Profiling cognition in fragile X syndrome: A psychophysiological and neuropsychological approach

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Chapter 3

Attentional set-shifting in fragile X syndrome

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Abstract

The ability to flexibly adapt to the changing demands of the environment is often reported as a core deficit in fragile X syndrome (FXS). However, the cognitive processes that determine this attentional set-shifting deficit remain elusive. The present study investigated attentional set-shifting ability in fragile X syndrome males with the well-validated Intra/Extra Dimensional Set-Shifting paradigm (IED) which offers detailed assessment of rule learning, reversal learning, and attentional set-shifting ability within and between stimulus dimensions. A novel scoring method for IED stage errors was employed to interpret set-shifting failure in terms of repetitive decision-making, distraction to irrelevance, and set-maintenance failure. Performance of FXS males was compared to typically developing children matched on mental age, adults matched on chronological age, and individuals with Down syndrome matched on both mental and chronological age. Results revealed that a significant proportion of FXS males already failed prior to the intra-dimensional set-shift stage, whereas all control participants successfully completed the stages up to the crucial extra-dimensional set-shift. FXS males showed a specific weakness in reversal learning, which was characterized by repetitive decision-making during the reversal of newly acquired stimulus-response associations in the face of simple stimulus configurations. In contrast, when stimulus configurations became more complex, FXS males displayed increased distraction to irrelevant stimuli. These findings are interpreted in terms of the cognitive demands imposed by the stages of the IED in relation to the alleged neural deficits in FXS.

3.1 Introduction

Fragile X Syndrome (FXS) is the most frequent inherited type of intellectual disability with a prevalence of 1:4000 in males and 1:8000 in females (Turner, Webb, Wake, & Robinson, 1996a, 1996b). FXS is most often caused by silencing of the fragile X mental retardation 1 (FMR1) gene, which results in reduced or absent FMR1 protein (FMRP) levels (Oostra & Chiurazzi, 2001; Verkerk et al., 1991). FMRP plays an important role in early brain development by regulating the translation of proteins important for cortical network formation (Greenough et al., 2001; Irwin et al., 2001; Oostra & Chiurazzi, 2001). FXS males are typically characterized by a general impairment in intellectual functioning (Dykens, Hodapp, & Leckman, 1987; Hagerman & Hagerman, 2002), as well as by pronounced attentional dysfunction (Cornish, Munir, & Cross, 2001; Cornish, Sudhalter, & Turk, 2004; Munir, Cornish, & Wilding, 2000b; Scerif, Cornish, Wilding, Driver, & Karmiloff-Smith, 2004, 2007; Scerif et al., 2005). In particular, FXS males show a weakness in the ability to flexibly respond to the rapidly changing demands of the environment (Munir et al., 2000b; Scerif et al., 2007; Wilding, Cornish, & Munir, 2002), also referred to as an impairment in cognitive flexibility or attentional set-shifting (Miller, 2000; Miller & Cohen, 2001). To date, the cognitive mechanisms that underlie this attentional set-shifting deficit in FXS remain poorly understood.

Attentional set-shifting is generally defined by two key aspects. The first aspect refers to the predisposition to selectively respond or attend to a particular stimulus dimension (e.g., the shape or color of a particular stimulus). This response bias has been established on the basis of reinforcing feedback, and is referred to as the stimulus-response ‘set’. The second aspect consists of the disengagement of attention from the previously correct stimulus dimension to the newly correct stimulus dimension, indicating an attentional ‘shift’ (Owen, Roberts, Polkey, Sahakian, & Robbins, 1991). Studies on attention in FXS have demonstrated that switching attention between alternating target stimuli is characterized by a weakness in inhibiting a previously successful response (Cornish et al., 2001; Munir et al., 2000b; Scerif et al., 2007; Wilding et al., 2002; Woodcock, Oliver, & Humphreys, 2009). For example, Cornish et al. (2001)

compared attentional set-shifting ability in FXS with that of intellectually disabled (Down syndrome) and typically developing adults, using the Wisconsin Card Sorting Test (WCST), a widely used neuropsychological measure of attentional set-shifting. Results showed that FXS males committed significantly more perseverative errors than the other control groups. This perseverative behavior has been interpreted to suggest an inability to refrain from responding to a previously learned sorting rule when it is no longer appropriate (Cornish et al., 2001; Cornish et al., 2004), indicative of a primary deficit in *shifting* attentional set in FXS individuals.

An important critique of the WCST, however, is that this task only assesses *extra-dimensional set-shifting* (ED) and fails to assess *intra-dimensional set-shifting* (ID set-shifting) (Owen et al., 1993; Owen et al., 1991). ED set-shifting refers to shifting an attentional set *between* stimulus dimensions (e.g., switching stimulus-response mappings from the stimulus dimension ‘color’ to ‘shape’), whereas ID set-shifting refers to the engagement of an attentional shift towards new stimuli within the same stimulus dimension (e.g., shape) (Owen et al., 1991). In addition, recent WCST investigations have found that besides perseverative behavior, impairments in attentional set-shifting can also reflect an underlying weakness in *maintaining* an attentional set (i.e., set-maintenance failure) (Barceló & Knight, 2002). In turn, this set-maintenance failure could interfere with continued responding to a previously reinforced stimulus (Barceló & Knight, 1999, 2002). Based on the substantial literature reporting working-memory deficits in FXS (Baker et al., 2010; Munir, Cornish, & Wilding, 2000a; Ornstein et al., 2008; Van der Molen et al., 2010; Wilding et al., 2002) it could be hypothesized that, next to perseverative behavior, set-maintenance failure contributes to the observed attentional set-shifting weaknesses in FXS males.

In addition to these rule-based types of attentional set-switching, Ravizza & Carter (2008) recently proposed that attentional set-shifting also comprises perceptual switching, which entails switching visuospatial attention between sets of features of presented stimuli. Recently, Kogan and colleagues (2009) investigated the more perceptual aspects of attentional set-shifting in males with FXS and Down syndrome (DS) employing a two-stimulus object discrimination-learning and reversal-learning paradigm. Results revealed that FXS males showed increased difficulty with learning the correct rule, as well as with reversal of the rule. In addition, the analysis of the committed error types in the object reversal stage showed that FXS males committed more random errors (i.e., performance on chance-level) than perseverative errors (Kogan et al., 2009). Interestingly, these findings indicate that attentional set-shifting abilities within a single-stimulus dimension show a different pattern of errors than across multiple stimulus-dimensions (Cornish, Scerif, & Karmiloff-Smith, 2007). Within this context, random errors suggested perceptual weaknesses (Ravizza & Carter, 2008), which subsequently interfere with efficient object discrimination, as well as impaired learning of stimulus-reward associations.

