al. 2006; Morton et al. 2006; Brigman and Rothblat 2008; Bartko et al. 2011; Talpos et al. 2009; Brigman, Mathur et al. 2010), using methods analogous to those employed in human and nonhuman primate experimental designs (Rapp et al. 1996; Buckmaster et al. 2004; Solomon et al. 2011). The innovative touchscreen equipment and software are highly analogous to

the CANTAB system from Cambridge Cognition, which is used to evaluate learning and memory in humans and nonhuman primates (Robbins et al. 1994, 1998; Fray and Robbins 1996; Spinelli et al. 2004, 2006). Bussey–Saksida touchscreen systems offer distinct behavioral advantages over manually performed odor discrimination tasks, including minimal demands on motor abilities, automated task parameters which ensure standardization, reduced investigator time and effort, and the use of similar equipment across species (Bussey et al. 2008, 2012; Brigman, Graybeal et al. 2010).

Our laboratory's first step was to design a touchscreen version of TI in mice. Second, we evaluated the hypothesis that the BTBR T^{+} tf/J (BTBR) mouse model of ASD, which displays deficits on conventional learning tasks, would exhibit inference impairments compared with control mice. The current study was designed to test the hypothesis that mice with good cognitive skills in other tasks can perform a touchscreen TI task, and that mice with impairments on other learning tasks will fail to perform a touchscreen TI task. For control testing and to validate our task design, we assessed performance in an inbred strain of mice with generally normal cognitive abilities, C57BL/6J (B6). Lastly, we aimed to determine if inferential deficiencies in a mouse model would be qualitatively analogous to the impairments observed in people with ASD. Specifically, impairments in the A > E inference but intact performance on the complex B > D inference have been observed in adults with ASD (Solomon et al. 2011).

Materials and Methods

Experimental Design

One independent cohort of B6 and BTBR was evaluated on a standard task of pairwise visual discrimination and reversal learning (Brigman and Rothblat 2008; Brigman et al. 2008; Brigman, Mathur et al. 2010). In another independent cohort, each strain was evaluated during serial training of the premise pairs (the A > B, B > C, C > D, D > E sequence) and 2 integrated retention tests (A > B > C > D > E), 1 in ordered presentation and 1 presented in a pseudorandom manner. At the end of training, both strains were tested using a TI probe test for the B > D transitive and the A > E end pair inference.

Mice

C57BL/6J (B6) and BTBR T+ tf/J (BTBR) mice were the offspring of breeding pairs purchased from The Jackson Laboratory (Bar Harbor, ME, USA). All mice were housed and bred in a conventional mouse vivarium at the National Institute of Mental Health (NIMH, Bethesda, MD, USA), using harem breeding trios. After 2 weeks with a male, females were separated into individual cages (Techniplast, Exton, PA, USA) before parturition. Pups were kept with the dam until weaning at postnatal day 21. After weaning, juveniles were group housed by sex and strain in standard plastic cages in groups not exceeding 4 per cage. Cages were maintained in ventilated racks in a temperature (20 °C) and humidity (~55%) controlled vivarium on a 12 h circadian cycle, lights on from 07:00 to 19:00 h. Standard rodent chow and tap water were available *ad libitum*. In addition to standard bedding, a Nestlet square, shredded brown paper and a cardboard tube were provided in each cage. Light levels were ~325 lux during the light phase. Background noise was 50–60 dB. Mice were 8 weeks of age at the start of testing. All subject mice were males. Mice were maintained on a restricted diet and kept at 90% of free-feeding body weight during behavioral testing, to ensure sufficient motivation to work for food reinforcement. Mice were fed upon return to the home cage after testing. Testing was conducted during the light phase of the light/dark cycle after mice were acclimated to the behavioral annex for 1 h. All procedures were approved by the NIMH Animal Care and Use Committee and the University of California Davis Institutional Animal Care and Use Committee.

Apparatus

Bussey–Saksida touchscreen equipment and components for mice were purchased from Campden Instruments Ltd/Lafayette Instruments (Lafayette, IL, USA). Operant chamber walls were trapezoidal, to enhance the subject's attentional focus. The chamber contained a perforated floor for cleaning, a nose-poke infrared sensitive touchscreen spanning the wider end of the trapezoid, and a calibrated peristaltic liquid reward pump connected to a trough well at the narrow end of the trapezoid. All components were enclosed in a sound attenuating cubicle (56.5 × 49.5 × 54.6 cm). A house light, noise generator and video cameras were mounted inside the chambers. The touchscreen was infrared with increased sensitivity and measured 12.1 inches landscape with a screen resolution of 800 × 600. The screen was covered by a black Plexiglas panel that had 2 7 × 7.5 cm windows separated by 0.5 cm and located at a height of 6.5 cm from the floor of the chamber. Stimuli presented on the screen were controlled by software (ABET II Touch Software, Lafayette Instruments) controlled and managed by the WhiskerServer Controller (Campden Instruments, UK) for every 4 chambers. Stimuli were visible through the windows (1 stimulus/window). Nosepokes at the stimuli were detected by the touchscreen and recorded by the software. Methods for training mice on the initial phases of the touchscreen task were adapted from the pioneering procedures published by the Bussey and Holmes laboratories (Izquierdo et al. 2006; Brigman et al. 2008; Brigman, Mathur et al. 2010; Bussey et al. 2012). Our laboratory modified the preprogrammed technology of the Bussey Laboratory (University of Cambridge) included in the ABET II software for the TI relational learning task.

