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A PSYCHOPHYSIOLOGICAL & NEUROPSYCHOLOGICAL APPROACH

Melle van der Molen
Profiling cognition in fragile X syndrome:
A psychophysiological and neuropsychological approach

Melle van der Molen
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Chapter 1

General Introduction
General Introduction

For quite some time, tracing back cognitive phenotypes to its genetic origin has been considered to provide new insights in the neurobiology of cognitive development. This challenge in the field of neuroscience is, however, complicated by the divide between molecular neuroscience, systems neuroscience and cognitive neuroscience. For example, as Jonathan Flint already accurately pointed out in 1999, “Success in finding mutations that give rise to mental retardation has not been matched by advances in our understanding of how genes influence cognition”. More than 10 years later, this argument still holds as scientific disciplines too often focus on either the molecular characterization of genetic alterations or the profile of cognitive/behavioral functions in particular neurodevelopmental disorders. According to Flint (1999), molecular neuroscience will always be a limited way of understanding cognitive processes if it divorces from systems neuroscience. I would like to complement this notion by arguing that in understanding the neurobiological basis of cognitive development, a multidisciplinary approach is needed that incorporates, bottom-up, neurobiological investigations (e.g., studies on genetics, molecular studies, and neuronal systems such as signal transduction pathways), as well as, top-down, neuropsychological investigations that study the profile of cognitive/behavioral functions in addition to their underlying information processing characteristics (see Figure 1 for the profiling characteristics of the bottom-up / top-down approach).

By employing a top-down approach, this thesis reports on the cognitive and psychophysiological profile of an etiologically well-defined neurodevelopmental disorder: fragile X syndrome (FXS). The major aim is to trace back cognitive functioning in this single-gene disorder to its underlying information processing characteristics in the brain. Instead of relying on surface manifestations of cognitive abilities (Bishop, 2006), this approach should further our understanding of the typical information processes that go astray in the FXS brain. Importantly, the results from these investigations may contribute not only to our understanding of the gene-brain-cognition relationship in FXS, but provide new insights into those processes involved in
normal cognitive development (Bishop, 2006; Karmiloff-Smith, 2009). The goal of the current chapter is to summarize current theoretical perspectives on normal brain development, and to provide an overview of FXS neurobiology and the alleged influence of single-gene silencing on brain development. This overview should provide an outline for the experiments presented in this thesis, and a framework for interpreting its findings.

**Figure 1.** The bottom-up and top-down profiling approach to study cognitive development. With courtesy of G.J.A. Ramakers, Netherlands Institute of Neuroscience (adapted version).

### 1.1 Cortical network development: advances in theoretical perspectives

Due to recent advances in structural and functional neuroimaging, the classic neuropsychological assumption that cognitive functions can be mapped onto (predetermined) localized brain regions has gradually changed into a different theoretical perspective – one that incorporates both genetic and environmental influences that dynamically alter brain structure and function in order to arrive at the highly connected cortical circuitry as that can be observed in the adult brain (Johnson, 2001; Johnson, 2011). This theoretical viewpoint, coined *interactive specialization* (Johnson, 2001), generally states that the responsive properties of a specific region in the brain are determined by connectivity patterns with other brain regions, and their current states of activity. One
important assumption of the interactive specialization account is that different regions and/or pathways in the brain become increasingly specialized as a result of their progressive recruitment during cognitive processing in developmental time. These architectural constraints (Elman et al., 1996) of brain development constitute that specific sensory input is more efficiently processed by particular neural circuits due to a process of gradual recruitment of these neural circuits – a process also referred to as progressive specialization (Johnson, 2000; Johnson, Halit, Grice, & Karmiloff-Smith, 2002). This is in line with the observation of widespread or diffuse brain activation during stimulus processing in a young infant, whereas later during development, brain activation in response to similar stimulus processing will be confined to more specific neural circuitries as a result of progressive specialization in the processing of this class of stimuli (Durston et al., 2006; Johnson, 2001; Johnson, 2011; Johnson et al., 2002).

These dynamic changes in cortical information processing during infancy are consistent with a process in which interregional interactions help to shape intraregional connectivity, such that several regions together come to support particular perceptual and cognitive functions (Supekhar, Musen, & Menon, 2009). Recent research on intrinsic network connectivity (i.e., brain activity not directly related to sensory or motor events) yielded interesting findings indicating that functional brain development can generally be characterized by diminishment of over-connectivity in the infant brain and increased pruning and rewiring of functional brain networks, as observed in the adult brain (Greicius, Supekhar, Menon, & Dougherty, 2009; Johnson, 2001; Johnson, 2011; Uddin, Supekhar, & Menon, 2010). More specifically, short-range functional connectivity (i.e., strong interregional interactions observed in the infant brain) gradually develops into the strengthening of long-range functional brain networks (i.e., strong intraregional interactions observed in the adult brain). This process of “Small-World” (Douw et al., 2011; Stam, 2004) functional brain organization agrees with the interactive specialization hypothesis mentioned earlier, and is purported to assist in the fast and dynamic processing of sensory information. An important task of the brain is
then to (rapidly) evaluate which stimulus-driven sensory information becomes linked to adaptive responses, and eventually becomes incorporated in the texture of cognition (Mesulam, 1998).

Building on recent developments in the field of systems neuroscience, neural networks have been demarcated that independently facilitate a variety of attentional processes (Crottaz-Herbette & Menon, 2006; Fox, Corbetta, Snyder, Vincent, & Raichle, 2006; Menon & Uddin, 2010). Although theoretical models differ in their functional description, separate neural mechanisms have been identified that facilitate stimulus-driven or bottom-up detection of salient information (i.e., a saliency network), top-down attentional control over lower-level sensory information processing to guide goal-directed behavior and decision-making (i.e., a central executive network), and a default-mode network, activated during periods of rest and posited to reflect a physiological baseline state of brain activity (Fox & Raichle, 2007; Menon & Uddin, 2010; Raichle et al., 2001; Raichle & Snyder, 2007). The salience network, which comprises the anterior insula and anterior cingulate cortex, has been argued to operate as a switching mechanism in the dynamic activation of the default-mode network (ventromedial prefrontal cortex) and the central executive network (dorsolateral prefrontal cortex) (Menon & Uddin, 2010). There is increasing evidence suggesting that aberrant functional connectivity between these networks can result in a variety of attentional impairments, for example as seen in ADHD (Fair et al., 2010), and Alzheimer’s Disease (Li et al., 2011), related to top-down and/or bottom-up attentional processing mechanisms.

1.2 Vulnerability of cortical network development: evidence from neurodevelopmental disorders

The fast and efficient processing of information is considered crucial for cognitive development and argued to be dependent on the quality and quantity of neuronal network formation (Ramakers, 2005; Van Galen & Ramakers, 2005), which can be modified for better or for worse depending on environmental constraints. For example, an enriched environment can enhance electrical activity in cortical circuits due to an increase in sensory stimulation,
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resulting in stimulated dendritic growth, or enhancement of synapse formation (Ramakers, 2005). In contrast, environmental constraints can also exert negative influences on neuronal network development. For example, visual deprivation studies in rodents have shown a decrease in dendritic spines, cortical dendrites and synapses in rodent cortical networks (Ramakers, 2005; but see Coleman & Riesen, 1968; Cragg, 1975). Similarly, in humans, sensory deprivation in the visual modality can cause long-term perceptual deficits, even after years of restoration of the sensory input (Johnson, 2001; Maurer, Lewis, Brent, & Levin, 1999). Among other environmental constraints (e.g., alcohol abuse during pregnancy, metabolic or infectious disease, premature birth, and anoxia), the effects of sensory deprivation can have detrimental consequences for structural and functional brain development, which could in turn manifest as a neurodevelopmental disorder.