This notion of a perceptual impairment in FXS is in accordance with recent electrocortical findings, showing exaggerated sensory responses to stimulus perception (Castrén, Paakkonen, Tarkka, Ryynanen, & Partanen, 2003; Ferri et al., 1994; Rojas et al., 2001; Van der Molen et al., 2011; Van der Molen et al., in press) as well as neuroimaging findings reporting dysfunction in a widespread neural network including the frontostriatal brain circuitry (Haas et al., 2009; Hallahan et al., 2011; Hessl, Rivera, & Reiss, 2004; Hoeft et al., 2007; Hoeft et al., 2008; Kwon et al., 2001; Lee et al., 2007; Lightbody & Reiss, 2009; Menon, Leroux, White, & Reiss, 2004; Reiss & Dant, 2003) and hippocampal formation (Hoeft et al., 2007; Lightbody & Reiss, 2009; Menon et al., 2004). These brain regions are frequently associated with stimulus discrimination and reversal learning, as well as attentional set-shifting (Kehagia, Murray, & Robbins, 2010; Rogers, Andrews, Grasby, Brooks, & Robbins, 2000; Schoenbaum, Chiba, & Gallagher, 2000).

Based on the findings of Cornish et al. (2001) and Kogan et al. (2009), weaknesses in attentional set-shifting ability in FXS males seem to be differentially expressed during discrimination learning and reversal (i.e., random search behavior), and extra-dimensional set-shifting (i.e., perseverative behavior). However, as different experimental paradigms were employed, caution is warranted when comparing results between these studies. Moreover, it remains elusive whether FXS males show attentional set-shifting deficits in ID set-shifting, and if so, what cognitive processes characterize these deficits. To address this question, a paradigm should be employed that could aid in investigating attentional set-shifting ability in the face of simple stimulus discrimination, as well as ID and ED set-shifting.

A paradigm widely used to investigate both ID and ED set-shifting, as well as simple discrimination learning and reversal, is the intra-extra dimensional set-shifting paradigm (IED). The IED is a subtest from the well validated Cambridge Neuropsychological Test Automated Battery (CANTAB) (De Luca et al., 2003; Lowe & Rabbitt, 1998; Robbins et al., 1998; Robbins et al., 1994), a neuropsychological assessment battery that has been successfully used in children from up to 4 years of age to adulthood (Luciana, 2003; Luciana & Nelson, 2002) and in a variety of neurodevelopmental disorders, including FXS (Van der Molen et al., 2010), Down syndrome (Visu-Petra, Benga, Tincas, & Miclea, 2007), and Williams syndrome (Rhodes, Riby, Matthews, & Coghill, 2011; Rhodes, Riby, Park, Fraser, & Campbell, 2010). The IED is administered via a computer touchscreen and comprises nine stages with increasing difficulty. The first two stages involve basic stimulus discrimination within a single stimulus dimension (e.g., shape), rule acquisition and reversal, as well as learning to benefit from feedback. Stages 3-to-5 assess the ability to ignore irrelevant multidimensional compound stimuli, while selectively responding to the previously reinforced stimulus dimension (e.g., shape). Two critical shifts are introduced at stages six and eight, which assess the ability to adequately shift attentional set to new stimuli from the same stimulus dimension (e.g., shape) (intra-dimensional shift), and to shift attentional set to new stimuli from a different stimulus dimension (e.g., lines) (extra-dimensional shift) (Downes et al., 1989; Roberts, Robbins, & Everitt, 1988).

The present study sets out to investigate the underlying cognitive processes that give rise to the weak attentional set-shifting abilities in FXS males by using the IED paradigm. To this end, the IED was considered a suitable paradigm for the following reasons: (1) the IED is a computerized attentional set-shift paradigm with an appealing stimulus configuration, and excludes concurrent scoring procedures; (2) the IED incorporates abstract patterns instead of meaningful stimuli, thereby minimizing the confound of focusing on detail. Preoccupation with parts of objects is frequently observed for persons with FXS (Hagerman & Hagerman, 2002) and presenting abstract rather than meaningful stimuli could minimize attentional bias towards specific (parts of) stimuli; (3) the IED includes stages with varying levels of difficulty, or cognitive demand. This allows for isolating both lower-level (e.g., visual-perceptual abilities, sustained visual attention) as well as higher-level cognitive processes (e.g., switching attention within or between stimulus dimensions) (Bertone, Hanck, Kogan, Chaudhuri, & Cornish, 2010; Ravizza & Carter, 2008) which could differentially impact on attentional set-shifting abilities in FXS; (4) the IED is a validated measure of attentional set-shifting ability at both a behavioral (Lowe & Rabbitt, 1998; Wild, Howieson, Webbe, Seelye, & Kaye, 2008) and neurological level (Owen et al., 1991; Rogers et al., 2000). Failure during specific stages of the IED could therefore be interpreted in terms of their well-established brain-behavior relationships.

Performance on the IED is commonly indexed by the number of stages successfully completed and by the number of errors committed on each stage (Luciana & Nelson, 2002; Owen et al., 1991). In the present study we refined the analysis of IED performance by discriminating between three error types of interest: (1) ‘repetition errors’, defined as repeated responding to an incorrect stimulus, (2) ‘maintenance errors’, due to set-maintenance failure, (3) ‘discrimination (or random) errors’, defined as errors committed due to switching to a wrong stimulus (from a similar or different dimension). This analysis was adopted from the revised scoring method developed by Barcélo et al. (1999) for the WCST (see also Somsen, 2007; Somsen, Van der Molen, Jennings, & van Beek, 2000), but included a few modifications to comply with the design of the IED[1]. Variables of interest in the present study were the

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[1] The WCST includes only extra-dimensional set-shifting, whereas the IED includes both intra-, and extra-dimensional set-shifting. Furthermore, stimulus-response mappings in the WCST are based on matching a response card to one of four stimulus cards, based on one out of three stimulus dimensions. In the IED, however, the stimulus configuration becomes more complex as the participants advances to the next stage. During the early stages, participants can only choose between two stimuli, whereas during later stages participants can choose between multiple stimuli.

number of stages successfully completed (attrition rate), and the proportion of repetition, set-maintenance, and discrimination errors committed in each stage.

Task performance of FXS participants was compared to that of (1) a chronological age-matched (CA) control group comprising typically developing adults, (2) a mental age-matched (MA) control group comprising typically developing children, and (3) an intellectually disabled control group comprising individuals with Down syndrome (DS) matched on both chronological and mental age. This latter group was included as DS has a distinct genetic origin from FXS and is the most frequent known cause of intellectual disability. In both groups, attentional function profiles have been described. For example, FXS males show disproportionate attentional control difficulties relative to MA controls (Cornish et al., 2001; Scerif et al., 2007; Scerif et al., 2005) whereas attentional function in DS has been reported to be comparable to their developmental level (Goldman, Flanagan, Shulman, Enns, & Burack, 2005). By comparing task performance to another intellectually disabled control group, attentional set-shifting deficits in FXS could be attributed to their specific etiology, rather than developmental delay.