Pretraining for Transitive Inference

Body weights of the mice were slowly reduced over 1 week of food restriction, then maintained at 90% free-feeding body weight by feeding ~2.0 g of rodent chow per mouse per day and weighing daily. Mice were acclimated to the chambers for 3 days in 40-min sessions with house light illumination (~60 lux). During this habituation phase, the reward trough was illuminated and a palatable liquid diet reinforcer (Strawberry Ensure Plus, Abbott, IL, USA), diluted 50% with water, was available to the mice (~35 µL) at the start of the sessions and was re-administered following head poke well entry. Trough entries and trial initiations increased over the course of the habituation training for all subjects. Mice that initiated trials were advanced to autoshaping, a progressive training phase that required subject initiation of trials and a correction training in which overhead lighting was turned off for 5 s (Bussey et al. 2001, 2008; Izquierdo et al. 2006; Morton et al. 2006; Brigman et al. 2008; Brigman, Mathur et al. 2010a; Brigman, Graybeal et al. 2010). Criterion under these conditions was defined by a completion of 23 of 30 trials with an accuracy of 80% or higher on 2 consecutive training days. Following successful pretraining, mice were moved onto TI.

Pretraining for Discrimination and Reversal

All procedures and phases were similar to the pretraining regimen described above, however, the overhead lighting (~60 lux) was absent during the training phases, and the liquid reinforcement was at full strength rather than diluted 1:1. During the final correction phase, if the mouse touched the blank side of the screen, a timeout period was triggered in which the image disappeared and the overhead lighting (~60 lux) was turned on for 5 s. The ITI of 20 s began and no liquid reinforcer was emitted from the dispenser. Criterion under these conditions was defined by a completion of all 30 trials with greater than 85% accuracy or higher on 2 consecutive training days. Following successful pretraining, mice were moved onto discrimination and reversal.

Discrimination

Two novel equiluminescent stimuli were presented in a spatially pseudorandomized manner over 30-trial sessions (20 s ITI) in the absence

of overhead lighting. Responses at the correct stimulus resulted in 7 μL reinforcer. Responses at the incorrect stimulus resulted in a 5-s timeout (signaled by the ~60 lux house light turning on), followed by a correction trial. Visual stimuli presented included the spider and airplane, provided in the Campden/Lafayette software. Stimuli remained on the screen until a response was made (Brigman et al. 2005; Brigman and Rothblat 2008; Brigman et al. 2008; Bussey et al. 2008; Brigman, Mathur et al. 2010). Designation of the correct and incorrect stimulus was counterbalanced across strain groups. To examine simple learning under a stringent definition, acquisition was assessed with a group performance criterion of an average of 90% or higher correct responses (excluding correction trials) over 2 consecutive sessions. The dependent variables for discrimination and reversal were sessions to criterion, summed number of trials over sessions and number of correction trials to criterion.

Reversal

Reversal learning procedures were similar to discrimination learning as described above, however during reversal, the reinforcement contingencies were reversed such that the previous correct image was incorrect and unrewarded, while the previous unrewarded image was correct and rewarded. The criterion for reversal learning was an average percent correct of 80% or higher, consistent with previous methods (Brigman and Rothblat 2008).

Transitive Inference Training and Probe Test

To evaluate the ability to integrate learned relationships and use them flexibly, we created a visual operant version of TI based on the olfactory paradigm originally developed for rats (Dusek and Eichenbaum 1997) and optimized for mice (DeVito et al. 2009, 2011; Devito, Kanter et al. 2010; DeVito, Lykken et al. 2010). After progression through the pretraining regimen, subjects were given a simple visual discrimination of 60 trials over a 60-min period, over 2 sessions per day. Responses at the incorrect stimulus resulted in a 5-s timeout (signaled by the ~60 lux house light turning on), followed by a correction trial. Visual stimuli presented included spider, airplane, pinwheel, marble array, and daisy shapes, provided in the Campden/Lafayette software. The images were selected based on their equi-luminescent properties in the touchscreen chambers, as evaluated by pilot studies in our laboratory, from personal communication with the Lafayette Instruments technical team, and from the published literature in the rat (Bussey et al. 2001, 2008). Stimuli were presented in left-right pseudorandomized manner over 60-trial sessions (20 s ITI) in the presence of overhead lighting. Animals were trained on a series of 4 overlapping discriminations: A rewarded over B, B rewarded over C, C rewarded over D, and D rewarded over E, to generate the A > B > C > D > E hierarchy. Mice were first trained with A > B until they reached criterion. The next trained discrimination was B > C, then C > D, then D > E. All images were counterbalanced in groups. This was followed by 2 retention stages. Retention test #1 (RT#1) presented all the images in serial order. Retention test #2 (RT#2) presented all of the premise pairs in pseudorandom order. A maximum of 40 sessions was provided to reach criterion. Previous publications reported that most subjects required no more than 30 sessions for inbred strains and 60 sessions for mutants (Brigman et al. 2008). In our testing chambers and experimental design, 40 sessions was slightly more than double the amount of sessions B6 control mice required to complete any given phase of training (unpublished observations). Table 1 illustrates the training sequence. Figure 1 illustrates the visual stimuli in hierarchical order.

After criterion or the cutoff maximum number of sessions in RT#2 was reached, mice were given a probe test during a single session in which the previously trained comparisons are presented in addition to 2 novel ones, A > E (anchored novel pairing) and B > D (the transitive novel pairing). Our expectation was that all mice capable of completing the training phase would prefer A over E, given previous findings in the literature that this is a trivial inference for most species. Mice choosing B over D would have demonstrated the ability to perform transitive inference.

