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Discrimination Learning and Attentional Set Formation in a Mouse Model of Fragile X

Kimberly S. Casten², Annette C. Gray², and Rebecca D. Burwell^{1,2}

¹Department of Cognitive, Linguistic, and Psychological Sciences, Brown University, Providence, RI 02912

²Department of Neuroscience, Brown University, Providence, RI 02912

Abstract

Fragile X Syndrome is the most prevalent genetic cause of mental retardation. Selective deficits in executive function, including inhibitory control and attention, are core features of the disorder. In humans, Fragile X results from a trinucleotide repeat in the *Fmr1* gene that renders it functionally silent and has been modeled in mice by targeted deletion of the *Fmr1* gene. *Fmr1* knockout (KO) mice recapitulate many features of Fragile X syndrome, but evidence for deficits in executive function is inconsistent. To address this issue, we trained wild-type and *Fmr1* KO mice on an experimental paradigm that assesses attentional set-shifting. Mice learned to discriminate between stimuli differing in two of three perceptual dimensions. Successful discrimination required attending only to the relevant dimension, while ignoring irrelevant dimensions. Mice were trained on three discriminations in the same perceptual dimension, each followed by a reversal. This procedure normally results in the formation of an attentional set to the relevant dimension. Mice were then required to shift attention and discriminate based on a previously irrelevant perceptual dimension. Wild-type mice exhibited the increase in trials to criterion expected when shifting attention from one perceptual dimension to another. In contrast, the *Fmr1* KO group failed to show the expected increase, suggesting impairment in forming an attentional set. *Fmr1* KO mice also exhibited a general impairment in learning discriminations and reversals. This is the first demonstration that *Fmr1* KO mice show a deficit in attentional set formation.

Keywords

prefrontal; cognition; transgenic mice; behavior

Fragile X Syndrome (FXS) is the most common known genetic cause of intellectual disability, affecting an estimated 1:4000 males and 1:8000 females (Turner, Webb, Wake, & Robinson, 1996). FXS patients suffer from dysmorphic facial features, macroorchidism, increased susceptibility to epilepsy, a general learning impairment, and specific cognitive-behavioral deficits distinct from other learning disabilities. Boys with FXS tend to be hyperactive; impulsive; resistant to change in stimuli, conditions, or environment; and hypersensitive and hyper-reactive to stimuli (Freund & Reiss, 1991; Hagerman, Amiri, &

Address correspondence to: Rebecca D. Burwell, Ph.D., Department of Cognitive, Linguistic, and Psychological Sciences, Brown University, 89 Waterman Street, Providence, RI 02906, phone: (401) 863-9209, FAX: (401) 863-1300, rebecca_burwell@brown.edu.

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Cronister, 1991; Kau, Reider, Payne, Meyer, & Freund, 2000; Levitas, 1996; Reiss & Freund, 1990). They also commonly exhibit impairments in social cognition and attention (K. Cornish et al., 2005; K. Cornish, Scerif, & Karmiloff-Smith, 2007; K. Cornish, Sudhalter, & Turk, 2004; K. M. Cornish, Munir, & Cross, 2001; Hooper et al., 2008; Scerif, Cornish, Wilding, Driver, & Karmiloff-Smith, 2007). In most cases of FXS, the *Fmr1* gene contains an expansion of trinucleotide (CGG) repeats in its 5' untranslated region that leads to hypermethylation of the *Fmr1* promoter, blocking expression of the fragile X mental retardation protein (FMRP) (Devys, Lutz, Rouyer, Bellocq, & Mandel, 1993; Pieretti et al., 1991; Verheij et al., 1993), which is expressed at high levels throughout the developing brain (Abitbol et al., 1993; Hinds et al., 1993). Studies of FXS patients have shown modest enlargement of the hippocampus, caudate nucleus, and fusiform gyrus and decreased volume of the superior temporal gyrus, amygdala, and cerebellar vermis (Gothelf et al., 2008; Hessler, Rivera, & Reiss, 2004; Reiss, Abrams, Greenlaw, Freund, & Denckla, 1995; Reiss, Aylward, Freund, Joshi, & Bryan, 1991). White matter, particularly in frontostriatal pathways, may also be abnormal in FXS patients (Haas et al., 2009; Hoeft et al., 2010; Hoeft et al., 2008). The degree of anatomical abnormality of the caudate and cerebellum may correlate with the degree of intellectual disability (Gothelf, et al., 2008; Mostofsky et al., 1998; Reiss, et al., 1995), highlighting the importance of these structures in cognition and the specific deficits found in FXS.