Taken together, the current study set out to investigate attentional set-shifting ability in FXS males in an experimental paradigm that examines two-stimulus discrimination learning, as well as ID and ED set-shifting ability. A novel approach to analyze stage-errors in terms of repetitive behavior, set-maintenance failure, or discrimination errors, could further our understanding on those specific cognitive processes amenable for the observed attentional set-shifting deficits. We examined the following hypotheses: (1) in terms of attrition rate, FXS males were expected to perform on par with the DS and MA groups, and to show larger attrition rates during the ID and ED set shift stages (stage 6 and 8) relative to the CA group; (2) based on the findings reported by Kogan et al. (2009), we expected FXS males to commit more discrimination errors than the CA, MA and DS participants during the first two IED stages, which are tapping two-stimulus discrimination and reversal learning to a single stimulus dimension; (3) based on the previously reported sensitivity to distraction in FXS (Scerif et al., 2007), we expected that FXS males would be more distracted by irrelevant stimuli during the compound discrimination stages than the CA, MA, and DS groups. This distraction would be reflected by larger proportions of discrimination errors; (4) based on the frequently described weakness in inhibiting prepotent responses (Cornish et al., 2001; Munir et al., 2000b; Scerif et al., 2007; Wilding et al., 2002; Woodcock et al., 2009), we expected FXS males to commit more repetition errors during the reversal stages than the control groups; and finally, (5) for all groups we expected that the level of intellectual functioning would significantly predict IED performance, as indexed by the number of stages successfully completed.

3.2 Method

3.2.1 Participants

This study comprised four groups, including 27 adult males (mean age = 27.82, SD = 7.08) with the FXS full mutation, 20 individuals with DS serving as an intellectually disabled control group (mean age = 22.42, SD = 3.56, 10 females), 31 typically developing adults (mean age = 27.26, SD = 8.08, 14 females) serving as a chronologically age-matched control group (CA) and 40 typically developing children (mean age = 5.70, SD = 1.15, 17 female) serving as a mental age-matched control group (MA). Chi-square analyses indicated that gender distribution differed significantly between groups, $\chi^2(3) = 19.51$, p = 001. This effect could solely be attributed to the FXS group, which only contained males. The effect of gender on the IED variables of interest was tested and yielded no significant differences (p's > .05).

FXS participants were recruited with the assistance of the Dutch fragile X syndrome parents support group. DS participants were recruited with the help of the Dutch organization of parents of children with DS. Confirmation of the FXS full mutation (FXS group) and trisomy of chromosome 21 (DS group) was based on prior genetic testing. For the purpose of developmental age-matching, developmental level of FXS and DS participants was assessed using the Snijders and Oomen Non-Verbal Intelligence Test (SON-R 2-7 & SON-R 5-17; Snijders, Tellegen, & Laros, 1998). Control participants from the MA and CA groups were administered the Raven Standard Progressive Matrices (Raven &

Court, 1998) to obtain an estimate of their non-verbal intelligence level. Based on the SON-R mental age scores, both FXS and DS groups did not differ from the MA group in terms of mental age ($p > .05$).

Children from the MA group were recruited by contacting schools in nearby communities. Primary caregivers provided informed consent for the participants within the FXS, DS, and MA groups. Adults from the CA group were recruited within proximity of the university and nearby communities. These participants provided signed informed consent and received either course-credits or a monetary compensation for participation. All FXS and DS participants were free from additional diagnosed psychiatric disorders, based on DSM-IV-TR classifications (American Psychiatric Association, 2002). All participants had normal or corrected-to-normal vision. The study was approved by the ethical committee of the university and complied with relevant laws and guidelines.

3.2.2 IED set-shift paradigm

The IED is a two-choice computerized attentional set-shifting paradigm, included in the CANTAB (Cambridge Cognition, 2002)[2] designed to assess the ability to learn stimulus-response mappings and to switch to different stimulus-response mappings when a predetermined response criterion has been reached. On each trial, four rectangular boxes appear on the computer screen that are aligned to the top/bottom and to the left/right of the center (see Figure 1). Two of these boxes contain two abstract patterns, which are either purple-filled shapes and/or white lines (each representing a different stimulus dimension). Participants have to choose one of the two options presented. Feedback is then provided on the correctness of their response by displaying a short ‘green-colored’ flash coupled with a high-pitched tone when correct, and a ‘red-colored’ flash coupled with a low-pitched tone when incorrect. After six consecutive correct responses, the response criterion is reached and the

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[2] The CANTAB is a widely used computerized tool for the assessment of frontal and medial temporal lobe dysfunctions. Normative data for these subtests have been extended by De Luca et al., 2003 and Luciana & Nelson, 2002. Indices of reliability have been reported by Lowe & Rabbitt, 1998. For a detailed description of the CANTAB subtests included in this study, see Luciana & Nelson, 1998.

participant proceeds to the next stage (without notification), with a maximum of nine stages. The test ends after successful completion of the ninth stage, or when a participant fails to reach criterion after the 50th trial of any given stage. Duration of the IED is approximately 7-to-10 minutes, depending on the performance of the participant. On every trial, stimuli are cleared from the screen after 1500 ms upon a touch-response provided by the participant. The inter-trial interval was set at 1000 ms.

Figure 1 displays a schematic illustration of the nine stages of the IED together with an example of the response criterion associated with each stage (marked by yellow squares). During the first stage, participants are presented with two patterns of a single dimension (i.e., purple-filled shapes) and have to choose which one of these two patterns is correct: simple discrimination (SD). During the second stage, stimuli remain the same, but now a *reversal* of the correctness of the stimuli is applied. That is, the previous incorrect stimulus now is the correct response criterion: simple discrimination reversal (SD-R). At the third stage, stimuli from the other dimension (white lines) are introduced and positioned next to the relevant stimulus dimension (shape), but the correct response criterion (i.e., shape) remains unchanged: compound discrimination (CD). During the fourth and fifth stages, the irrelevant dimension (i.e., white lines) is superimposed on the relevant dimension (i.e., purple-filled shapes), with the white lines presented in the foreground at all times. First, participants have to ignore the superimposed dimension and remain responding to same stimulus as during the previous stage: compound discrimination imposed (CD-I). Next, participants have to apply a reversal of stimulus-response mappings within the same stimulus dimension. That is, the purple-filled shapes remain the correct dimension, only the other stimulus type now is correct: compound discrimination reversal (CD-R). At stage six, new stimuli are introduced for both dimensions, while the dimension of these stimuli remains similar (i.e., purple-filled shapes and white lines). Participants have to switch responding to these new stimuli, but again to the stimuli of the same dimension (purple-filled shapes) as during the previous stages: intra-dimensional set-shift (ID). At stage seven, a simple reversal within the same dimension (e.g., purple-filled shapes) has to be applied: intra-dimensional shift reversal (ID-R). At stage eight, new stimuli of the same dimension are again introduced: extra-dimensional set-shift (ED). Participants now have to switch responding from the previous correct stimulus dimension (purple-filled shapes) to the new correct stimulus dimension (white lines). At the ninth and final stage, participants have to apply a simple reversal of stimulus-response mappings within the same stimulus dimension of the previous stage (e.g., white lines): extra-dimensional reversal (ED-R).