Two daily sessions were conducted with a minimum interval of one hour between the end of the first training session and the beginning of the second. Each subject was trained until they reached the criterion of 75% correct. To reach this criterion, the mice must demonstrate an average of 75% correct, and completion of at least 30 trials per session, in 2 training sessions, occurring either in the same day or on successive days. Upon reaching this criterion, each subject proceeded to the next image pair comparison, or to the retention stage. For example, if a mouse performing A > B achieves 72% correct on one day and 79% the next day, in its next training session it will receive B > C. In the probe test, the stimulus pairs were randomly intercalated, with an even load

Table 1
Testing design and training schedule for visual transitive inference in mice

Transitive inference sequence

Stages	Training schedule
Serial training	A > B, 60 trials per 60 min session until criterion B > C, 60 trials per 60 min session until criterion C > D. 60 trials per 60 min session until criterion D > E. 60 trials per 60 min session until criterion
Retention test #1	A > B. B > C, C > D, D > E (15 trials each in order)
Retention test #2	B > C, O > E, A > R C > D (15 trials each pseudorandom)
Probe test	C D, B > C, D > E, A > B (7 trials each pseudorandom) A > E. B > D (15 trials each pseudorandom)
Criterion	30 trials or more >75% correct averaged in 2 sessions over 2 days

The serial order of the sessions was designed in accordance with methods used for the transitive inference task in nonhuman primates and rodents. Two sessions a day were administered throughout training. Sessions consisted of repeated presentations of the same pair of visual stimuli. Reinforcement was contingent on selecting the image that was correct for the pair (A > B). Criterion was 75% correct responses. The pair then progressed to B > C until 75% or higher correct responses, then C > D until 75% or higher correct responses, and lastly D > E until 75% or higher correct responses were achieved. Retention test #1 (RT#1) consisted of 15 trials of each premise pair for a total of 60 trials per session. The 4 premise pairs were presented in the original training order (A > B > C > D > E) until 75% or higher correct responses. Retention test #2 (RT#2) consisted of 15 pseudorandom presentations of trials of each premise pair. The 4 premise pairs were intermixed and presented in a pseudorandom order until 75% or higher correct responses. The day after reaching criterion on the pseudorandom RT#2, subjects were given a probe test for the transitive (B > D) pairing and for the end-pair (A > E). Probe trials represented novel choices between items that had not previously been presented together. Fifteen probe trials of BD and 15 probe trials of AE were intermixed within presentations of the 7 trials of each of the premise pairs.

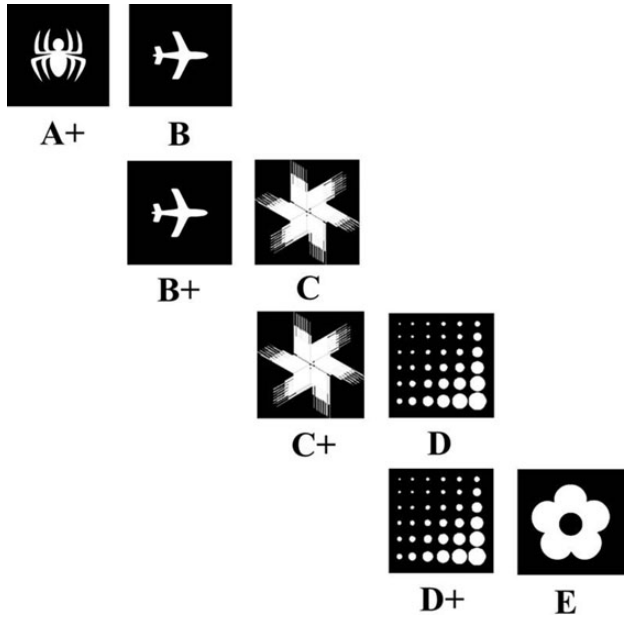

Figure 1. Schematic of the visual stimuli used for the transitive inference hierarchy. This visual task was modified from a nonoperant olfactory version in rats (Dusek and Eichenbaum 1997).

of A > E and B > D trials (15× per session). The remaining 30 trials consisted of random presentation of the A > B, B > C, C > D, D > E pairs (30× per session) for a total of 60 trials (Table 1).

An index of preference was calculated as correct selection – incorrect selection/correct selection + incorrect selection, to normalize for total selections of each image, as previously described (Eichenbaum et al. 1996; DeVito et al. 2009; Eichenbaum and Fortin 2009). All trials during probe testing were unrewarded in concordance with previous literature (DeVito et al. 2009, 2011; Solomon et al. 2011). Data from each training session were analyzed daily to avoid overtraining. Additional comparisons were made after adjusting for multiple measures with the Bonferroni correction when appropriate.

Results

Cohort 1 ($N = 7$ B6 and $N = 7$ BTBR) completed simple discrimination and reversal. One mouse from the original ($N = 8$) of each strain was dropped due to poor performance in the pretraining regimen. Cohort 2 ($N = 7$ B6 and $N = 7$ BTBR) was used for the TI assay. One mouse from each strain was dropped due to poor performance in the pretraining regimen resulting in a total ($N = 6$) of each strain for the TI assay. All remaining mice progressed through training of premise pairs and completed the integrated retention tests in fewer than 40 sessions or were truncated at the 40 session maximum, and were then tested in the probe trial for inferential judgments.