Neurodevelopmental disorders are typically characterized by a general impairment in intellectual functioning, as well as impairments in adaptive behavior (e.g., social or communicative functioning) (American Psychiatric Association, 2002; Chiurazzi & Oostra, 2000), and have been argued to primarily result from structural abnormalities in neuronal network formation – also referred to as the Network Hypothesis of Mental Retardation (Ramakers, 2002). Due to abnormal development and/or plasticity of structural network connectivity (e.g., disturbed neuronal migration, altered dendritic morphology) the efficiency of information processing within the network is compromised (Van Galen & Ramakers, 2005). This hypothesis is driven by the observation that neuronal network development is characterized by abnormal spine densities and dendritic complexity in several neurodevelopmental disorders (e.g., Down Syndrome, Rett Syndrome, fragile X syndrome; Kaufmann & Moser, 2000). As neurodevelopmental disorders display a constellation of impairments in cognitive functioning (Zigler & Hodapp, 1991), studying the neurobiological mechanisms that go astray in these disorders can further our understanding of those processes involved in typical and atypical cognitive development (Karmiloff-Smith, 2009).
In pursuit of investigating the neurobiological basis of cognitive development, neurodevelopmental disorders with known genetic origin have received increased interest during the last few decades (Ropers & Hamel, 2005). That is, due to genetic malformations, such as chromosome duplication (e.g., Down syndrome) or genetic deletions (e.g., Williams Syndrome), neuroanatomical and neurophysiological abnormalities can be inferred from their specific genetic etiology. Of particular interest are those neurodevelopmental disorders caused by single-gene mutations, as these neurodevelopmental disorders can be mimicked in rodents by inactivating (“knocking out”) the gene that has been identified to cause the disorder in humans (Flint, 1999).

The most common neurodevelopmental disorder caused by a single-gene mutation is fragile X syndrome (FXS). FXS is caused by a mutation in the fragile X mental retardation gene (FMR1)\(^1\) (Verkerk et al., 1991), and has been well-defined on a neurobiological level due to the development the \(fmr1\) knockout mouse model (Bakker et al., 1994). In FXS, a bottom-up approach in understanding cognitive development can be employed, which allows for investigating the consequences of genetic alterations on a cellular, network, and brain level (see Figure 1). This line of research incorporates methods such as molecular analyses, electrophysiological single-cell recordings, and tracing early trajectories of brain development in knockout models that mimic the human variant of a particular neurodevelopmental disorder. The bottom-up approach can reveal important information on the function of the gene in terms of neuronal network development. To complement the bottom-up approach in its limitations in terms of cognitive profiling, the top-down approach allows for investigating the cognitive and behavioral features that can then be traced back to their underlying information processing characteristics, and the structural and functional properties of brain networks (Figure 1). From the top-down perspective, research has primarily focused on cognitive and behavioral abnormalities in individuals with FXS. Although, to a great degree, these to-

\(^1\) FMR1 refers to the human gene, while \(fmr1\) refers to the non-human gene.
down investigations have contributed to our understanding how specific genetic malformations can result in cognitive dysfunction, the specific information processing characteristics that contribute to the major cognitive deficits remain elusive. Therefore, the current thesis offers a detailed top-down profiling approach, in which both neuropsychological and psychophysiological investigations aim to investigate those (lower- and higher-level) cognitive processes most significantly affected by FMR1 silencing.

1.3 Background of fragile X syndrome

FXS is the most common inherited type of mental retardation and occurs approximately in 1:4000 males and in 1:8000 females (Turner, Webb, Wake, & Robinson, 1996). FXS derived its name from the constricted or ‘fragile’ site along the long arm of the X chromosome, first identified by Lubs (1969). From this point, molecular analyses led to the delineation of the neurobiological origin of FXS, namely, the expansion of a polymorphic CGG repeat in the 5’ untranslated region of FMR1 gene, located at Xq27.3 of the X-chromosome (Fu et al., 1991; Verkerk et al., 1991). In the normal population, CGG repeats in the FMR1 gene vary between 5 and 50 copies, whereas CGG repeat expansions between 50 and 200 are associated with the FXS premutation in male and female carriers (Fu et al., 1991; Willemsen, Levenga, & Oostra, 2011). These CGG repeats in the premutation range are unstable and can expand to larger numbers when transmitted from parent to child. Abnormally long CGG repeat expansions (>200) are found in the FXS full mutation and cause transcriptional silencing of the FMR1 gene, leading to reduced expression or absence of the Fragile X Mental Retardation Protein (FMRP) (Koukoui & Chaudhuri, 2007; Oostra & Chiurazzi, 2001).

In premutation carriers, there is evidence of increased transcription of the FMR1-gene – resulting in elevated FMR1 mRNA levels – which can be linked to weaknesses in cognition and behavior, including executive functioning deficits, balance problems, tremors, and dementia (Grigsby et al., 2008; Willemsen, Mientjes, & Oostra, 2005). The constellation of symptoms has been referred to as Fragile X Tremor Ataxia Syndrome, and mostly affects males with
the FXS premutation in late adulthood (Loesch et al., 2003; Loesch, Churchyard, Brotchie, Marot, & Tassone, 2005). In the case of the FXS full mutation, the FMR1-gene is silenced, resulting in reduced or absent FMRP levels and more severe cognitive and behavioral impairments (Bailey, Hatton, Tassone, Skinner, & Taylor, 2001; Dykens, Hodapp, & Leckman, 1987; Hagerman & Hagerman, 2002). As the FMR1 gene mutation is X-linked, males with the FXS full mutation generally exhibit more severe effects in cognitive/behavioral functioning than females, corresponding to moderate-to-severe levels of intellectual disability (Dykens et al., 1987; Hagerman & Hagerman, 2002).

1.4 The neurobiology of fragile X syndrome: function of FMRP

Although there is still ongoing debate on the specific function of FMRP, it has been suggested that FMRP acts as a RNA binding protein involved in cytoskeletal protein synthesis in neurons (Weiler et al., 2004). A frequently purported function of FMRP relates to the active transporting of mRNA from the nucleus to the dendrites and spines (Bagni & Greenough, 2005). Lack or absence of FMRP is linked to dysregulation of cytoskeletal protein expression, dysmorphogenesis of dendritic spines, and abnormal synaptic plasticity (Greenough et al., 2001; Huber, Gallagher, Warren, & Bear, 2002; Irwin et al., 2001; Oostra & Chiurazzi, 2001). A number of studies have demonstrated immature (longer and thinner) spines in the *fmr1* knockout mice (Galvez, Gopal, & Greenough, 2003; McKinney, Grossman, Elisseou, & Greenough, 2005; Weiler et al., 2004), and in human FXS brains (Irwin et al., 2001), indicative of an abnormal dendritic pruning process compromising normal brain development through aberrant synaptic plasticity (Pfeiffer & Huber, 2007, 2009).

A possible explanatory mechanism for the observed dendritic spine dysgenesis is that FMRP inhibits group-1 metabotropic glutamate receptor (mGluR) dependent protein synthesis (Bear, Huber, & Warren, 2004; Huber, 2007; Koukoui & Chaudhuri, 2007). Normally, the activation of mGluR receptors plays a key role in protein synthesis-dependent synaptic plasticity by triggering long-term depression (Huber, 2007), a neuronal mechanism that
decreases synaptic strength (Cooke & Bliss, 2006). In the absence of FMRP, however, mGluR activation is enhanced, resulting in exaggerated long-term depression (Huber et al., 2002). Normally, both long-term depression and long-term potentiation (the latter enhancing synaptic strength) work in concert in response to neural signal transmission mechanisms that enable synaptic plasticity, a key process during brain development (Cooke & Bliss, 2006). In FXS, however, these neurobiological processes seem to be compromised.