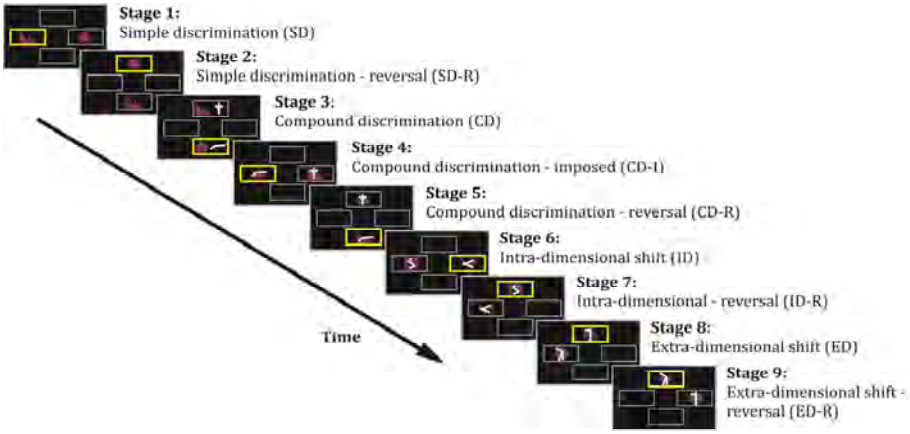

Figure 1. Schematic drawing of the nine stages of the IED attentional set-shifting paradigm.

The variables of interest in this study were the number of stages completed successfully, the number of trials needed to reach criterion for each stage, and the errors committed at each stage. For the scoring of errors, we defined the following error types (Barceló & Knight, 1999, 2002; Somsen, 2007; Somsen et al., 2000): (1) ‘warning errors’, defined as errors committed on the first trial of a reversal stage (i.e., the SD-R, CD-R, and ID-R stages) or on the first trial of stages displaying a new stimulus configuration (i.e., ID and ED stages). These error types were scored separately, as they notify the participant of a change of the correct criterion and cannot be anticipated, but were not included in the analysis; (2) ‘maintenance errors’, defined as the first error committed after a series of 3-to-5 consecutive correct trials on any given stage before a criterion was reached (thereby excluding warning errors). This error type should reflect a failure to maintain responding according to the previous learned criterion; (3) ‘discriminative errors’, defined as errors committed due to switching to a wrong stimulus of either the same or different stimulus dimension, which excluded maintenance errors, but included errors committed on the first trial of a non-reversal stage (i.e., CD, CD-I, and CD-R stages), as well as errors committed on later trials showing a different stimulus configuration as on the previous trial (with the exception of errors committed on the first trial of a reversal stage, which were interpreted as warning errors); and (4) ‘repetition errors’, defined as errors committed on trials directly after a warning, discriminative or maintenance error with a stimulus configuration identical to the one presented in the previous trial (i.e., type or the combination of stimuli, not their location on the screen).

3.2.3 Procedure

The IED was administered individually to the participants in a silent room. Participants were seated at approximately 70 cm from a 12-inch Paceblade Slimbook Tablet PC (©Paceblade Technology), running on the Windows XP operating system. Instructions to the participants were derived from the CANTAB manual (Cambridge Cognition, 2002), which briefly states that the participant is told that he/she will see two patterns on each trial. The participant has to touch the pattern he/she thinks is correct. After each touch (or choice), the computer provides feedback on the correctness of the choice made. The participant can follow a rule to be sure to make the correct choice. After completing the first rule, the participant progresses to a new stage in which the computer will apply a similar or different rule (depending on the stage) (for a detailed instruction of the IED see Downes et al., 1989; Robbins et al., 1998).

3.2.3 Data-analysis

Group differences in the proportion of participants failing at a particular stage (attrition rate) were investigated with Likelihood ratio analysis for contingency tables. Proportions of each error type of interest (repetition, maintenance, and discrimination error) were calculated based on the number of total errors committed during a particular stage. These proportions were then square root transformed and submitted to a repeated measures analysis of variance (ANOVA), with Error Type (three levels: repetition, maintenance, discrimination) and Stage (eight levels: SD, SD-R, CD, CD-I, CD-R, ID, ID-R, ED) as within-subjects factors, and Group (four levels: FXS, DS, MA, CA) as between-subjects factor. The ED-R stage was excluded from the analysis, as most participants in the FXS, DS, and MA groups did not succeed in completing the ED stage. A discriminant function analysis was carried out separately for the IED stages (except for the ED-R stage), with Error Type (repetition, maintenance, discrimination) as predictor variables and Group (FXS, DS, MA, and CA) as dependent variables, to investigate which error type best discriminated the groups for each of the IED stages. Finally, stepwise linear regression analyses were conducted to investigate whether chronological age and intelligence-level (SON-R MA values in DS and FXS groups, and Raven percentile in MA and CA groups) could predict IED performance, as indexed by the number of stages successfully completed. All analyses were performed using the Statistical Package of Social Sciences version 17 (SPSS Inc, 2008). Post-hoc significance testing was performed using Bonferroni correction, and alpha was set at .05.

3.3 Results

3.3.1 Intellectual functioning and overall IED performance

Table 1 presents participant characteristics, mental age-level (FXS and DS participants), Raven percentile scores (CA and MA participants), and the average number of IED stages completed. Non-parametric Mann-Whitney tests revealed that the CA group completed significantly more stages than the FXS (Z = -5.32, $p < .0001$), MA (Z = -5.37, $p < .0001$), and DS groups (Z = -3.69, *p*

<.0001). The DS group completed more stages than the FXS group (Z = -2.14, *p* = .03), the MA group did not differ from the FXS and DS groups (*p* > .05).

Table 1. Demographic, intelligence, and overall IED performance characteristics of the participant groups.

	FXS	DS	MA	CA	*Significant difference*
Participants (n)	27	20	40	31	n.s.
Age in years	27.81 (7.08)	22.42 (3.55)	5.75 (1.15)	27.26 (11.32)	MA* < FXS, DS, CA
Gender (Male/Female)	27/ 0	10 / 10	22/18	17 / 14	FXS*
SON-R Mental age	5.54 (1.17)	5.66 (1.08)	-	-	n.s.
Raven Percentile	-	-	77.63 (17.2)	69.29 (21.1)	n.s.
IED stages completed	6.85 (1.51)	7.80 (0.95)	7.53 (0.78)	8.74 (0.68)	CA > FXS, DS, MA**** DS > FXS*

Note. Except for participants (number), data represent mean (standard deviation) values. Abbreviations: FXS = fragile X syndrome, MA = mental age control group, DS = down syndrome, CA = chronological age control group. SON-R = Snijders Oomen Non-Verbal Intelligence Test – Revised. * significant at *p*<.05, **** significant at *p*< .0001.

Table 2 presents group differences in the number of trials needed and the total number of errors committed per stage. Non-parametric Mann-Whitney tests revealed that FXS participants needed more trials and committed more errors during all IED stages than the CA group (all *p*'s < .05), except during the compound discrimination stage for the number of committed errors. Based on overall performance (total errors and trials), IED performance of FXS participants was particularly impaired during the reversal of the simple discrimination (SD-R) and compound discrimination (CD-R) stages. FXS males committed more errors (SD-R, CD-R) and needed more trials (CD-R) to succeed in these stages relative to all control groups (*p*'s < .05). During the compound discrimination stages (CD, CD-I), and ED reversal stage, FXS males committed more errors (CD-R, ED-R) and needed more trials (CD, CD-I, ED-R) than DS participants (*p*'s < .05), but performed on par with MA controls. These findings suggest that attentional set-shifting ability in FXS males is particularly characterized by a deficit in reversal learning.