Habituation to the Operant Touchscreen Apparatus in B6 and BTBR

Table 2 lists the scores from the habituation phase at the start of the touchscreen procedure. These values were used to confirm that the reinforcement aliquots of liquid diet were consumed and trials were completed. B6 and BTBR mice steadily increased the number of trials completed and progressed simultaneously to discrimination learning (B6: $F_{1,7} = 19.73$, $P < 0.001$, BTBR: $F_{1,7} = 39.53$, $P < 0.001$). No strain differences ($F_{1,14} = 0.50$, $P > 0.05$) or interactions ($F_{3,14} = 0.63$, $P > 0.05$) were detected.

Table 3 illustrates the correct image selection versus blank screen using a 20-s timeout for correction. Performance in B6 and BTBR mice improved over time, with both strains reaching the criterion of 30 trials with an 85% or higher choice accuracy on 2 consecutive training days (B6: $F_{1,7} = 8.72$, $P < 0.0001$, BTBR: $F_{1,7} = 11.11$, $P < 0.0001$). No strain differences ($F_{1,14} = 0.52$, $P > 0.05$) or interactions ($F_{7,14} = 0.20$, $P > 0.05$) were detected.

Discrimination and Reversal Learning Using a Pairwise Visual Task in B6 and BTBR

Figure 2*A* illustrates visual discrimination to a stringent 90% criterion using the nutrient fortified liquid reinforcer, Ensure strawberry milkshake, 7 µL. Control B6 mice required a mean of 21.9 ± 2.74 sessions. BTBR mice required a mean of 31.6 ± 4.6 sessions. Both strains of mice reached criterion (B6: $F_{6,27} = 4.75$, $P < 0.001$; BTBR: $F_{6,32} = 4.49$, $P < 0.001$).

The trend for a slower rate of acquisition learning by BTBR compared with B6 did not reach statistical significance on percent correct across training sessions ($F_{1,28} = 4.1$, $P = 0.08$) or days to reach 90% criterion ($t_{(1,12)} = -1.95$, $P > 0.05$).

Table 2
Habituation in B6 and BTBR

	Habituation trials completed (mean ± SEM)		
	C57BL6/J ($N = 7$)	BTBR T + tf/J ($N = 7$)	P value
Day 1	40.25 ± 15.1	55.87 ± 6.9	NS
Day 2	63.5 ± 19.9	95.7 ± 8.58	NS
Day 3	90.75 ± 38.4	90.12 ± 13.06	NS
Day 4	156.25 + 27.4	155.62 + 24.06	NS

Scores presented are the number of trials completed during the habituation portion of pretraining for discrimination and reversal in the operant touchscreen procedure.

Table 3
Correction training in B6 and BTBR

	Pretraining time out correction phase % correct of image selection versus blank (mean ± SEM)		
	C57BL6/J ($N = 7$)	BTBR T + tf/J ($N = 7$)	P value
Day 1	0.55 ± 0.050	0.59 ± 0.04	NS
Day 2	0.77 ± 0.03	0.81 ± 0.03	NS
Day 3	0.74 ± 0.04	0.75 ± 0.03	NS
Day 4	0.68 ± 0.03	0.70 ± 0.06	NS
Day 5	0.S4 ± 0.04	0.80 ± 0.04	NS
Day 6	0.87 ± 0.04	0.78 ± 0.02	NS
Day 7	0.91 ± 0.01	0.78 ± 0.04	NS
Day 8	0.82 ± 0.05	0.81 ± 0.04	NS

Progressive percentages of correct responses achieved by mice during the final phase of pretraining for discrimination and reversal, which assessed correct image selections versus blank screen selections. A correction time out (20 s ITI) followed incorrect responses.

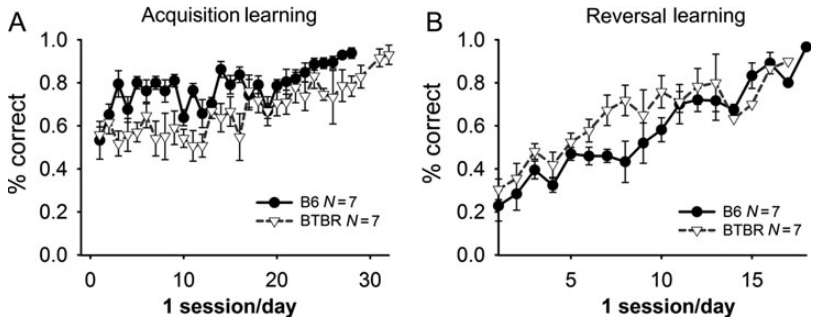

Figure 2. Percentages of choice accuracy for discrimination and reversal learning across training session days in B6 and BTBR. Acquisition of visual discrimination of luminescent-balanced stimuli in B6 and BTBR inbred strains of mice. (*A*) Simple discrimination learning and (*B*) simple reversal learning were normal in both strains, as demonstrated by similar accuracy in the percent correct responses in B6 and BTBR. Both strains reached the stringent criteria of 90% choice accuracy discrimination and 80% choice accuracy for reversal. Both strains exhibited normal acquisition curves, that is, increased correct responses across training sessions. Data are mean ± standard error of the mean. $N = 7$ per strain.