FXS is associated with selective deficits in executive function, including impaired inhibitory control, working memory, attention, and planning, that become more pronounced as cognitive demands increase (Hooper, et al., 2008; Lanfranchi, Cornoldi, Drigo, & Vianello, 2009; Munir, Cornish, & Wilding, 2000a, 2000b; Ornstein et al., 2008; Scerif, et al., 2007; Wilding, Cornish, & Munir, 2002). Brain regions affected in FXS include those implicated in executive function, including prefrontal areas (Lie, Specht, Marshall, & Fink, 2006; Monchi, Petrides, Petre, Worsley, & Dagher, 2001). Moreover, functional MRI investigations show that FXS patients fail to recruit additional resources in these areas as working memory and inhibitory control tasks become more difficult (Hoeft et al., 2007; Kwon et al., 2001; Lightbody & Reiss, 2009; Menon, Kwon, Eliez, Taylor, & Reiss, 2000; Menon, Leroux, White, & Reiss, 2004; Rivera, Menon, White, Glaser, & Reiss, 2002; Tamm, Menon, Johnston, Hessler, & Reiss, 2002). FXS individuals are also known to have deficits in attentional set shifting (Hooper, et al., 2008), which depends on prefrontal dopamine (Robbins & Arnsten, 2009).

The present study was designed to further examine deficits in executive function in the mouse model of FXS, *Fmr1* knockout (KO) mice (Consortium, 1994). The *Fmr1* KO mouse expresses a number of endophenotypic markers similar to core defects found in FXS, including macroorchidism, increased susceptibility to epileptic seizures, hyperactivity, increased sensitivity to sensory stimuli, and deficits in learning and memory (Chen & Toth, 2001; Consortium, 1994; Kooy, 2003; Qin, Kang, & Smith, 2002; Zhao et al., 2005). As in humans with FXS, neurons in *Fmr1* KO mice have immature dendritic spines and an increased density of spines (Comery et al., 1997; Irwin et al., 2001; McKinney, Grossman, Elisseou, & Greenough, 2005; Nimchinsky, Oberlander, & Svoboda, 2001). *Fmr1* KO mice show subtle deficits in spatial learning and reversal learning (Consortium, 1994; D'Hooge et al., 1997; Paradee et al., 1999) and display impaired inhibitory control and attention in a five-choice serial reaction time task (Moon et al., 2006), suggesting that the model is useful for studying deficits in executive function. FXS patients have difficulty with the Wisconsin Card Sort Task (WCST) and other tasks that require inhibition of a previously learned rule or target and a shift in attention to a new rule or target (K. M. Cornish, et al., 2001; Hooper, et al., 2008; Mostofsky, et al., 1998). Thus, we hypothesized that *Fmr1* KO mice would show similar deficits on a task that requires attentional set shifting.

In this study, we used an attentional set-shifting task originally developed for rats (Birrell & Brown, 2000). Subjects learned discriminations between two stimuli that differed in two of three perceptual dimensions (texture, odor, and digging medium). Only one dimension predicted the location of a food reward. Thus, subjects were required to attend to one perceptual dimension to select the appropriate target and obtain a food reward. Subsequently, subjects were trained to shift attention to novel targets within the same perceptual dimension (intra-dimensional shift, IDS) or to targets within a previously irrelevant dimension (extra-dimensional shift, EDS). In general, IDSs are easier than EDSs. The cost in performance for EDSs is commonly used as an indicator of the formation of an attentional set. Previous studies have failed to demonstrate a difference between IDS and EDS performance in mice (Brigman, Bussey, Saksida, & Rothblat, 2005; Colacicco, Welzl, Lipp, & Wurbel, 2002). To facilitate formation of an attentional set in our paradigm, mice were trained on multiple IDS problems followed by reversals before presentation of the EDS. As predicted, wild-type mice exhibited the expected shift effect, demonstrating that mice can form an attentional set to a perceptual dimension. Importantly, *Fmr1* KO mice did not show evidence of attentional set formation. This is the first demonstration of an attentional set-shifting deficit in *Fmr1* KO mice.