Table 2. Comparison of the number of trials needed and the number errors committed for the participants attempting a stage.

Stage	FXS	MA	DS	CA	Significance
	M (SEM)	M (SEM)	M (SEM)	M (SEM)	
SD					
Trials (n)	10.37 (1.16)	10.15 (0.93)	8.65 (1.02)	6.65 (0.13)	FXS > CA
errors (n)	1.78 (0.62)	1.63 (0.47)	0.76 (0.37)	0.06 (0.04)	FXS > CA
Participants (n)	27	40	20	31	
SD-R					
Trials (n)	12.04 (1.13)	11.15 (0.82)	9.80 (1.42)	7.77 (0.32)	FXS > DS, CA
errors (n)	4.19 (0.66)	2.38 (0.28)	2.80 (1.11)	1.26 (0.12)	FXS > MA, DS, CA
Participants (n)	27	40	20	31	
CD					
Trials (n)	13.52 (2.14)	9.03 (0.61)	7.10 (0.42)	7.74 (0.55)	FXS > DS, CA
errors (n)	3.00 (1.00)	1.20 (0.24)	0.65 (0.25)	0.71 (0.21)	n.s.
Participants (n)	27	40	20	31	
CD-I					
Trials (n)	10.19 (1.32)	8.23 (1.00)	6.15 (0.11)	6.06 (0.04)	FXS > DS, CA
errors (n)	1.73 (0.57)	1.00 (0.48)	0.20 (0.12)	0.13 (0.08)	FXS > DS, CA
Participants (n)	27	40	20	31	
CD-R					
Trials (n)	16.50 (2.49)	11.30 (1.54)	11.15 (1.97)	7.42 (0.23)	FXS > MA, DS, CA
errors (n)	5.69 (1.17)	2.50 (0.63)	3.00 (1.97)	1.10 (0.10)	FXS > MA, DS, CA
Participants (n)	26	40	20	31	
ID					
Trials (n)	9.13 (1.43)	8.68 (0.63)	9.40 (1.11)	6.39 (0.12)	FXS > CA
errors (n)	1.43 (0.26)	1.33 (0.21)	1.30 (0.40)	0.35 (0.10)	FXS > CA
Participants (n)	23	40	20	31	
ID-R					
Trials (n)	13.55 (2.52)	10.45 (0.92)	11.85 (1.84)	7.23 (0.26)	FXS > CA
errors (n)	4.55 (1.32)	2.13 (0.28)	3.30 (0.90)	17.74 (2.66)	FXS > CA
Participants (n)	22	40	20	31	
ED					
Trials (n)	39.71 (3.38)	40.60 (2.35)	39.25 (3.61)	17.74 (2.66)	FXS > CA
errors (n)	20.57 (2.12)	26.07 (2.99)	20.05 (2.33)	5.80 (1.29)	FXS > CA
Participants (n)	21	14	9	27	
ED-R					
Trials (n)	27.86 (2.59)	26.07 (2.99)	15.44 (2.01)	6.22 (0.16)	FXS > DS, CA
errors (n)	22.14 (2.59)	19.07 (2.77)	12.89 (3.28)	1.22 (0.14)	FXS > CA
Participants (n)	7	7	7	27	

Note. Abbreviations: FXS = fragile X syndrome, MA = mental age, DS = Down syndrome, CA = chronological age, SD = simple discrimination, SD-R = simple discrimination reversal, CD = compound discrimination, CD-I = compound discrimination imposed, CD-R = compound discrimination reversal, ID = intra-dimensional set-shift, ID-R = intra-dimensional set-shift reversal, ED = extra-dimensional set-shift, ED-R = extra-dimensional set-shift reversal, M = mean, SEM = standard error of the mean. Significance testing at $p < .05$.

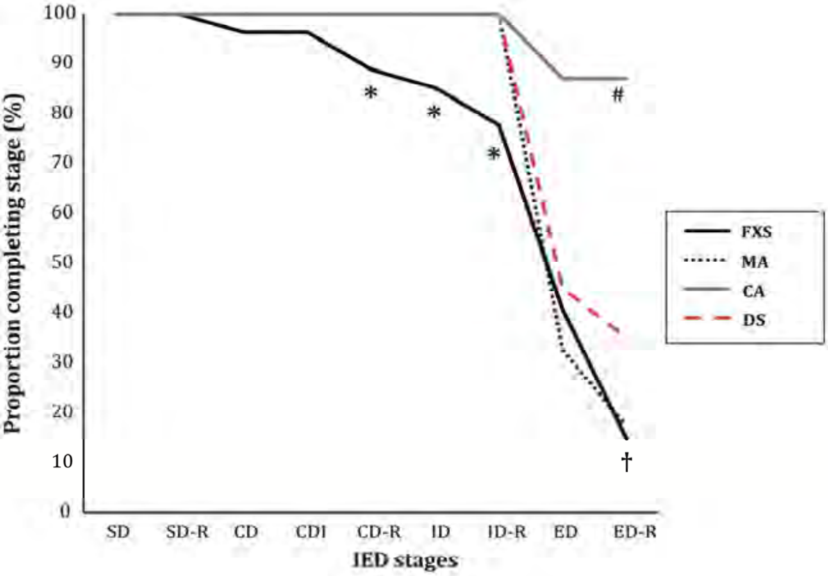

Figure 2. Attrition rate in the nine stages of the IED. Attrition rate is significantly larger for FXS males relative to the MA, CA and Down Syndrome groups in the compound discrimination reversal, intra-dimensional set-shift and reversal stages (*). In the extra-dimensional set-shift stage, attrition rate is significantly smallest in the CA group (#), whereas attrition rate is largest in both FXS and MA control groups (†). Abbreviations: SD = Simple Discrimination, SD-R = Simple Discrimination Reversal, CD = Compound Discrimination , CD-I = Compound Discrimination Imposed , CDI-R = Compound Discrimination Imposed Reversal, ID = Intra-Dimensional Set-Shift, ID-R = Intra-Dimensional Reversal, ED = Extra-Dimensional Set-Shift, ED-R = Extra-Dimensional Set-Shift Reversal.

3.3.2 IED attrition rate

Figure 2 shows the percentage of participants from the four groups successfully completing each stage of the IED paradigm. As expected, the CA group was most successful on the IED, as 87.1% of the participants successfully completed all nine stages, relative to 14.8%, 35.0%, and 20.0% of the participants from the FXS, DS and MA groups, respectively. Group differences were examined using Likelihood ratio analyses. Results showed that attrition rates were significantly larger for the FXS group relative to the CA, MA, and DS groups for the compound discrimination reversal (CD-R), $\chi^2(3) = 9.12$, $p = .03$, ID set-shift (ID), $\chi^2(3) = 12.29$, $p = .006$, and ID set-shift reversal (ID-R) stages, $\chi^2(3) = 18.83$, $p = .001$. During the ED set-shift (ED) and reversal (ED-R) stages, attrition rates of the FXS, DS, and MA groups were significantly larger than those observed for the CA group, $\chi^2(3) = 23.79$, $p = .001$. Together, these findings suggest that the weak reversal learning abilities and enhanced distractibility to irrelevant stimuli (see SD-R and CD-I performance of FXS males in Table 2) leads to failure of a significant proportion of FXS males during the reversal of the compound discrimination stage (CD-R). In addition, the observed impairment in FXS males during the ID set-shift suggests enhanced difficulties with shifting attentional set for recently or novel reinforced stimulus-reward associations *within* a single stimulus dimension.