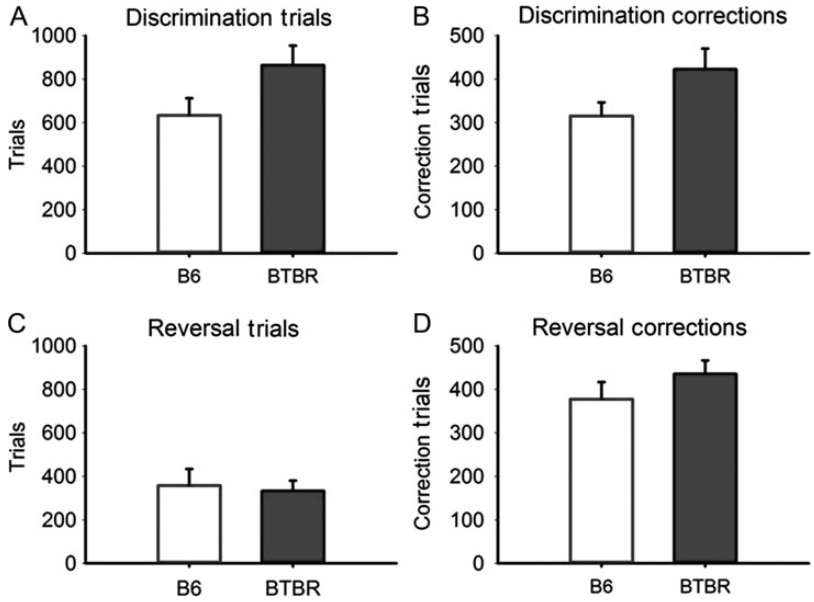

Figure 3. Trials to criterion and correction errors for pairwise discrimination and reversal learning in B6 and BTBR. Visual discrimination acquisition and reversal learning were normal in both B6 and BTBR mice. (*A*) Total number of trials completed demonstrated a trend for more trials required by BTBR when compared with B6; however, this trend failed to reach statistical significance ($P = 0.08$), when summed to reach a stringent 90% criterion of correct responses. (*B*) Number of correction error trials did not differ between B6 and BTBR. (*C*) Total number of trials for reversal learning did not differ between BTBR and B6, when summed to reach the 80% criterion of correct responses for reversal. (*D*) Number of correction error trials during reversal did not differ between B6 and BTBR. Data are mean ± standard error of the mean. $N = 7$ per strain.

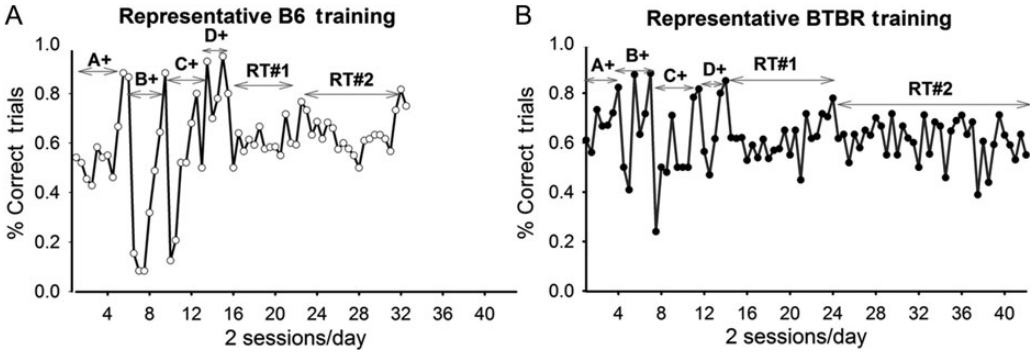

Figure 4. Representative image stimuli and representative transitive inference training learning curves in B6 and BTBR. Mice were trained on a sequence of premise image pair discriminations in which the + sign indicated the rewarded image. Representative training progression of (*A*) B6 mouse and of (*B*) a BTBR mouse, in learning the series of premise pairs (early session numbers, illustrated on the left of each graph, A+, B+, C+, D+) and reaching criterion on 2 retention tests (later session numbers, illustrated on the right of each graph, ordered RT#1, pseudorandom RT#2). BTBR acquired premise pair discriminations at a rate similar to B6, but required more sessions to reach criterion during integrated retention tests.

Figure 2*B* illustrates correct responses to the valued image for reversal learning. Control B6 mice reached criterion in 14.5 ± 0.95 sessions. BTBR mice reached criterion in 11.3 ± 3.44 sessions. B6 and BTBR did not differ on percent correct across training sessions ($F_{1,16} = 0.65$, $P > 0.05$). Days to reach the 80% criterion did not differ by strain ($t_{(1,12)} = 1.96$, $P > 0.05$). Both strains reached the reversal criterion (B6: $F_{6,17} = 8.44$, $P < 0.001$; BTBR: $F_{6,16} = 6.75$, $P < 0.001$).

Figure 3 illustrates pairwise visual discrimination and reversal learning in B6 and BTBR. Number of trials to the discrimination criterion (Panel *A*, $t_{(1,12)} = 2.11$, $P = 0.08$) and number of correction errors (Panel *B*, $t_{(1,12)} = -1.72$, $P > 0.05$) did not differ by strain. After the criterion was reached for pairwise discrimination learning, correct images were reversed. B6 and BTBR did not differ on number of trials to reach reversal criterion (Panel *C*, $t_{(1,12)} = 1.04$, $P > 0.05$), nor on number of correction errors (Panel *D*, $t_{(1,12)} = 0.06$, $P > 0.05$).