Additionally, several lines of evidence have reported reduced expression of γ-aminobutyric acid type A (GABA\textsubscript{A}) receptor in the \textit{fmr1} mouse model (D’Hulst et al., 2006; D’Hulst & Kooy, 2007; Kooy et al., 1996). GABA\textsubscript{A} receptors are the main inhibitory receptors in the brain, and decreased activation has been observed in the cortex, hippocampus, and brainstem of \textit{fmr1} knockout mice (for a review, see D’Hulst & Kooy, 2007). Although the causal mechanism for reduced GABA\textsubscript{A} activity remains elusive, abnormal functioning of the GABA\textsubscript{A} receptor has been linked to the clinical signatures in full mutation FXS individuals (e.g., epileptic activity, hypersensitivity; D’Hulst & Kooy, 2007).

Together, absence of FMRP significantly alters normal brain development as reflected by an immature cortical network, imbalance between long-term potentiation and long-term depression that subsequently results in aberrant synaptic plasticity, and an imbalance in the main inhibitory (GABA) and excitatory (glutamate) neurotransmitter systems in the brain. Based on these findings, it could be suggested that the observed impaired intellectual functioning and atypical behavior can be traced back to abnormal neural network connectivity and increased ratio of neural excitation/inhibition, which potentially alters the processing of sensory information in the brain.

1.5 Brain pathology in fragile X syndrome

To investigate the effects of FMRP depletion on structural and functional brain development, the last decade has known an increase in neuroimaging studies which examined in detail the structural brain abnormalities in FXS. There is increasing evidence that specific neural structures are more affected due to
silencing of the FMR1 gene than others (Hallahan et al., 2011; Zangenehpour, Cornish, & Chaudhuri, 2009). For example, Abitbol et al. (1993) reported higher FMR1 expression in the cholinergic neurons of the nucleus basalis magnocellularis and the pyramidal neurons of the hippocampus, the latter structure frequently associated with memory and learning (Sable, Low, Maclin, Fabiani, & Gratton, 2004; Squire, 1992; Sutherland & McNaughton, 2000). The importance of FMR1 expression in these cortical structures was thereafter corroborated by Greicius, Boyett-Anderson, Menon, and Reiss (2004), who were able to demonstrate that during visual memory encoding, participants with FXS showed a significant reduction of brain activation in the basal forebrain and hippocampus related to FMR1 expression levels, while activation in other cortical regions revealed no differences. Additionally, Gothelf and coworkers (2008) aimed to reveal which brain structures best distinguished children and adolescents with FXS from typically developing controls. Results showed that FXS individuals could be best differentiated from controls based on enlarged volumes of caudate nucleus and reduced amygdala volumes in FXS individuals. Together, these findings show abnormalities in brain structures that are associated with memory and learning (e.g., associative and reinforcement learning), as well as regulation of social-emotional behavior (Bickart, Wright, Dautoff, Dickerson, & Barrett, 2011; Bressler & Menon, 2010; Pare, Collins, & Pelletier, 2002; Uddin et al., 2010).

Due to recent advances in neuroimaging technology, methods became available to study patterns of functional brain connectivity in FXS. For example, by using Diffusion Tensor Imaging (DTI), white matter tracts can be visualized in the brain that reveal important information on the integrity of structural cortical networks (Cascio, Gerig, & Piven, 2007). Recent DTI studies found aberrant patterns of white matter connectivity in the sensorimotor pathways, as well as the ventral frontostriatal pathways of FXS individuals (Barnea-Goraly et al., 2003). In particular, the observation of aberrant frontostriatal white matter connectivity seems to be a robust finding, as it can be observed in children (Haas et al., 2009; Hoefl et al., 2007; Hoefl et al., 2010), as well as adolescents (Hallahan et al., 2011; Hoefl et al., 2007) with the FXS full mutation.
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Additionally, functional brain imaging studies demonstrated abnormal activity in females with the FXS full mutation in frontostriatal and frontoparietal networks during respectively inhibition (go/no-go) (Menon, Leroux, White, & Reiss, 2004) and working memory tasks (Menon, Kwon, Eliez, Taylor, & Reiss, 2000). In particular, reduced brain activity was observed in right lateralized frontostriatal networks, and the observed activity patterns were positively correlated with FMRP expression levels (Menon et al., 2004). Interestingly, in both investigations a reduction in right lateralized brain activity was compensated by an increase of left lateralized brain activity, postulated to assist in task performance. This observation is in keeping with the interactive specialization hypothesis of functional brain development (Johnson, 2001; Johnson, 2011), which states that cognitive functions can be governed by different cortical circuitries as a result of progressive specialization of these networks in the processing of a particular class of stimuli. Moreover, similar compensatory left lateralized frontoparietal brain activity associated with top-down attentional control mechanisms have been reported in typically developing children when performing a conflict task (Konrad et al., 2005). This increase in left lateralized brain networks was interpreted to reflect immature attentional networks. Based on the observation of immature spine morphology in FXS (Irwin et al., 2001), it could be argued that normal development of the functional brain networks is constrained due to FMR1 silencing.

In sum, the pattern of findings derived from these neuroimaging studies suggests that in the absence of FMRP, large-scale neural network connectivity in frontostriatal, frontoparietal and sensorimotor tracts are significantly altered. These neural networks are important for a host of attentional and cognitive functions, from the early processing of sensory information (sensory-motor regions) to the ability to adaptively modulate information processing in different sensory modalities to accomplish goal-directed behavior (Bressler & Menon, 2010; Uddin et al., 2010).
1.6 Cognitive and behavioral profile in fragile X syndrome

From a top-down perspective (see Figure 1), profiling cognitive abilities in a single-gene disorder like FXS could reveal important information on the gene-brain-cognition relationship. A direct relation between FMRP and cognitive development may not be so straightforward, however, due to the cascading neurobiological modifications as a result of FMRP depletion (Irwin et al., 2001). In conjunction with environmental constraints, these neurobiological processes could differentially impact on cortical network development (Johnson, 2001; Karmiloff-Smith, 2009), and subsequently result in the cognitive and behavioral profile observed in FXS.

Generally, cognitive dysfunction in FXS is characterized by global cognitive impairment plus disproportional weaknesses in working memory (Baker et al., 2010; Munir, Cornish, & Wilding, 2000a), inhibitory control (Cornish, Munir, & Cross, 2001; Scerif, Cornish, Wilding, Driver, & Karmiloff-Smith, 2007; Scerif et al., 2005) and abilities to plan and organize behavioral schemes to accomplish goal-directed behavior (Cornish et al., 2001). In addition, FXS males can be characterized by a profile of striking attentional weaknesses, including a weakness in the ability to flexibly shift attention from one concept to another, as well as focused attention to currently relevant tasks, while ignoring competing or irrelevant environmental demands (Scerif, Cornish, Wilding, Driver, & Karmiloff-Smith, 2004; Scerif et al., 2007; Scerif et al., 2005). Opposed to these cognitive and attentional weaknesses, visual-perceptual abilities (Dykens et al., 1987; Maes, Fryns, Van Walleghem, & Van den Berghe, 1994), long-term memory for meaningful information (Dykens et al., 1987; Maes et al., 1994), as well as simple verbal abilities (e.g., receptive and expressive vocabulary) (Backes et al., 2000; Maes et al., 1994) are less affected due to FMR1 silencing.

At the behavioral level, males with FXS typically show hyperactive and avoidant behavior, as well as stereotypic or repetitive movements (e.g., hand-flapping of hand-biting) (Backes et al., 2000; Hagerman & Hagerman, 2002). FXS males furthermore display hypersensitivity or hyperarousal to sensory stimulation, which is observed for all sensory modalities (Miller et al., 1999).
Although the manifestation of these atypical behaviors is subject to high levels of heterogeneity (Hagerman & Hagerman, 2002), the behavioral phenotype shows high overlap with atypical behaviors commonly seen in autism, ADHD, and oppositional defiant disorder (Backes et al., 2000; Hagerman & Hagerman, 2002). Taken together, the observed cognitive-behavioral profile in FXS is marked by weaknesses that compromise attentional control and efficient goal-directed behavior, necessary for adaptive functioning in daily live settings. To date, however, the underlying information processing characteristics in the brain amenable for these attentional and cognitive weaknesses remain unclear.