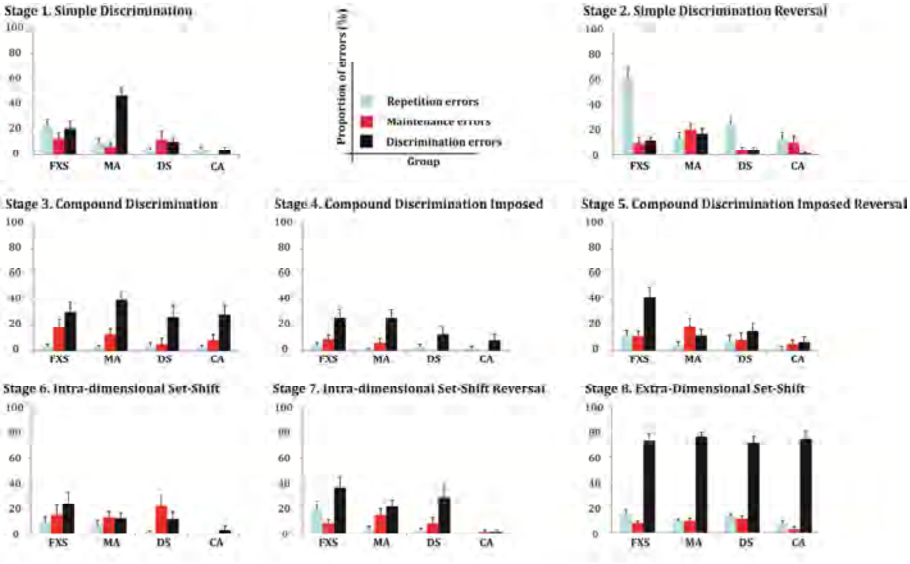

Figure 3. Proportion of committed error types (repetition, maintenance, discrimination) in all IED stages (except for the extra-dimensional set-shift stage) for the participants in the FXS, DS, MA, and CA groups.

3.3.3 Error type analysis

Overall results. Figure 3 depicts the error types of interest (repetition, discrimination, and set-maintenance errors) committed for each group for the IED stages up to the ED set-shift stage[3]. The ANOVA yielded main effects for Error type, $F(2, 216) = 83.22$, $p < .0001$, $\eta^2 = .44$, and Stage, $F(7, 756) = 38.04$, p

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[3] 'Warning errors' are not reported as the proportions of these error types could only be committed on the first trial of a reversal stage and were similar between groups per IED stage (all p's < .05).

$< .0001$, $\eta^2 = .26$. Participants committed more discrimination errors than repetition and maintenance errors ($p < .05$). As expected, most errors were committed during the ED set-shift stage. Error proportions were smallest for the compound discrimination imposed (CD-I) and reversal (CD-R) stages (p's $< .05$). The significant main effect of Group, $F(3, 108) = 22.83$, $p < .0001$, $\eta^2 = .39$, revealed that error rates were significantly lower in the CA group relative to the FXS, MA and DA groups ($p < .05$). In addition, FXS and MA participants committed significantly more errors relative to the DS group (p's $< .05$). The analysis furthermore yielded a significant three-way interaction of Error Type by Stage by Group, $F(42, 1512) = 2.26$, $p < .0001$, $\eta^2 = .06$, which is plotted in Figure 3. This interaction will be examined in further detail below using Bonferonni corrected alpha levels.

Repetition errors. The proportion of repetition errors per group and per stage is presented in Figure 3. Post-hoc comparisons revealed that FXS males committed significantly more repetition errors in the simple discrimination stage than participants within the CA ($p = .005$) group. In the reversal of the simple discrimination and ID stages, FXS males committed significantly more repetition errors than participants within the MA ($p < .0001$), DS ($p = .001$), and CA groups ($p < .0001$). In the IDR stage, FXS males committed significantly more repetition errors than the MA ($p = .001$) and CA ($p < .0001$) groups, whereas in the ED stage, the proportion of repetition errors in FXS males significantly exceeded those observed in the CA group ($p = .003$). As expected, FXS males showed increased repetitive decision-making relative to the control groups, already during the reversal of attentional set *within* a single stimulus dimension.

Maintenance errors. The proportion of maintenance errors per group and per stage is presented in Figure 3. Post-hoc comparisons revealed that FXS males did not differ from the other control groups in terms of failing to maintain attentional set in the IED stages up to the ED-shift (all p's $> .05$). Like the MA and DS groups, FXS males committed more set-maintenance errors in the ED stage relative to the CA group, however, these differences only reached levels of significance between the FXS and CA groups ($p = .003$). Together, these findings suggest that attentional set-shifting abilities in FXS males cannot be characterized by a specific failure in maintaining attentional set.

Discrimination errors. The proportion of discrimination errors per group and per stage is presented in Figure 3. Post-hoc comparisons revealed that during the simple discrimination (SD) stage, a significantly larger number of discrimination errors was observed in the MA group compared to DS (p = .001) and CA groups (p < .0001). During the reversal of the SD stage, MA participants committed significantly more discrimination errors than participants within the CA group (p = .006). Interestingly, during the reversal of the compound discrimination (CD-R) stage, FXS males committed a significantly larger number of discrimination errors than participants within the MA (p< .0001), DS (p< .004), and CA (p< .0001) groups. Finally, in the reversal of the ID stages, FXS males committed significantly more discrimination errors than CA participants (p< .0001). These findings suggest that FXS males show enhanced distractibility during reversal learning when faced with non-reinforced stimuli from a different stimulus dimension (CD-R and ID-R).

Discriminant analysis on the error types. A discriminant function analysis was performed to investigate whether groups could be accurately discriminated from each other based on error types. The analysis yielded two significant discriminant functions. The first function explained 49.5% of the variance, canonical R^2 = .55, whereas the second function explained 36.3% of the variance, canonical R^2 = .48. In combination these discriminant functions significantly differentiated the participant groups Λ = .17, $\chi^2(72)$ = 168.80, p < .0001. Subsequent analyses revealed that repetition errors associated with the reversal of the simple discrimination (SD-R) (r = .41) and ID set-shift (ID-R) (r = .39) stages loaded highest on the first function, whereas discrimination errors associated with the simple discrimination (SD) stage (r = .54) loaded highest on the second function. As can be seen in Figure 4, the first function discriminated the FXS group from the other groups whereas the second function discriminated the MA group from the other groups. The discriminant analysis correctly classified 76.6% of the participants. These findings provide additional support for the FXS males showing increased repetitive decision-making, which is specifically evoked during reversal learning stages.