Proficiency During Training on the Visual Transitive Inference Task in B6 and BTBR Mice

Figure 4 illustrates representative learning progression of a B6 (Panel *A*) and BTBR (Panel *B*) mouse during the series of premise pairs and criterion on both phases of tests.

Figure 5 illustrates performance on the relational transitive premise pairs and integrated retention stages in B6 and BTBR mice. BTBR did not differ from B6 on the acquisition of the AB, BC, CD, and DE premise pairs (Panel *B*, between strain repeated measures ANOVA, $F_{1,12} = 2.14$, $P > 0.05$), nor was an interaction of strain by learning significant ($F_{3,27} = 1.73$, $P > 0.05$). Average days to criterion across the premise pairs also confirmed no statistical difference between B6 and BTBR in premise learning ($t_{(1,11)} = 1.46$, $P > 0.05$). Both B6 and BTBR required more days to reach criterion in the retention tests from the final premise pair DE (ordered RT#1, $t_{(1,5)} = -7.09$, $P < 0.0009$; pseudorandom RT#2, $t_{(1,5)} = -5.22$, $P < 0.005$).

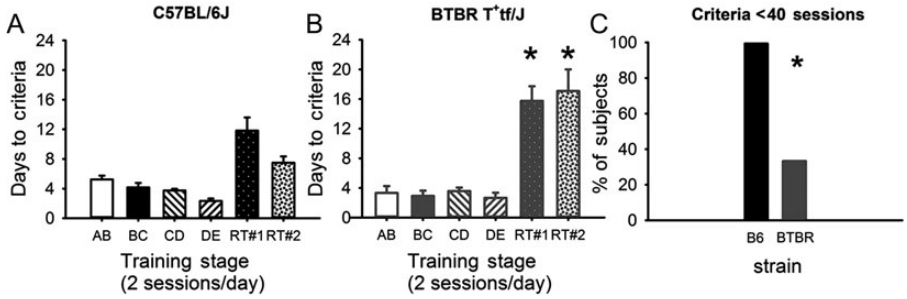

Figure 5. Performance on the relational premise pairs (A > B, B > C, C > D, D > E) in B6 and BTBR mice. The criterion for progression to the next training stage was an average of 75% or greater correct responses over 2 consecutive sessions. (*A*) Total number of days to criterion across stages of training in B6 mice. Higher number of days to criterion was observed for the more complex ordered RT#1 and pseudorandom RT#2 stages when compared with performance on the last premise pair DE. (*B*) Total number of days to criterion across all stages of training in BTBR mice. Similar numbers of sessions were needed to acquire the premise training pairs, when compared with B6. BTBR also required significantly more sessions to reach criterion on the 2 retention tests, when compared with performance on the premise pair DE. BTBR required more days to reach criterion than B6 on both ordered RT#1 and pseudorandom RT#2. Data are mean ± standard error of the mean. $N = 6$ per strain. *$P < 0.05$, days to reach criterion in BTBR versus B6. (*C*) Percentages of B6 and BTBR that completed each stage of training. Mice were given up to 40 sessions to reach criterion on each training stage. Mice that did not reach criterion by the end of 40 sessions were assigned a 40 session maximum and advanced to the next training stage.

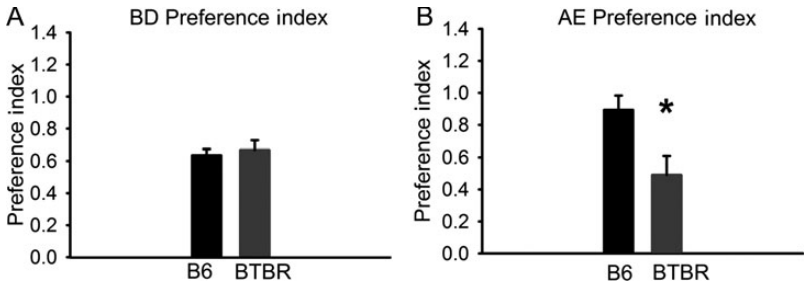

Figure 6. Performance on the transitive pair BD and the end pair simple inference AE in B6 and BTBR mice. An index of preference was calculated as described in the Materials and Methods. (*A*) Comparable strain performance was exhibited on the transitive intermediate pair B > D preference index, which requires a high level of inferential judgment. (*B*) Strain comparisons using the preference index revealed impaired performance in BTBR on the anchored end pair A > E. *$P < 0.05$, lower preference index for A versus E in BTBR versus B6. $N = 6$ per strain.

On retention phases (Fig. 5*A* and *B*), BTBR reached criterion more slowly than B6 (ordered RT#1: $t_{(1,11)} = -2.60$, $P < 0.05$; pseudorandom RT#2: $t_{(1,11)} = -2.89$, $P < 0.05$). B6 completed ordered RT#1 in 10.4 ± 1.3 sessions and pseudorandom RT#2 in 7.5 ± 0.85 sessions to reach criterion (Panel *A*). BTBR completed ordered RT#1 in 17.1 ± 2.9 sessions and pseudorandom RT#2 in 15.7 ± 1.9 sessions to reach criterion (Panel *B*). 100% of the B6 mice tested completed each stage of TI training and probe testing. Only 33% of BTBR completed all training stages. Specifically, some BTBR but no B6 failed to complete the integrated retention test sessions while all of the BTBR and B6 subjects learned the simple discriminations (Fig. 5*C*).