1.7 The importance of lower-level information processing characteristics for interpreting cognitive functioning

In terms of gene-brain-cognition relationships it is often claimed that FMR1 silencing results in a cascade of neurobiological dysfunctions that alter brain development in such a way that neural circuits are predominantly affected that normally enable higher-level cognitive operations (Munir et al., 2000a; Munir, Cornish, & Wilding, 2000b; Wilding, Cornish, & Munir, 2002). A related assumption is that the consequences of FMRP depletion on cognitive functioning can be interpreted in terms of ‘intact’ versus ‘impaired’ abilities, with pronounced impairments in higher-level cognitive operations, whereas perceptual or lower-level functions are relatively spared due to FMR1 silencing.

Basically, two fundamental objections can be raised against these interpretations. First, consider the aforementioned interactive specialization account on functional brain development (Johnson, 2001; Johnson, 2011; Johnson et al., 2002). This notion rejects the assumption that the function of a single gene can be linked to regional specific changes in brain development. Therefore, the absence of FMRP cannot directly be linked to impaired higher-level cognitive functioning. In contrast, genetic mutations in neurodevelopmental disorders cause more global cognitive impairment, and are therefore more likely to affect widespread neural circuitries in the cortex as opposed to specific neural (sub) systems (Johnson et al., 2002), a notion in keeping with the Network Hypothesis of mental retardation (Ramakers, 2002).
In terms of the FXS gene-brain-cognition relationship, it may well be that neural circuitries are affected that normally mediate higher-level cognitive functioning, however, aberrant lower-level perceptual processes may also play an important role in these observed cognitive dysfunctions (Bertone, Hanck, Kogan, Chaudhuri, & Cornish, 2010a, 2010b).

The second objection relates to the methodological criticism on interpreting cognitive abilities in neurodevelopmental disorders in terms ‘intact’ versus ‘impaired’ abilities (Karmiloff-Smith, 2009). For example, when performance on a cognitive task does not differ between FXS participants and chronologically age-matched controls, this particular cognitive ability is frequently considered to be intact. However, caution is warranted when expressing such absolute statements (Karmiloff-Smith, 2009), as the underlying information processing demands may well be different between groups. That is, FXS participants may differ in terms of basic processing speed, or recruit different neural circuitries. These underlying cognitive operations are frequently overlooked, but may be highly informative in terms of between-group comparisons, as well as for elucidating the gene-brain-cognition relationship in FXS.

Both stimulus-driven and cognitive information processing can be investigated with high temporal accuracy by measuring event-related brain potentials (ERP). Typically, electrocortical scalp voltage deflection is measured time-locked to the onset of a particular stimulus of interest (e.g., a tone or a picture). ERP components can then be identified that characterize different stages of stimulus representation in the brain. Frequently investigated ERP components include the N1, the Mismatch Negativity (MMN), and P3, each associated with different aspects of (pre)attentive information processing. For example, the N1 is the earliest, sensory component of the ERP and is associated with detecting the physical aspects (e.g., intensity) of a stimulus, and can be identified at approximately 100 ms after stimulus onset (Nääätänen & Picton, 1987). The N1 typically habituates (i.e., shows a reduction in amplitude) in response to repeated presentation of the same, standard, stimulus, a process related to the refractoriness of the neural populations generating the N1
response (Budd, Barry, Gordon, Rennie, & Michie, 1998; May & Tiitinen, 2010; Näätänen & Picton, 1987). However, upon presentation of a different, or deviant, stimulus, the N1 recovers to its initial activity as observed for the first presented standard stimulus. This restored N1 generation is argued to reflect sensory or pre-attentive deviance detection, and is best observed in an ERP difference wave (Duncan et al., 2009; Schröger, Bendixen, Trujillo-Barreto, & Roeber, 2007) derived from subtracting the ERP to a standard stimulus from the ERP to a deviant stimulus. This early ‘deviance detection mechanism’ is also referred to as the MMN (Näätänen, Paavilainen, Rinne, & Alho, 2007). Importantly, the MMN can only be observed when the neural circuitry generating the N1 has established a neural representation, or a memory trace, of the repetitive aspects of the standard stimulus. That is, at least a sufficient number of standard stimuli have to precede a deviant stimulus in order to generate the MMN. Based on this observation, the MMN is postulated to index pre-attentive sensory memory formation created by the repetitive aspects of the standard stimulus (Cowan, Winkler, Teder, & Näätänen, 1993).

When deviant stimuli can be sufficiently distinguished from standard stimuli, the MMN is usually followed by the P3 component of the ERP. Basically, the P3 component is argued to reflect the outcome of a decision-making process (Nieuwenhuis, Aston-Jones, & Cohen, 2005) and exists of two sub-components: the P3a and the P3b (Polich, 2007). The P3a is the early component (peaking around 250-350 ms) and is associated with the involuntary triggering of attention (Escera, Alho, Winkler, & Näätänen, 1998; Escera, 2007), whereas the P3b is the late component (peaking around 300-450 ms) and is associated with the active direction of attention to stimuli and decision-making (Nieuwenhuis et al., 2005; Nieuwenhuis, De Geus, & Aston-Jones, 2010; Polich, 2007; Polich & Kok, 1995). For example, in atypical development, studies frequently demonstrate reduced P3b amplitudes, as well as prolonged peak latencies in a variety of neurodevelopmental disorders (e.g., Rett and Prader-Willi syndrome) (Stauder, Brinkman, & Curfs, 2002; Stauder, Smeets, van Mil, & Curfs, 2006). These findings suggest aberrant attentional functioning and reduced processing speed, contributing to understanding of
how abnormalities at the attentional and cognitive level are represented at the network level. Therefore, tracing back cognitive impairments to its information processing characteristics may yield important findings that contribute to elucidating the gene-brain-cognition relationship in FXS.

1.8 Remaining questions and outline of this thesis
To investigate the implications of a single-gene mutation on brain development and cognition, FXS provides an excellent model. FXS has been well defined at the neurobiological level, and cognitive functioning in FXS has been extensively investigated. However, to date, one important aspect of the gene-brain-cognition trajectory remains understudied in FXS; that is, information processing in the FXS brain. More specifically, whereas cognitive functioning in FXS has been extensively characterized, little is known about the underlying information processing characteristics in the brain that could contribute to the observed profile of cognitive impairments. To date, it remains uninvestigated whether these cognitive impairments are similar for FXS males of various intellectual performance levels. This latter question is particularly important to answer, as intellectual functioning in FXS is subject to high levels of heterogeneity (Backes et al., 2000; Hagerman & Hagerman, 2002), and similarity or dissimilarity between cognitive profiles of high vs. low performing FXS males could reveal important information both in term of gene-brain-cognition trajectories, as well as of educational and intervention purposes.

This thesis comprises four experimental investigations that aim to profile cognition in adult males with the FXS full mutation in a top-down profiling manner in order to better understand the underlying information processing mechanisms associated with cognitive dysfunction in FXS. Chapter 2 addresses the question whether adult males with the FXS full mutation can be characterized by a profile of relative strengths and weaknesses in cognitive abilities. Moreover, as heterogeneity in intellectual functioning is often reported in FXS (Abbeduto, Brady, & Kover, 2007; Bailey et al., 2001; Dykens et al., 1987; Loesch, Huggins, & Hagerman, 2004; Mazzocco, 2000), it was examined whether cognitive profiles would be similar for individuals of high
and low intellectual functioning. In addition, this investigation addresses the important methodological issue of how choosing a reference measure could affect the interpretation of a cognitive ability as a relative ‘strength’ or ‘weakness’. In Chapter 3, the focus is directed to one of the most striking weaknesses in the FXS phenotype – attentional function. More specifically, in this chapter the underlying processes are investigated that contribute to the frequently observed attentional set-shifting deficit in FXS males (Cornish et al., 2001; Woodcock, Oliver, & Humphreys, 2009). In order to characterize attentional set-shifting deficits specific to the FXS etiology, performance of FXS males is compared to that of several control groups matched on chronological and mental age, as well as intellectual level. The goal of the investigations presented in Chapters 4 and 5 is to examine the information processing characteristics associated with pre-attentive (chapter 4) and active (chapter 5) change detection mechanisms using ERPs. These ERP studies should further our understanding on the information processing characteristics that go astray in FXS during the sensory or bottom-up stadia of information processing associated with involuntary and voluntary attentional processes. Finally, Chapter 6 will provide a summary of the experiments described in this thesis, and will address recommendations for further research that could bridge the divide between FMR1 gene-silencing and the FXS phenotype.
Publications

All empirical chapters of this thesis have been published or submitted for publication in international, peer-reviewed journals. To acknowledge the important contribution of the co-authors, a list of references to these chapters is presented below.