Methods

Subjects

All subjects were bred in the Brown University Animal Care Facility and kept on a 12:12 hour light:dark cycle. Subjects were 6-7 week-old male F1 generation wild-type (WT; n=9) and *Fmr1* KO mice (n=7) bred from male 129P3/J mice and female C57BL/6J mice heterozygous for *Fmr1*, obtained from Jackson Laboratories (Bar Harbor, ME). Pilot studies indicated that the behavior of interest was most robust in the F1 hybrid. In addition, it has been suggested that behavioral phenotypes of different hybrid crosses should be more alike than phenotypes of different inbred strains (Silva et al., 1997). Genotyping was carried out using PCR on tail DNA extracts. One week before habituation, mice were put on feeding schedules and received 2.9-3.1 grams of LabDiet 5008 Formulab (PMI Nutrition International, LLC, St. Louis, MO) each day. Meals were adjusted to maintain weights at 85-90% ad lib feeding weights. All methods involving the use of live animals conformed to NIH guidelines and were approved by the Brown University Institutional Animal Care and Use Committee.

Apparatus and experimental stimuli

Training and testing sessions were completed in a black plexiglas box (51.5L × 27W × 18H cm), open on the top. For all sessions, two small clay flower pots, (4.3 cm in diameter, 3.7 cm in height) were placed side by side at one end of the apparatus. Food rewards were hidden in one of the two containers presented simultaneously, which could be distinguished by odor, outside texture, and digging medium. The food reward, an ice cream sprinkle (Betty Crocker Carousel Mix), was buried underneath the digging medium. The bottom of each pot was filled with melted wax, which was allowed to harden, to weight the containers for greater stability. When odor stimuli were required, the wax was scented. Habituation pots contained unscented wax, bedding was the digging medium, and there was no added outside texture. Exemplar pots represented only one of the three perceptual dimensions. Testing pots represented all three dimensions: the bottom half of the pot was filled with scented wax; the top half of the pot was filled with one type of digging medium; and a texture stimulus covered the outer surface, including the lip. Pilot studies revealed that covering the outer surface with textured material was insufficient for learning, and extending the texture over the lip of the cup was necessary.

Behavioral training and testing

Each animal completed preliminary training that included habituation, exemplar discrimination, single discrimination (SD), compound discrimination (CD), and reversal. Subsequent testing consisted of three IDSs, each followed by a reversal, an EDS, and a final reversal. Mice were allowed to correct mistakes during the first four trials, after which they were allowed to dig in only one pot. Criterion for advancing to the next phase of the task was eight correct out of 10 consecutive trials. During each phase of testing, one probe trial was given in which the stimuli were presented without a food reward to rule out the possibility that animals used smell of the reward to perform the task. If the mouse chose the correct pot, the experimenter dropped the food reward on the top of the digging medium.

Habituation—Habituation sessions lasted ten minutes, and the position of the containers alternated from one end of the apparatus to the other for each presentation. Mice were trained to consistently dig through the bedding material to the bottom of the containers for food rewards.

Exemplar discrimination—Animals were required to discriminate between successive pairs of exemplar containers that presented and differed in only one of the three perceptual dimensions. One member of the exemplar pair contained the food reward. Mice were exposed to exemplars for each of the three stimulus dimensions: textures were plain or shiny ribbon; odors were juicy melon or cherry blossom; and digging media were dark or light colored tissue paper. Testing stimuli for subsequent phases of the task are presented in Table 1. Order of presentation of perceptual dimensions and the rewarded stimulus were counterbalanced across groups.

Simple and compound discriminations—After reaching criterion on the exemplar discriminations, animals began SD. Animals were presented with stimulus containers that represented all three perceptual dimensions, but differed on only one of the dimensions. For example, the rewarded container would have Texture 1, Medium 1, and Odor 1, whereas the unrewarded container would exhibit Texture 2, Medium 1, and Odor 1 (Table 1, right column). After reaching criterion on SD, mice began CD, during which the same stimulus dimensions were presented, but the containers now varied in two dimensions. The previously relevant dimension remained relevant, and the second varying dimension did not predict the location of the reward. The third dimension did not vary. For example, the rewarded container would have Texture 1, Medium 1 or 2, and Odor 1, whereas the unrewarded container would exhibit Texture 2, Medium 1 or 2, and Odor 1. After reaching criterion on the CD, the animals were presented with a reversal in which the previously unrewarded stimulus was rewarded.