Performance on the BD Transitive Pair and the AE End Pair in B6 and BTBR Mice

Figure 6*A* and *B* illustrates performance during TI probe testing for untrained pairs assessed by preference index. B6 and BTBR demonstrated engagement in the task by selecting images that required inferential judgments. B6 selected image *A* in 13.2 ± 0.80 choices, over image *E* in 0.80 ± 0.58 choices, in the new end pair inference choice pair A > E ($t_{(1,5)} = 11.06$, $P < 0.0001$). BTBR selected image *A* in 10.3 ± 0.98 choices, over image *E* in 3.5 ± 0.84 choices, in the new end pair inference choice pair A > E ($t_{(1,5)} = 3.93$, $P < 0.01$). B6 exhibited transitivity by selecting image *B* in 9.2 ± 0.49 choices over image *D* in 5.60 ± 0.50 choices in the new transitive inference choice pair B > D ($t_{(1,5)} = 4.6$, $P < 0.005$). BTBR exhibited transitivity by selecting image *B* in 8.83 ± 1.01 choices over image *D* in 3.83 ± 0.53 choices in the new transitive inference choice pair B > D ($t_{(1,5)} = 3.34$, $P < 0.05$). Repeated-measure ANOVAs detected a significant interaction of strain during the A > E pairings ($F_{1,10} = 7.84$, $P < 0.02$) but not during the B > D pairings ($F_{1,10} = 0.36$, $P > 0.05$). Performance on the transitive BD probe test did not differ significantly between B6 and BTBR (Panel *B*, $t_{(1,10)} = -0.78$, $P > 0.05$). Although both strains displayed choice accuracy on all phases, performance on the anchored pairing A > E was significantly lower in BTBR when compared with B6 (Panel *A*, $t_{(1,10)} = 2.93$, $P < 0.02$).

Discussion

The primary aim of the present study was to validate an automated touchscreen assay for mice that maximizes procedural analogies to human testing equipment, in a sophisticated task that assesses TI, in the B6 inbred strain of mice that performs well in most cognitive and social tasks, and the BTBR inbred mouse model of ASD with known cognitive deficits. Our results demonstrate that both B6 and BTBR performed the simple discrimination and reversal tasks at levels comparable to those in the literature for B6, BTBR, DBA/2J, and BALB/cJ strains of mice, for touchscreen, Pavlovian instrumental learning and olfactory discrimination (Lederle et al. 2011; Andre et al. 2012; Rutz and Rothblat 2012; Yang et al. 2012). Both

strains exhibited successful acquisition of the TI premise pairs with visual stimuli, similar to performance in control, ASD, and schizophrenia clinical populations (Titone et al. 2004; Coleman et al. 2010; Solomon et al. 2011). B6 and BTBR mice both accomplished the most challenging transitive inference, that B > D, and exhibited intact preference index ratios. However, while both strains performed the trivial anchored item inference A > E, scores were significantly worse for BTBR than B6. Despite typical performance on acquisition and reversal tasks, BTBR required extended sessions when the task retention tests were introduced, complexity was increased, and flexible integrations were required. Our findings are consistent with a larger literature in BTBR on other cognitive tasks, which described impairments in probabilistic learning, contextual rule shift response inhibition, novel object recognition memory, and complex social learning (Amodeo et al. 2012; Rutz and Rothblat 2012; Lipina and Roder 2013; Silverman et al. 2013; Yang et al. 2012).

One strategy for TI learning is based on relational flexibility and relies on stimulus hierarchical encoding. Logical inferences are made from learned information stored about the premise pairs. The HPC, entorhinal cortex and PFC appear to be essential for flexible access to representative associations acquired during initial training and support explicit memorization of the premise pair relationships by binding together their individual elements during early learning in rodent and nonhuman primate models (Bunsey and Eichenbaum 1996; Rapp et al. 1996; Dusek and Eichenbaum 1997; Buckmaster et al. 2004; DeVito, Lykken et al. 2010). Another strategy for TI involves associative strengths of the premise pairs (Frank et al. 2003, 2005; Van Elzakker et al. 2003). This strategy has been validated through the use of computational models (Van Elzakker et al. 2003; Frank et al. 2003). Inferences are made by associations of positive and negative feedback and values transferred to the representations of the stimuli. A gradient of unequal associative strengths is formed, which supports TI. Cortico-striatal dopaminergic circuitry appears to be essential for the formation of associative strengths (Frank et al. 2005). Specifically, the hierarchy of associative weights is supported by the basal ganglia, PFC (Jog et al. 1999; Aizenstein et al. 2004; Daw et al. 2005; Graybiel 2008; Doll et al. 2009;) and the orbitofrontal cortex (OFC), for rapid and flexible updating of representations of expected value and to store working memories of the values of premise pairs (Schoenbaum and Roesch 2005).

Anatomical and structural imaging literature predicted that BTBR would exhibit impaired transitive inference, based on the absence of corpus callosal connections of their left and right cortical hemispheres, and reduced size of their entorhinal cortex, dentate gyrus, nucleus accumbens, frontal, and parietotemporal lobes (Wahlsten et al. 2003; Kusek et al. 2007; Wahlsten 2012; Ellegood et al. 2013; Jones-Davis et al. 2012). In humans, functional imaging showed regional activation of the posterior parietal lobe during a 3-point inference task (Goel and Dolan 2001; Waechter et al. 2013). A TI network has been described, that highlighted the bilateral PFC, premotor area, insular, and posterior parietal cortex using an 11-point task (Acuna et al. 2002). Additional recent studies discovered that damage to the ventromedial PFC resulted in a deficit in the ability to use TI (Koscik and Tranel 2012).