Chapter 2

Profiling fragile X syndrome in males:

Strengths and weaknesses in cognitive abilities

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Abstract

The present study examined the cognitive profile in Fragile X Syndrome (FXS) males, and investigated whether cognitive profiles are similar for FXS males at different levels of intellectual functioning. Cognitive abilities in non-verbal, verbal, memory and executive functioning domains were contrasted to both a non-verbal and verbal mental age reference. Model-based cluster analyses revealed three distinct subgroups, which differed in level of functioning, but showed similar cognitive profiles. Results showed that cognitive performance is particularly weak on measures of reasoning and performal abilities confined to abstract item-content, but relatively strong on measures of visuo-perceptual recognition and vocabulary. Further, a significant weakness was found for verbal short-term memory. Finally, these results indicated that the choice of an appropriate reference is critically important in examining cognitive profiles. The pattern of findings that emerged from the current cognitive profiling of FXS males was interpreted to suggest a fundamental deficit in executive control.
2.1 Introduction

Fragile X Syndrome (FXS) is the most frequently reported inherited type of mental retardation in males (Turner, Webb, Wake, & Robinson, 1996), and is most often caused by transcriptional silencing of the Fragile X Mental Retardation 1 (FMR1) gene (Fu et al., 1991; Oostra & Chiurazzi, 2001; Verkerk et al., 1991). In the FXS full mutation this single-gene defect results in reduced or absent FMR1 protein (FMRP) expression (Koukou & Chaudhuri, 2007). FMRP is argued to be specifically involved in synaptic and dendritic refinement during early brain development (Christie, Akins, Schwob, & Fallon, 2009). Absence of FMRP is primarily associated with abnormal maturation of synaptic connectivity (Oostra & Chiurazzi, 2001) and is argued to be the primary cause of the cognitive deficits frequently observed in FXS (Loesch, Huggins, & Hagerman, 2004; Visootsak, Warren, Anido, & Graham, 2005). Although the cognitive profile of FXS males has been extensively studied over the years, little is known about the relation between performance level and cognitive profile. The goal of the present study was: (a) to examine the cognitive profile of FXS full mutation males of different performance levels over a wide range of cognitive abilities; and (b) to investigate whether such a cognitive profile would be similar for FXS males of different levels of performance.

Over the years, the neuropsychological phenotype of full mutation FXS has been well documented and is characterized by a general impairment in cognitive performance, with some cognitive abilities more strongly affected than others (Cornish, Turk, & Hagerman, 2008; Hodapp, Dykens, Ort, Zelinsky, & Leckman, 1991; Maes, Fryns, Van Walleghe, & Van den Berghe, 1994). Relative strengths in cognitive performance are frequently reported for vocabulary capacity (Dykens, Hodapp, & Leckman, 1987; Maes et al., 1994; Philofsky, Hepburn, Hayes, Hagerman, & Rogers, 2004), visuo-perceptual abilities (Cornish, Munir, & Cross, 1999; Hodapp et al., 1992; Maes et al., 1994), and the processing and recall of simultaneous and meaningful information (Backes et al., 2000; Dykens et al., 1987; Freund & Reiss, 1991; Maes et al., 1994; Munir, Cornish, & Wilding, 2000a; Powell, Houghton, & Douglas, 1997).
In contrast, consistent deficits have been reported for verbal short-term memory (Freund & Reiss, 1991; Munir et al., 2000a), visuo-spatial memory (Munir et al., 2000a), linguistic processing (Abbeduto, Brady, & Kover, 2007; Abbeduto & Hagerman, 1997; Ferrier, Bashir, Meryash, Johnston, & Wolff, 1991), selective and divided attention (Munir, Cornish, & Wilding, 2000b; Scerif, Cornish, Wilding, Driver, & Karmiloff-Smith, 2007; Wilding, Cornish, & Munir, 2002) and the processing of sequential and abstract information (Dykens et al., 1987; Freund & Reiss, 1991; Powell et al., 1997).

Accumulating evidence suggests a fundamental deficiency in executive control (Cornish, Sudhalter, & Turk, 2004a; Cornish et al., 2004b; Wilding et al., 2002). That is, those processes that provide top-down guidance for orchestrating the more basic cognitive processes to accomplish goal-directed behavior. Executive control exerts its influence on cognition by modulating information processing in different cognitive modalities, driven by a prefrontal neural network (Miller & Cohen, 2001; Posner & Petersen, 1990; Posner & Rothbart, 2007). Importantly, deficits in executive control have their repercussions for performance across a wide range of cognitive abilities. Illustrative in this respect is that within the domain of executive function FXS males show difficulties in inhibiting pre-potent responses (Cornish, Scerif, & Karmiloff-Smith, 2007; Hooper et al., 2008; Loesch et al., 2003; Munir et al., 2000b; Wilding et al., 2002), impaired cognitive flexibility (e.g., task-switching; Cornish, Munir, & Cross, 2001; Hooper et al., 2008; Woodcock, Oliver, & Humphreys, 2009), and weak problem-solving abilities (Hooper et al., 2008; Maes et al., 1994). In addition, within the domain of working memory, deficits have been attributed to a general limitation in working memory capacity (Munir et al., 2000a; Ornstein et al., 2008). That is, the amount of attention available to maintain and manipulate information, mediated by executive control processes. Furthermore, the pattern of deficits reported for more complex verbal abilities (e.g., perseverations in speech; Abbeduto et al., 2007) and non-verbal reasoning abilities (e.g., processing of abstract information; Maes et al., 1994), seem to implicate a specific deficit for cognitive abilities
relying on executive control. Together, these findings point to inefficient executive control as a core-deficit in FXS males.

The observed pattern of strengths and weaknesses in FXS cognitive functioning may suggest a specific cognitive profile for FXS. That is, the cognitive profile in FXS might well be different from cognitive profiles seen in other mental retardation syndromes (Cornish et al., 2007; Cornish et al., 2008). However, syndrome-specificity of a cognitive profile is also determined by heterogeneity in cognitive performance levels. More specifically, FXS males functioning at higher performance levels may be characterized by a different cognitive profile compared to FXS males functioning at lower performance levels. Such differences constrain the notion of syndrome-specific cognitive profiles. For example, FXS is characterized by an increased heterogeneity in the level of intellectual functioning, corresponding to moderate-to-severe levels of mental retardation (Abbeduto et al., 2007; Bailey, Hatton, Tassone, Skinner, & Taylor, 2001; Dykens et al., 1987; Loesch et al., 2004; Mazzocco, 2000). The question arises whether high-functioning FXS males show similar or distinct strengths and weaknesses in cognitive performance relative to low-functioning FXS males.

The primary objective of the present study was to examine the relative strengths and weaknesses in a wide range of cognitive abilities in FXS full mutation males functioning at different performance levels. More specifically, we investigated whether such a cognitive profile would differ between FXS males of different performance levels. Cognitive performance was examined in a large sample of FXS males for the following cognitive domains: non-verbal (reasoning and performat) abilities, verbal abilities, memory performance, and aspects of executive function. Test results were converted into mental age (MA) equivalents, which allowed for comparing between cognitive abilities within, as well as between participants. To interpret the cognitive abilities in terms of relative strengths and weaknesses, each performance measure was contrasted to a non-verbal and verbal MA reference measure (NVMA and VMA respectively). Two reference measures were employed to avoid interpretation bias resulting from comparison to a single reference measure. For example,
reference to a single measure of intelligence can result in serious interpretation problems, as this reference or cognitive ability could reveal as a significant strength or weakness (see Mottron, 2004, for a detailed discussion on this issue).