IDS and reversals—After reaching criterion on the SD, CD, and reversal, animals were tested on three intra-dimensional shift (IDS) problems, in which the same two perceptual dimensions varied, the relevant dimension was the same, but a novel set of stimuli were presented. For example, the rewarded container would exhibit Texture 3, Medium 3 or 4, and Odor 3, whereas the unrewarded container would exhibit Texture 4, Medium 3 or 4, and Odor 3 (Table 1, right column). A reversal was performed after animals reached criterion (REV1). Mice were tested on two more IDS problems and reversals.

EDS—After reaching criterion on three subsequent IDSs and reversals, animals were then presented with an EDS, in which novel stimuli were presented and the previously irrelevant, varying dimensions became the relevant dimension. For example, the rewarded container would exhibit Texture 5 or 6, Medium 5, and Odor 5, whereas the unrewarded container would exhibit Texture 5 or 6, Medium 6, and Odor 5 (Table 1, right column). It is expected

that normal animals form an attentional set for the stimulus dimension rewarded in earlier phases of training and testing, resulting in increased number of trials needed to reach criterion on the EDS. Following testing on the EDS, animals performed a final reversal (REV4). Relevant dimensions and rewarded stimuli were counterbalanced across groups.

Data Analysis

Trials to Criterion (TTC, 8 correct in 10 consecutive trials) was the main variable of interest and was primarily analyzed by univariate analysis of variance (ANOVA) or repeated measures ANOVA (rANOVA). Genotype (WT and *Fmr1* KO) was the between subject independent variable for all analyses. The control variables (SD TTC, CD TTC, and initial reversal TTC) and probe trials (Percent Correct) were analyzed by Univariate ANOVA. The IDS problems were analyzed by rANOVA with Problem (1, 2, or 3) and Condition (discrimination or reversal) as the within-subject independent variables. Performance on the EDS and the final reversal were analyzed by Univariate ANOVA. Because *Fmr1* KO mice demonstrate hyperactivity, we also analyzed Latency to Choose in order to clarify whether deficits were due to impulsivity/hyperactivity.

Because the TTC variable exhibited a skewed distribution, we used nonparametric approaches to test differences in the ability to form an attentional set. By convention, a significant increase in trials to criterion when a rule is changed is considered indication that an attentional set had been formed. For each group, we used the Wilcoxon Signed Rank Test to determine whether there was a significant increase in TTC between the mean IDS and the EDS. We also tested the effect size for each group using the *r* equivalent (Rosenthal & Rubin, 2003) and Cohen's *d* (Rosnow, Rosenthal, & Rubin, 2000). The *r* equivalent is a measure of effect size that is useful for nonparametric tests and for repeated measures tests. Cohen's *d* is a standard test for effect size and also can be corrected for dependent samples. In addition, we calculated the variable, Shift Effect, by subtracting the mean TTC for the IDS problems from the TTC for the EDS. Group differences in Shift Effect were analyzed by the Wilcoxon Ranked Sums Test with Genotype (WT and *Fmr1* KO) as the between subject independent variable.

Data were analyzed using SPSS (V17, SPSS, Inc.) or SAS (V. 9.1.3, Sas Institute, Inc.) with a significance level of $p < 0.05$.

Results

WT and *Fmr1* KO mice were not different in acquisition of the training discriminations. ANOVA of TTC confirmed no effects of Genotype for SD (WT, 8.1 ± 0.1 ; *Fmr1* KO, 8.3 ± 0.2 ; $p > 0.28$), CD (WT, 8.0 ± 0 ; *Fmr1* KO, 8.3 ± 0.2 ; $p < 0.17$), or the initial reversal (WT, 10.0 ± 0.4 ; *Fmr1* KO, 9.6 ± 0.4 ; $p < 0.45$). Thus, both groups were able to learn and perform the basic elements of the task equally well, and there was no indication that *Fmr1* KO mice learned the initial discriminations differently from WT mice.

Both groups of mice exhibited good performance on probe trials, indicating that odor cues were not responsible for accurate performance in the task. Each mouse completed 12 probe trials with the exception of one WT mouse that omitted two probe trials and one *Fmr1* KO mouse that omitted three probe trials. Probe trial accuracy did not differ between WT and *Fmr1* KO mice (91.5 ± 2.4 vs. 87.0 ± 2.8 , $p > 0.24$).