Performance of BTBR mice was worse than B6 on ordered and pseudorandom retention tests. These integrated sessions require increased memory load for concurrent discriminations and are impaired following prefrontal lesions (DeVito, Lykken et al. 2010). Our results corroborate the results of that used the olfactory version in lesioned mice. BTBR has low frontal lobe volume and low prefrontal cholinergic transmission (Ellegood et al. 2013; McTighe et al. 2013). BTBR's poor performance in integration may also be related to its unusual hippocampal formation, reduced hippocampal commissure, or abnormal white matter connectivity (Wahlsten et al. 2003; Ellegood et al. 2013). Hippocampal lesioned rats are impaired in interleaving distinct experiences according to their overlapping elements to form a relational network and make correct predictions (Dusek and Eichenbaum 1997) while studies on humans have similarly indicated a selective role for the HPC in supporting inferences from memory (Heckers et al. 2004; Preston et al. 2004). A detailed histological investigation of brain structures involved during retention phases may lead to precise neural substrates to be examined in future directions. However, despite significant volume reductions in BTBR in regions implicated in TI, BTBR were capable of making the difficult B > D inference. Remarkably, BTBR were not proficient in the anchored, easier end pair judgment A > E. This finding is striking because adults with ASD similarly demonstrated impairments in the A > E inference, while the B > D selectivity remained intact (Solomon et al. 2011). The types of deficits we detected in BTBR, therefore, were highly analogous to the types of deficits detected in adults with autism.

The touchscreen methodology has been employed in mice and rats for tasks of visual discrimination and reversal (Bussey et al. 2001; Izquierdo et al. 2006; Brigman, Mathur et al. 2010; Graybeal et al. 2011; Rutz and Rothblat et al. 2012), paired associative learning (Talpos et al. 2009; Bartko et al. 2011), location memory and pattern separation (McTighe et al. 2009; Talpos et al. 2010), visuospatial attention (Botly and De Rosa 2012), dimensional set shifting (Brigman et al. 2005), and 5 choice serial reaction time (Bartko et al. 2011; Romberg et al. 2011; McTighe et al. 2013). Rodent models of neuropsychiatric diseases that have been evaluated in touchscreen tasks include a genetic model of Alzheimer's disease β-amyloid pathology (Romberg et al. 2011, 2013), Huntington's disease (Morton et al. 2006) addiction and reward abnormalities (Izquierdo et al. 2006; Lederle et al. 2011), schizophrenia (Brigman et al. 2006, 2008, 2009; Brigman, Mathur et al. 2010), and lesion studies of memory function (Brigman and Rothblat 2008; Clelland et al. 2009; McTighe et al. 2009). Previous rodent TI research employed odor discriminations and time spent digging in media (Dusek and Eichenbaum 1997; DeVito et al. 2009, 2011; DeVito, Kanter et al. 2010; DeVito, Lykken 2010; Andre et al. 2012) or a visual discrimination that used graphical images mounted to the lateral walls of a T-maze (Van der Jeugd et al. 2009). Our mouse TI task successfully demonstrated that the Bussey–Saksida touchscreen operant system is useful for assessing higher order cognitive demand tasks. It is the first TI design to use computer generated graphic images, serial premise pair training and automated output by touchscreen technology. Our findings are consistent with BTBR deficits on the contextual rule shift touchscreen assay of response inhibition (Rutz and Rothblat 2012) and slower initial rates to touchscreen acquisition (McTighe et al. 2013). BTBR may be employing an alternative learning strategy from control B6 mice, since BTBR was normal on visual discrimination and reversal, and retained the ability to make inferential choices, while failing at more complex integration of discriminations.

These results point the way to the future development and evaluation of challenging, cognitive tasks for mouse models of neurodevelopmental disorders. Rodent assay methods and equipment that are analogous to human tasks may enhance preclinical approaches to therapeutic discovery (Chudasama and Robbins 2006; Robbins and Arnsten 2009; Keeler and Robbins 2011; Bussey et al. 2012). This is critical given that multiple forms of higher cognition including executive functions, memory, and learning are impaired in individuals with autism, ADHD, schizophrenia, and other neurodevelopmental disorders (Cassidy et al. 2000; Zaroff et al. 2004; Carter et al. 2008, 2011; Solomon, Ozonoff, Carter et al. 2008; Solomon, Ozonoff, Cummings 2008; Barch, Braver et al. 2009, Barch, Carter 2009; Sevin et al. 2009; Barnett et al. 2010; Woodcock et al. 2010, 2011; Berger-Sweeney 2011; Yang, Chan et al. 2012). Analogous behavioral assays that assess cognition in animal models of neurodevelopmental disorders would contribute an important new component to the preclinical toolbox, which could enhance preclinical discoveries that inform clinical investigations.

Funding

This work was supported by the National Institute of Mental Health Intramural Research Program and the MIND Institute, University of California Davis School of Medicine.

Notes

The authors are grateful to Drs Andrew Holmes, Henriette Van Praag, and Michelle Potter for their assistance and guided tours of their operant touch screen equipment and procedures, at the National Institute of Alcohol Abuse and Alcoholism (NIAAA) and the National Institute of Aging (NIA), respectively. They express sincere gratitude to Dr Jonathan Brigman (University of New Mexico) for his knowledgeable suggestions on the design of our novel task and on general operant learning procedures. We thank Mr Matthew Croxall of Lafayette Instruments for his technical advice. *Conflict of Interest*: None declared.

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