Since full mutation males share the same genetic cause of mental retardation, we expected that FXS males functioning at different performance levels would show similar cognitive profiles. In addition, based upon previous studies on cognitive functioning in FXS males, we anticipated FXS males to be more impaired on tasks that require higher levels of executive control. Thus, specific deficits were expected for cognitive abilities relying on executive functions and working memory processes. These deficits were expected to be evident for all FXS males, regardless of cognitive performance level. In contrast, relative strengths were expected for visuo-perceptual recognition (i.e., Gestalt closure) and vocabulary.

2.2 Method

2.2.1 Participants

The present study included 43 adult males, ranging in age from 18 to 48 years (mean age = 28.7, SD = 8.5). Participants were recruited through the Dutch Fragile X Parent Network. The FXS full mutation was established by DNA testing. All participants were free from additional diagnosed psychiatric disorders, based on DSM-IV-TR classifications (American Psychiatric Association, 2002) and had normal or corrected-to-normal vision. None of the participants were taking (prescribed) medication. Informed consent was obtained from parents or legal guardians. The protocol for this study was reviewed and approved by the ethical review committee of the university.

2.2.2 Measures

A test battery was assembled from neuropsychological and intelligence tests for children to assess non-verbal (reasoning and performal) abilities, verbal abilities, memory performance, and aspects of executive function. Tests were selected based upon the following criteria: (1) tests should preferably cover a
wide (MA) age range to enable assessment of both higher- and lower-functioning individuals; (2) tests (with the exception of verbal measures) should preferably be independent of verbal output by the participants to enable inclusion of individuals with limited or absent speech. Raw scores were converted into MA equivalents, and, unless indicated otherwise, the variable of interest was the number of items answered correctly. A schematic overview including psychometric characteristics of the test battery is presented in Table 1.

### 2.2.2.1 References for non-verbal and verbal mental age

**Non-verbal mental Age.** The total score of the Snijders and Oomen Non-Verbal Intelligence Test (SON-R 2½-7 & SON-R 5½-17; Snijders, Tellegen, & Laros, 1998) was employed as a measure of NVMA\(^1\). The SON-R has well documented indices of reliability and concurrent validity (Harris, 1982; Moore, O’Keefe, Lawhon, & Tellegen, 1998), and correlates highly with the more widely used intelligence batteries, such as the Stanford Binet (Thorndike, 1973) and the Wechsler Preschool and Primary Scale of Intelligence (WPPSI; Harris, 1982; Moore et al., 1998; Wechsler, 1963). Also, the SON-R is rated as “good” on reliability by the Committee On Test Affairs Netherlands (COTAN; Evers, 2001; Evers, Van Vliet-Mulder, & Groot, 2000). Furthermore, the SON-R has proven to be particularly useful to assess non-verbal intelligence in children with (mental) disabilities (Janke & Petermann, 2006; Jenkinson, Roberts, Dennehy, & Tellegen, 1996). The SON-R subtests do not require verbal output by the participant, which enables inclusion of participants with limited or absent speech.

**Verbal mental age.** The Dutch version of the Peabody Picture Vocabulary Test, third edition (PPVT-III-NL; Schlichting, 2004), was used to index receptive vocabulary and to provide a reference for VMA. The PPVT has proven to be an adequate and reliable instrument for assessing verbal ability.

\(^1\)The SON-R total score is the weighted average of performance on the six SON-R subtests. The SON-R total score was considered as a representative contrast value for subtest comparison, based on the finding that strengths and weaknesses within the SON-R profile remain identical whenever the SON-R total score was employed as a contrast value subtest comparison, or when the SON-R subtest were contrasted against each other (i.e., with the SON-R total score excluded as contrast value).
and it accurately predicts full scale IQ and verbal IQ measures on the Wechsler Adult Intelligence Scale (Bell, Lassiter, Matthews, & Hutchinson, 2001). Moreover, although verbal responses are not required, the PPVT can still provide an estimate of verbal ability. The PPVT has been frequently used as an indicator of VMA in developmental studies (Cornish et al., 1999; Kogan et al., 2009).

2.2.2.2 Non-verbal reasoning abilities

Concrete reasoning. The SON-R subtest “Situations” was employed as a measure of concrete non-verbal reasoning ability. Drawings of specific (ecologically relevant) situations were presented. These drawings consist of a blank square, leaving out a specific part of a situation or object. Participants are required to choose the correct (i.e., the most sensible) picture from multiple options.

Abstract sorting. The SON-R subtest “Analogies” was employed as a measure of abstract reasoning ability. Participants are required to sort geometric colored tokens according to shape, color or size, or on more difficult items, according to a transformation rule that has to be detected.

Concrete sorting. The SON-R subtest “Categories” was employed as a measure of sorting ability confined to ecologically relevant objects. The participant is required to sort cards depicting objects, according to a category rule (e.g., tables versus chairs).

Concept formation. Concept formation or implicit reasoning was measured using the subtest “Conceptual Thinking” from the Kaufman Assessment Battery for Children, second edition (K-ABC II; Kaufman & Kaufman, 1983a; Kaufman & Kaufman, 1983b). Participants have to identify (by pointing) the odd object amongst a series of objects that belong to the same class or category.

Visuo-perceptual recognition. The subtest “Picture Recognition” of the Revised Amsterdam Children Intelligence Test (RAKIT; Bleichrodt, Drenth, Zaal,
2. Strengths and weaknesses in cognitive abilities

& Resing, 1987; Bleichrodt, Drenth, Zaal, & Resing, 1984)² was employed to assess visuo-perceptual abilities (i.e., Gestalt closure). Participants are instructed to denominate incompletely drawn pictures of objects. Reliability and validity of the RAKIT have been rated as “good” by the COTAN.

2.2.2.3 Performal abilities

Concrete object assembly. The SON-R subtests “Puzzles” was used to assess the ability to copy or rearrange visual meaningful stimuli. Participants are required to assemble a puzzle depicting a meaningful picture.

Abstract object assembly. The SON-R subtest “Mosaics” was used to assess the ability to copy abstract visual patterns using colored blocks.

Abstract drawing. The SON-R subtest “Patterns” was employed to assess visuo-constructive abilities involved in drawing geometric shapes. Participants are asked to copy geometric shapes with increasing difficulty.

2.2.2.4 Verbal abilities

Expressive vocabulary. Expressive vocabulary was measured with the subtest “Word Production” from the Dutch Language Test for Children (Taaltest voor Kinderen (TVK); Van Bon, 1982)³. While looking at a picture from a booklet, a prompting sentence is provided related to that picture. The participant is required to complete this sentence with a correct word.

Sentence production. The TVK subtest “Sentence Construction” was used to assess grammatical abilities necessary for correct sentence construction. While looking at a drawing (e.g., a picture of a cat on a car), participants are provided with a corresponding, but incongruent sentence (e.g., “the car is on the cat”). The participant has to change word order to form the correct sentence (e.g., “the cat is on the car”).

²The RAKIT (Bleichrodt et al., 1987; Bleichrodt et al., 1984) is a Dutch intelligence test for children aged 4-12 years, and is based on Thurstone’s (1938) theory of cognitive abilities. The RAKIT has high concurrent validity (.86) with the WISC-R for total IQ.
³The TVK (Van Bon, 1982) is a Dutch language assessment battery for children aged 4-9 years. The TVK comprises subtests which assess a broad range of receptive and productive verbal abilities. The TVK taps phonological, morphological, syntactical, and semantic aspects of grammatical competence. Appropriate reliability has been reported for the subtests “vocabulary production test” and “sentence production test” (Resing, Evers, Koomen, Pameijer, & Bleichrodt, 2005).
Verbal fluency. The RAKIT subtest “Production of Ideas” was used to assess semantic fluency. Participants are required to produce as many words as possible within one minute related to a specific category (e.g., “what can you find in a store?”).