During testing, *Fmr1* KO mice were impaired relative to WT mice (Figure 1), as indicated by significantly higher TTC across IDSs and reversals, $F(1,16)=4.67$, $p < 0.046$. Reversals were more difficult for both groups of mice as confirmed by a significant effect of

Condition, $F(1,16)=15.29$, $p<0.001$. There was no main effect of Problem ($p>0.96$) and no interaction of Genotype with Problem ($p>0.87$) or Condition ($p>0.37$).

As shown in Figure 1, WT mice required a greater number of trials to reach criterion in the EDS as compared to the IDS problems. This pattern is consistent with formation of an attentional set during the three IDS and Reversal problems. In contrast, *Fmr1* KO mice exhibited similar TTC for mean IDS and the EDS, suggesting the lack of attentional set formation to the original dimension. To determine whether the two groups differed on the measure of increased TTC for the EDS, for each mouse we calculated a Shift Effect (TTC for the EDS minus mean IDS). Seven of 9 WT mice exhibited a positive Shift Effect as compared to 2 of 9 *Fmr1* KO mice. Moreover, *Fmr1* KO mice were significantly less likely to exhibit a Shift Effect, as confirmed by a Wilcoxon rank-sum test, ($W = 64$, $p < 0.032$, $r_{\text{equivalent}} = 0.42$, $d = 0.99$). An $r_{\text{equivalent}}$ of 0.42 and Cohen's d of 0.99 are both considered to indicate a large effect size. The Wilcoxon Signed Rank Test indicated that the increase in TTC between the mean IDS and the EDS was significantly different for the WT mice ($S=17$, $p < 0.0156$, $r_{\text{equivalent}} = 0.75$, $d = 2.37$). The $r_{\text{equivalent}}$ indicates a large effect size, and the Cohen's d is considered quite large. In contrast, the difference between the final IDS and the EDS was not significant for the *Fmr1* KO mice ($p>0.719$).

Latency to choose did not differ by Problem or Genotype ($p>0.22$) for the IDSs and reversals. There was, however, a trend toward faster latencies for the *Fmr1* KO mice for the EDS and subsequent reversal, compared to the WT mice, $F(1,16)=4.162$, $p<0.058$. Shorter latencies of the *Fmr1* KO mice when the relevant stimuli represent a new perceptual dimension further supports the interpretation that *Fmr1* KO mice had not formed an attentional set.

Discussion

The present study examined attentional set formation in a mouse model of FXS. *Fmr1* KO and WT mice learned the initial and complex discriminations equally well, indicating they were able to acquire the basic components of the task and to process each of three perceptual dimensions. Our evidence suggests that WT mice formed an attentional set as they exhibited the expected increase in TTC following a shift of the relevant perceptual dimension. In comparison, *Fmr1* KO mice exhibited no difference in TTC between the mean IDS and the EDS. This result is consistent with an impairment in attentional set formation, and is the first demonstration of such deficits in *Fmr1* KO mice.

Previous studies failed to reveal expected effects of attentional set formation in WT mice (Bissonette et al., 2008; Brigman, et al., 2005; Colacicco, et al., 2002). In these studies, tasks involved only two perceptual dimensions, and there were fewer IDS problems prior to the EDS. Thus, subjects may not have formed attentional sets when learning the original discrimination. One group reported a decrease in performance during EDS, but the shift occurred in both perceptual dimension (odor or texture) and presentation mode (Young, Powell, Geyer, Jeste, & Risbrough, 2010). Odor was presented as scented bedding in a ceramic container, but texture was presented on the platform on which the container was positioned. This effect is more similar to a task shift, as opposed to a shift in a perceptual dimension. Another group reported a set-shifting effect when a third perceptual dimension was introduced in the EDS (2006). Thus, subjects were required to integrate a completely novel dimension into their perceptual set, while inhibiting attention to familiar dimensions and shifting to the novel dimension. This could bias performance in mouse strains that are hyper-reactive to unfamiliar conditions, such as *Fmr1* KO mice (Chen & Toth, 2001). In our task, three perceptual dimensions were presented throughout testing, one relevant, one irrelevant and varying, and one not varying. In addition, the order of presentation of

perceptual dimensions and the particular stimuli used were counterbalanced. Finally, we introduced multiple IDSs and reversals to encourage formation of attentional set. This paradigm resulted in the expected shift effect in the WT mice and was also amenable for use with *Fmr1* KO mice.