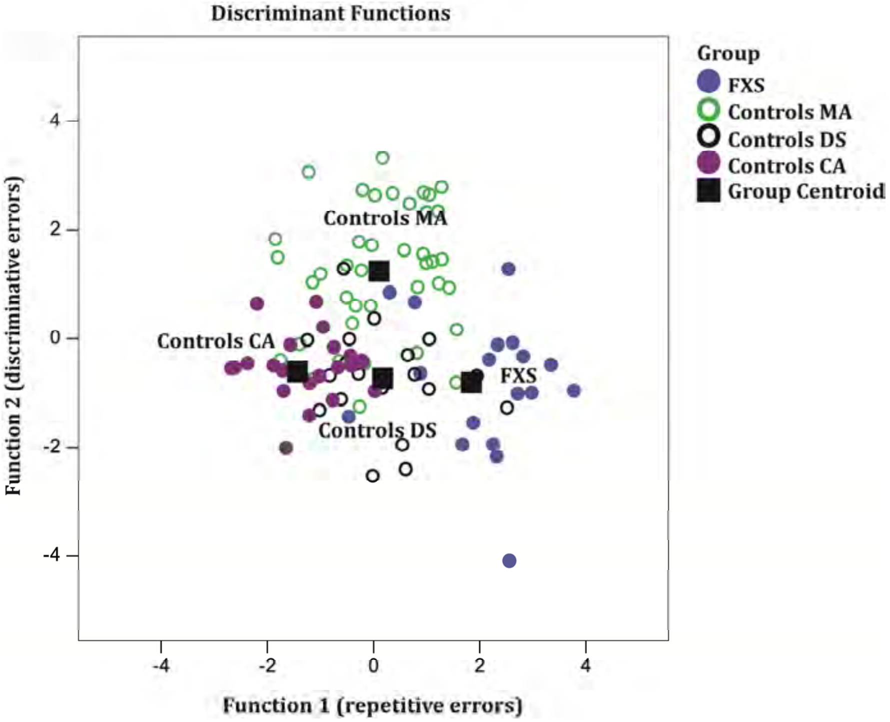

Figure 4. Discriminant function (variate) scores plotted for each participant according to group membership. Function 1, with largest contributions to repetition errors, differentiates FXS males from the other groups. Function 2, with largest contribution to discrimination errors, differentiates MA controls from the other groups.

3.3.4 Predictors of IED performance.

We examined whether level of intellectual ability (SON-R non-verbal mental age in FXS and DS groups; Raven percentiles in CA and MA groups) and chronological age could predict IED performance (i.e., the number of stages successfully completed). Stepwise regression analysis indicated that for the FXS group, intellectual ability (SON-R non-verbal mental age) significantly predicted IED performance, $F(1, 25) = 7.85$, $p < .01$, explaining 21% of the variance. As can be seen from the correlations in Table 3, the predictor variables failed to predict IED performance in the other groups.

Table 3. Pearson correlations between chronological age and intellectual performance level with the number of IED stages successfully completed in the FXS, DS, CA, and MA participant groups. ** significant at the $p < .01$ level (two-tailed).

	Number of IED stages successfully completed			
	FXS	MA	DS	CA
Chronological Age	-0.09	0.06	0.04	0.10
Intellectual Level	0.49**	0.04	0.08	0.24

Note. Abbreviations: FXS = fragile X syndrome, DS = Down syndrome, MA = mental age, CA = chronological age.** significant at $p < .01$.

3.4 Discussion

The present study was designed to investigate underlying cognitive processes that explain attentional set-shifting weaknesses in FXS males, using the IED set-shifting task of the CANTAB. A major finding that differentiated FXS males from the control groups was that IED performance in FXS males is already compromised before an intra-dimensional attentional set-shift had to be engaged. In contrast with our initial expectation, a significant proportion of FXS males already failed during the reversal of the compound discrimination stage (CD-R), whereas all participants from the MA, DS, CA control groups successfully completed the IED stages up to the ED set-shift. In addition, FXS males could best be differentiated from the other groups in terms of the larger proportion of repetition errors committed during the simple discrimination and reversal stages (SD, SD-R), and during the reversal of the ID set-shifting stage (ID-R). Finally, FXS males committed a significantly larger proportion of discrimination errors during the reversal of the compound discrimination stage (CD-R), indicating that the type of deficits in discrimination learning and rule reversal is dependent on stimulus complexity. These findings will be discussed vis-à-vis the cognitive demands imposed by the IED stages and their neural correlates.

Based on overall IED stage performance, FXS males displayed a specific weakness during the IED *reversal* stages prior to the ID set-shift. That is, FXS males needed more trials on the reversal of the simple discrimination (SD-R) and compound discrimination (CD-R) stages, relative to all control groups. In agreement with previous studies (Cornish et al., 2001; Munir et al., 2000b; Scerif et al., 2007; Wilding et al., 2002), FXS males are particularly deficient in redirecting attention from a previously correct to a previously incorrect stimulus. Putatively, the severity of this reversal learning deficit is enhanced by the presence of distractor stimuli, as attrition rates did not differ between groups on the simple discrimination reversal stage (SD-R).

To investigate the underlying cognitive processes that determine attentional set-shifting deficits in FXS, we performed a detailed analysis of the nature of errors committed during the IED stages. Discriminant analysis showed that FXS males could be best distinguished from the other control groups based on the proportion of repetition errors committed during the reversal of the simple discrimination (SD-R) and intra-dimensional set-shift (ID-R) stages. This finding is in line with our expectation of increased repetitive decision-making during the reversal stages of the IED, however, contrasts with recent findings on object discrimination and reversal learning in FXS males (Kogan et al., 2009). That is, during two-stimulus object reversal learning, these authors reported enhanced 'chance-level performance' (discrimination errors) in FXS that was attributed to side preferences of the stimulus display. This interpretation of impaired reversal learning in FXS is challenged by the current findings, as the IED randomly presents stimuli at four possible locations on the computer screen. In contrast, the observed repetitive decision-making in the current study more likely reflects a failure to disengage attention from a previously reinforced stimulus that becomes irrelevant (i.e., perseverative behavior), which is in line with the notion that FXS males show a pronounced weakness in inhibiting prepotent responses (Cornish et al., 2001; Scerif et al., 2007). Alternatively, FXS males could also show an impairment in the ability to redirect attention to a previously irrelevant stimulus that has become relevant, a phenomenon coined 'learned irrelevance' (Mackintosh, 1975). Future investigations should preferably employ more detailed experimental measures (Maes, Damen, & Eling, 2004; Maes, Eling, Wezenberg, Vissers, & Kan, 2011;

Maes, Vich, & Eling, 2006) to investigate whether perseveration or learned irrelevance is more likely to explain these reversal learning deficits in FXS.