Verbal reasoning. The RAKIT subtest “Telling Stories” was used to assess verbal reasoning and expressive speech by means of two pictures presented on an A4 sized sheet (e.g., a scene in a garden and in a living room). Participants are required to denominate as many objects as possible, as well as verbalize object relations and the plot of the story.

2.2.2.5 Memory

Verbal STM. Verbal STM for sequential information was assessed using the subtest “Number Recall” (digit span forward), adopted from the Kaufmann Assessment Battery for Children, second edition (K-ABC II; Kaufman & Kaufman, 1983a).

Visuo-spatial STM (sequential). Visuo-spatial STM for sequential information was assessed using the subtest “Spatial Span” of the Cambridge Neuropsychological Test Automated Battery (CANTAB; Strauss, Sherman, & Spreen, 2006)\(^4\). During this computerized analogue to the Corsi Block Tapping test (Corsi, 1972), participants are required to correctly copy a sequence of highlighted squares on a computer touch screen. After correctly copying a sequence of squares, the subsequent sequence is increased with an additional square. The variable of interest is the maximum length of the correctly recalled sequence of squares.

Visuo-spatial STM (simultaneous). The K-ABC subtest “Spatial Memory” (Kaufman & Kaufman, 1983b) was used to assess visuo-spatial STM capacity for the location of meaningful information. During each trial, a sheet is presented for five seconds, depicting several objects/animals within a specific spatial

\(^4\)The CANTAB is a widely used computerized tool for the assessment of frontal- and medial temporal lobe dysfunctions. The CANTAB subtests included in this study were derived from the ‘child battery’, appropriate for children aged 4-to-12 years. Normative data for these subtests have been extended by De Luca (2003) and Luciana and Nelson (2002). Indices of reliability have been reported by Lowe and Rabbit (1998). For a detailed description of the CANTAB-subtests included in this study, the reader is referred to Luciana and Nelson (1998).
arrangement. After the five seconds, an empty matrix is presented, and participants have to point to the location of the recalled stimuli within the matrix. A trial is scored correct when the location of all stimuli is recalled correctly.

*Working memory (self-ordered search).* The CANTAB subtest “Spatial Working Memory” (SWM) was employed to assess the ability to retain and update visuo-spatial information in working memory. On this subtest, analogous to the self-ordered pointing task of Petrides and Milner (Petrides & Milner, 1982), participants are required to search for tokens (hidden in boxes) by a process of search and elimination. Participants are instructed to collect the tokens by opening the boxes, and are told that a box remains empty once a token has been collected from that box. Therefore, to employ an efficient search strategy, participants have to keep in mind the boxes in which they have previously found a token. The variable of interest is the number of errors (total errors) committed.

*Visuo-spatial recognition memory (sequential).* The CANTAB subtest “Spatial Recognition Memory” (SRM) was used to assess visuo-spatial recognition memory. Participants are required to carefully look at a sequence of five squares presented in different locations on a touch-screen. Thereafter, two squares are presented simultaneously, and participants have to indicate which square was positioned at the exact location of one of the previous squares. The variable of interest is the percentage of correctly recalled locations.

*Visual recognition memory (simultaneous).* The CANTAB subtest “Pattern Recognition Memory” (PRM) was administered to assess visual recognition memory. Twelve geometric patterns are presented sequentially. Subsequently, two patterns are presented and the participant is asked to point to the pattern previously seen (recall mode). The recall mode is presented either immediately (PRM-I) or with a 20-minute delay (PRM-D). Both versions were used in this study. The PRM-I was taken to provide an index of short-term recognition memory, whereas the PRM-D was taken as a measure of long-term
recognition memory. For both versions, the variable of interest is the percentage of correctly recalled patterns.

**Associative learning.** The RAKIT subtest “Name Learning” was used to assess associative learning capacity. Participants are presented with a booklet containing pictures of cats or butterflies. For each picture a corresponding name is provided, which has to be memorized by the participant. The variable of interest is the number of correctly recalled objects.

### 2.2.2.6 Executive function

**Cognitive flexibility.** The CANTAB subtest “Intra-Extra Dimensional Set-Shift” (IED) was used to assess rule acquisition and reversal (cognitive flexibility). This subtest consists of a total of nine stages, assessing various set-shifting paradigms (e.g., simple discrimination, simple reversal, intra-dimensional set-shift (and reversal), or extra-dimensional set-shift (and reversal). The variables of interest are the number of completed stages, the total number of errors committed, and the number of trials completed.

**Planning.** Spatial planning and problem solving abilities were assessed with the CANTAB subtest “Stockings of Cambridge” (SOC). The SOC is a computerized analogue to the “Tower of London” (Shallice, 1982), and consists of two visual displays containing three colored balls placed as stacks in stockings. The goal situation (upper half of the screen) has to be copied by moving balls around in the experimental display (lower half of the screen). Items differ in the level of difficulty, as the number of moves required to copy the goal situation increases. The variable of interest is the number of problems solved with the minimum number of moves needed.

### 2.2.3 Procedure

Parents and primary caregivers of the participants were contacted by telephone to screen for possible behavioral or medical problems of the participant group. All participants were scheduled for the complete neuropsychological assessment. Four test sessions (with a duration of maximally 1.5 hours each) were scheduled for administration of the test
battery. Participants were tested individually in a quiet room. The SON-R test was administered to all participants during the first test session. CANTAB subtests were administered during the final test session on a 12-inch Paceblade Slimbook Tablet PC, running the Windows XP operating system. The remaining subtests were administered in a second and third test session. The order of test administration was equal for all participants. Based on the participants’ performance level (e.g., performance at a subtests’ floor level), assessment could be shortened to two or three sessions.

2.2.4 Data analysis

Data analysis was performed in three steps: (1) Participants performing below the subtests’ floor level were excluded from the analyses. The proportion of participants performing below floor level is presented in Table 1 for each test; (2) To determine whether the sample consisted of one or more subgroups, we performed a model-based cluster analysis (Fraley & Raftery, 2002). This analysis compares several ways of clustering by means of the Bayesian Information Criterion (BIC; Fraley & Raftery, 2003). The solution with the lowest BIC value yields the best description of the data. Model-based clustering compares solutions with different numbers of subgroups, as well as solutions with different constraints on the covariance matrices between subgroups. These constraints may both pertain to the equality of covariance matrices between subgroups as well as to the covariance structure within subgroups; (3) In order to examine relative strengths and weaknesses in FXS cognitive functioning, repeated-measures analyses of variance (ANOVA) were conducted in which all measures were contrasted to the NVMA or VMA reference measures. The cognitive profile was established by using a simple contrast method, which compares each cognitive ability to the two reference measures.

Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS Inc, Version 15.0, 2006) Greenhouse-Geisser correction was used whenever the assumption of sphericity was violated, but uncorrected dfs are reported for transparency. A Bonferroni correction was employed to
reduce the likelihood of Type 1 errors. In view of the large number of statistical tests performed, effects were considered significant at $p < .01$.

Table 1. Summary of the test battery, proportions of floor performance observed and average mental age and chronological age per subtests.