In the present study, *Fmr1* KO mice showed deficits in discrimination and reversal learning and in the formation of an attentional set. FXS individuals also have deficits in discrimination and reversal learning (Kogan et al., 2004). With regard to attentional set formation, the comparison is less straightforward. FXS individuals exhibit deficits in executive function, but the severity of impairment varies depending on the task. For example, working memory deficits tend to emerge when the attentional load exceeds attentional resources (Munir, et al., 2000a). Patients with FXS have difficulty shifting from one rule to another in tasks, for example in the WCST on which they make significantly more perseverative errors than control subjects (K. M. Cornish, et al., 2001; Mazzocco, Pennington, & Hagerman, 1993; Mostofsky, et al., 1998). Set-shifting deficits were also demonstrated on a subtest of the Contingency Naming Test (Hooper, et al., 2008). In the WCST and the CNT, FXS individuals form attentional sets as demonstrated by increased perseverative errors when the rule shifts, consistent with formation of an attentional set and difficulty in shifting attention. This contrasts with our results in the *Fmr1* KO. The materials in the WCST and CNT (shape, color, number) may be sufficiently simple to support the formation of an attentional set, as the rules are all within the same modality. Moreover, FXS individuals exhibit relatively intact visual-perceptual abilities in terms of form and color (Kogan, et al., 2004). To our knowledge, there has been no parametric examination of the role of complexity or perceptual modality in attentional set formation in FXS.

In rodent attentional set-shifting tasks, the perceptual dimensions differ in modality, an identified difference from primate models of attentional set formation (reviewed in (Robbins & Arnsten, 2009)). Thus, one possibility is that the requirement to shift modalities exceeded the attentional resources of the *Fmr1* KO mice. We suggest this is the basis of the impairment in attentional set formation. Alternatively, it is possible that, despite impairment in learning the discriminations, the *Fmr1* KO were facilitated in shifting to the new dimension. If that were the case, however, facilitated reversal learning would also be expected, which was not the case. We also observed a trend of the *Fmr1* KO mice to demonstrate shorter latencies to choose at the EDS and the final reversal as compared to WT mice. We assert this provides further evidence for impaired set formation. In addition, although not conclusive, the trend suggests increased impulsivity, another prominent feature of FXS.

Our findings are consistent with impaired executive function and inhibitory control in a mouse model of FXS, confirming the utility of this mouse model for investigation of the neuroanatomical and neurophysiological basis for FXS deficits in executive function.

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Figure 1.

Performance of WT and *Fmr1* KO mice during IDS, EDS, and reversal trials. TTC was 8 correct out of 10 consecutive trials. Three intradimensional shifts (IDS) and reversals (REV) were followed by the single EDS assessment and final REV. Non-parametric analyses indicated that there was no difference between WT and *Fmr1* KO mice on the IDS problems, but WT mice showed the expected increase in TTC between the IDS and EDS discriminations. Importantly, *Fmr1* KO mice failed to show this effect. Data are means $\pm$ standard error.

Table 1
Stimulus materials for each phase of training and testing

Phase	Texture	Medium	Odor	Example Order of Testing
SD/CD/RV	thick yarn thin yarn	large beads small beads	black cherry lavender	thick/ <i>large or small beads/</i> cherry thin/ <i>large or small beads/</i> cherry
IDS1/RV1	flat cord round cord	round beads triangular beads	strawberry ocean mist	flat / <i>round or triangular/</i> strawberry round/ <i>round or triangular/</i> strawberry
IDS2/RV2	plain pipe cleaner metallic pipe cleaners	small fuzzy balls large fuzzy balls	pumpkin spice rose petals	plain/ <i>small or large fuzzy/</i> pumpkin metallic/ <i>small or large fuzzy/</i> pumpkin
IDS3/RV3	cork texture paper raised dotted paper	large felt squares small felt squares	woodlands pralines&cream	cork/ <i>large or small felt/</i> woodland raised/ <i>large or small felt/</i> woodland
EDS/RV4	smooth paper textured paper	plain paper shiny paper	mango vanilla	<i>smooth or textured/</i> plain/ mango <i>smooth or textured/</i> shiny/ mango

Stimulus materials are shown for training which included a simple discrimination (SD), a compound discrimination (CD), and the initial reversal (RV) and for testing which included three intradimensional shifts (IDS) and an extradimensional shift (EDS) each followed by a reversal. Only the stimuli in bold were presented for the SD. In the right column are representative example stimuli. The relevant dimension is shown in bold type, the irrelevant in italics, and the unvarying in regular type. Rewarded stimuli and order of dimensions presented were counterbalanced.