Results furthermore demonstrated that this repetitive decision-making is particularly evoked when FXS males need to apply a reversal of a *recently* learned stimulus-reward association. That is, in contrast to an expected *general* reversal learning deficit in FXS, repetitive decision-making was most obvious during the reversal of the simple discrimination stage (SD-R), where participants had to apply a reversal of newly formed stimulus-response mappings. However, during the reversal of the compound discrimination stage, where a similar reversal of attentional set had to be applied (i.e., other stimulus from the dimension 'shape'), this repetitive decision-making in FXS was significantly decreased. Putatively, reversal learning in FXS is most problematic when a reversal has to be applied in the face of recently learned stimulus response mappings, possibly indicative of a underlying weakness in novelty processing.

Although the IED paradigm is not optimally suited to investigate novelty processing, there is neurophysiological evidence to support the notion of impaired change detection processes in FXS males. For example, exaggerated event-related cortical responses have been found in response to stimulus detection in FXS males (Castrén et al., 2003; Rojas et al., 2001; Van der Molen et al., 2011; Van der Molen et al., in press) and have been suggested to interfere with the efficiency of allocating attentional resources to potential important stimuli (Van der Molen et al., 2011; Van der Molen et al., in press). In support for this notion, FXS males show attenuated electrocortical markers of sensory memory formation (i.e., mismatch negativity), the triggering of involuntary attention (i.e., the P3a) and decision-making (i.e., the P3b) (Van der Molen et al., 2011; Van der Molen et al., in press), both important information processing components of the event-related potential, and key-aspects in change detection and attentional set-shifting (Barceló & Knight, 1999; Barceló, Munoz-Cespedes, Pozo, & Rubia, 2000; Menon & Uddin, 2010). Furthermore, neuroimaging studies consistently show dysfunction of frontal-striatal neural circuitry in FXS (Haas et al., 2009; Hoeft et al., 2010; Hoeft et al., 2007; Hoeft et al., 2008; Menon et al., 2004; Tamm, Menon, Johnston, Hessl, & Reiss, 2002) including prefrontal cortex, cingulate cortex, insula, caudate nucleus, and amygdala. Integrity of these frontal-striatal circuits is essential for a multitude of attentional and cognitive processes, such as saliency detection (Menon & Uddin, 2010), learning stimulus-reward associations (Rogers et al., 2000), and attentional set-shifting (Barceló & Knight, 1999; Rogers et al., 2000). Aberrant functional connectivity in these attention networks (Menon & Uddin, 2010) could be specific to the FXS neurobiology, as absence of FMRP results in a cascade of neurological alterations that impact on normal brain development (e.g., abnormal dendritic refinement) and neurotransmission (Bear, Huber, & Warren, 2004; D'Hulst & Kooy, 2007; Greenough et al., 2001; Huber, 2007; Irwin et al., 2001). Together, these neurodevelopmental changes resulting from FMRP depletion could have critically altered the functionality of basic stimulus processing in the FXS brain, which could subsequently hinder change detection processes and the generation of appropriate stimulus-response mappings.

A similar deficit in early stimulus processing in the FXS brain could possibly explain the observed attentional set-shifting deficits during the compound discrimination and ID set-shifting stages in FXS males. That is, during these more complex, multidimensional stages, additional stimuli were introduced from a different stimulus dimension (i.e., lines). Although participants were still required to respond to the same stimulus dimension as during the previous stages (i.e., shapes), FXS males now committed a significantly larger proportion of discrimination errors. Moreover, the proportion of discrimination errors significantly exceeded those observed for the other control groups during the reversal of the compound discrimination stage (CD-R). Instead of relapsing into repetitive behavior (as seen on the SD-R stage), FXS males now seemed to be distracted by the stimuli from the irrelevant stimulus dimension (i.e., lines), and engaged in trial-and-error behavior by responding to stimuli of both dimensions (i.e., shapes and lines). This augmentation in distraction errors confirms our hypothesis of enhanced distractibility to irrelevant stimuli in FXS, and putatively reflects impulsive responding due to the aforementioned deficits in stimulus perception and discrimination.

The alleged abnormalities in early stimulus perception and discrimination processes in FXS males could interfere with generating efficient stimulus-reward associations. As stimulus detection in FXS has been associated with hypersensitive neural responses (e.g., augmented N1 component of the event-related potential) (Castrén et al., 2003; Rojas et al., 2001; Van der Molen et al., 2011; Van der Molen et al., in press) this augmented neural activity could hinder the later processing of novel stimuli and the classification thereof. This is in accord with electrocortical findings demonstrating impaired stimulus classification, reflected by the P3b component of the event-related potential in FXS males (St Clair, Blackwood, Oliver, & Dickens, 1987; Van der Molen et al., in press). Subsequently, a stimulus could be incorrectly classified as target stimulus, due to prior information processing deficiencies, which results in noisy conditions for network-level decision-making (Gold & Shadlen, 2007; Theodoni, Kovacs, Greenlee, & Deco, 2011). In turn, this could result in increased responding to irrelevant or distractor stimuli as demonstrated by our current IED results.

This interpretation of an impairment of stimulus classification processes in FXS is in line with atypical search behavior reported in FXS children. For example, Scerif and colleagues (2004) found that children with FXS were more affected by distractors with a high resemblance to targets. Our current results add to these findings by showing that adult FXS males exhibit enhanced distractibility to irrelevant stimuli when overlapping on relevant stimuli. Importantly, this enhanced distractibility differentiates FXS males from MA and DS controls. That is, our current findings revealed that the mental age-matched and intellectually impaired controls performed relatively well during the compound discrimination and intra-dimensional set-shift stage, as opposed to FXS males. Performance in these control groups declined during the crucial ED-shift, which is in line with expected performance based on their mental age (for normative IED data, see Luciana & Nelson, 2002), whereas a fair number of FXS males already failed during the compound discrimination stages. Finally, these syndrome-specific impairments in attentional set-shifting (repetitive decision-making and enhanced distractibility during reversal learning) were likely to impair overall IED performance in FXS, which was predicted by general intelligence level. This suggests a syndrome-specific constellation of attentional weaknesses mediated by overall intellectual functioning.

In conclusion, the current study adds an important dimension to our understanding of attentional set-shifting deficits in FXS. In line with previous findings (Cornish et al., 2001; Wilding et al., 2002; Woodcock et al., 2009), FXS males can be differentiated from MA and intellectually impaired controls based on a key deficit in reversal learning. Our current findings suggest that the manner in which this attentional deficit is expressed depends on the cognitive constraints imposed by the environment. That is, by differentiating between error types in the IED paradigm, reversal learning deficits in FXS could be characterized by both repetitive decision-making, as well as random search behavior. Repetitive decision-making was predominantly observed during reversal learning within a single stimulus dimension, which was likely the result of an impaired ability to disengage attention from newly learned stimulus-response mappings. In contrast, the random search behavior was observed during more complex stimulus configurations, suggestive of enhanced distractibility to irrelevant stimuli. Importantly, linking these specific error types to task demands (i.e., IED stages) allows for a better understanding of the different cognitive processes that go astray in attentional set-shifting (i.e., within or between stimulus dimensions). This knowledge is crucial to the development of behavioral interventions as neurodevelopmental disorders may perform similarly on a particular task based on overall performance, however, the underlying cognitive and neural deficits may well be different between disorders.