<table>
<thead>
<tr>
<th>Measures administered</th>
<th>Cognitive ability assessed</th>
<th>Descriptives</th>
<th>Floor effects</th>
<th>MA (SD)</th>
<th>CA (SD)</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Reference measures</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SON-R total score</td>
<td>Non-verbal intelligence</td>
<td></td>
<td>0 %</td>
<td>4.86 (1.32)</td>
<td>28.91 (8.70)</td>
<td>43</td>
</tr>
<tr>
<td>PPVT-III</td>
<td>Verbal Ability</td>
<td></td>
<td>0 %</td>
<td>7.87 (2.63)</td>
<td>28.91 (8.70)</td>
<td>43</td>
</tr>
<tr>
<td><strong>Non-verbal measures</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reasoning abilities</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SON-R Situations</td>
<td>Concrete reasoning</td>
<td></td>
<td>0 %</td>
<td>6.05 (2.55)</td>
<td>28.91 (8.70)</td>
<td>43</td>
</tr>
<tr>
<td>SON-R Analogies</td>
<td>Abstract sorting</td>
<td></td>
<td>0 %</td>
<td>4.11 (1.15)</td>
<td>28.91 (8.70)</td>
<td>43</td>
</tr>
<tr>
<td>SON-R Categories</td>
<td>Concrete sorting</td>
<td></td>
<td>0 %</td>
<td>5.23 (1.74)</td>
<td>28.91 (8.70)</td>
<td>43</td>
</tr>
<tr>
<td>K-ABC Conceptual Thinking</td>
<td>Concept formation</td>
<td></td>
<td>0 %</td>
<td>5.27 (1.59)</td>
<td>28.91 (8.70)</td>
<td>43</td>
</tr>
<tr>
<td>RAFT Picture Recognition</td>
<td>Visuo-perceptual recognition</td>
<td></td>
<td>0 %</td>
<td>9.61 (2.60)</td>
<td>28.91 (8.70)</td>
<td>43</td>
</tr>
<tr>
<td><strong>Performal abilities</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SON-R Puzzles</td>
<td>Concrete object assembly</td>
<td></td>
<td>0 %</td>
<td>5.53 (1.27)</td>
<td>28.91 (8.70)</td>
<td>43</td>
</tr>
<tr>
<td>SON-R Mosaics</td>
<td>Abstract object assembly</td>
<td></td>
<td>0 %</td>
<td>4.27 (1.31)</td>
<td>28.91 (8.70)</td>
<td>43</td>
</tr>
<tr>
<td>SON-R Patterns</td>
<td>Abstract drawing (copying)</td>
<td></td>
<td>0 %</td>
<td>4.48 (1.07)</td>
<td>28.91 (8.70)</td>
<td>43</td>
</tr>
<tr>
<td><strong>Verbal measures</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>TüK Word Production</td>
<td>Expressive vocabulary</td>
<td></td>
<td>4.8 %</td>
<td>8.21 (1.51)</td>
<td>28.03 (7.52)</td>
<td>41</td>
</tr>
<tr>
<td>TüK Sentence Construction</td>
<td>Grammar (sentence production)</td>
<td></td>
<td>33 %</td>
<td>6.06 (1.74)</td>
<td>28.21 (7.61)</td>
<td>29</td>
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<tr>
<td>RAFT Production of Ideas</td>
<td>Verbal fluency</td>
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<td>35 %</td>
<td>6.18 (2.67)</td>
<td>28.11 (8.59)</td>
<td>28</td>
</tr>
<tr>
<td>RAFT Telling Stories</td>
<td>Verbal association and reasoning</td>
<td></td>
<td>44 %</td>
<td>4.75 (1.61)</td>
<td>27.04 (7.45)</td>
<td>24</td>
</tr>
<tr>
<td><strong>Memory measures</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K-ABC II Number Recall</td>
<td>Verbal STM</td>
<td></td>
<td>30 %</td>
<td>3.70 (1.16)</td>
<td>28.03 (7.52)</td>
<td>30</td>
</tr>
<tr>
<td>CANTAB Spatial Span</td>
<td>Visuo-spatial STM (sequential)</td>
<td></td>
<td>53 %</td>
<td>5.50 (1.12)</td>
<td>27.55 (8.19)</td>
<td>20</td>
</tr>
<tr>
<td>K-ABC I Spatial Memory</td>
<td>Visuo-spatial STM (simultaneous)</td>
<td></td>
<td>68 %</td>
<td>6.60 (1.93)</td>
<td>26.85 (7.79)</td>
<td>13</td>
</tr>
<tr>
<td>CANTAB SWM</td>
<td>Working memory (self-ordered search)</td>
<td></td>
<td>47 %</td>
<td>5.02 (1.43)</td>
<td>26.43 (6.51)</td>
<td>23</td>
</tr>
<tr>
<td>CANTAB SRM</td>
<td>Spatial recognition memory</td>
<td></td>
<td>63 %</td>
<td>4.53 (0.59)</td>
<td>30.13 (8.97)</td>
<td>16</td>
</tr>
<tr>
<td>CANTAB PRM-I</td>
<td>Recognition memory (instant recall)</td>
<td></td>
<td>53 %</td>
<td>5.97 (1.56)</td>
<td>25.65 (7.41)</td>
<td>20</td>
</tr>
<tr>
<td>CANTAB PRM-D</td>
<td>Recognition memory (delayed recall)</td>
<td></td>
<td>44 %</td>
<td>6.42 (1.57)</td>
<td>27.79 (8.70)</td>
<td>24</td>
</tr>
<tr>
<td>RAFT Learning Names</td>
<td>Associative learning</td>
<td></td>
<td>16 %</td>
<td>7.38 (2.83)</td>
<td>27.89 (7.55)</td>
<td>36</td>
</tr>
<tr>
<td><strong>Executive Function measures</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CANTAB IED</td>
<td>Cognitive flexibility</td>
<td></td>
<td>35 %</td>
<td>7.38 (2.13)</td>
<td>27.36 (8.69)</td>
<td>28</td>
</tr>
<tr>
<td>CANTAB SOC</td>
<td>Planning</td>
<td></td>
<td>63 %</td>
<td>7.44 (3.61)</td>
<td>28.63 (8.25)</td>
<td>16</td>
</tr>
</tbody>
</table>

Note. Abbreviations: STM = short-term memory; MA = Mental age; CA = Chronological age; SD = standard deviation from the mean.

2.3 Results

2.3.1 Floor performance

Exploration of the data was conducted for all participants to ensure that statistical analyses were performed only on the data for participants performing above floor level. Table 1 presents the proportion of participants performing below floor level for each subtest.
It can be seen that performance below floor level is most frequent for STM, working memory and EF subtests. Inspection of the data indicated that none of the participants performed above floor level on all of the tests administered. The analyses were confined to the participants who performed above floor level.

2.3.2 Subgroup differences

2.3.2.1 Model-based cluster analysis
To determine whether our sample of FXS participants consisted of two or more subgroups (e.g., high- versus low-performers) a model-based cluster analysis was performed. The analysis was restricted to those subtests that provided within-range MA data for all participants (N=43); that is, Concrete Reasoning, Abstract Sorting, Concrete Sorting, Concept Formation, Visuo-Perceptual Recognition, Concrete Object Assembly, Abstract Object Assembly, Abstract Drawing, and Receptive Vocabulary (VMA). Results indicated that our sample could best be described by three distinct subgroups (BIC= -1346.88, BIC of one-group model with unrestricted covariance -1358.11). In this solution the data are modeled as uncorrelated within subgroups.

Next, we examined whether these subgroups could be differentiated in terms of level of performance, and we investigated whether the cognitive profiles differed between subgroups. Hence, two mixed design repeated measures ANOVAs were performed with Subtests (10) as within-subjects factor and Subgroup (3) as between-subjects factor. ANOVAs were performed using either NVMA or VMA as reference measure (simple contrast). Chronological age was included as a covariate to determine whether differences in functioning are better explained by chronological age than by Subgroup.

2.3.2.2 Between subgroup analysis
First, between-subject analyses were analyzed in order to determine significant differences in MA performance levels between subgroups on the subtests. Results showed a main effect of Subgroup, $F(2, 39) = 123.94, p < .0001, \eta_p^2 = .86$. MA performance levels differed significantly between subgroups for all
subtests (all p's < .01), except for Concrete Object Assembly and Abstract Sorting (no significant differences between Subgroup 2 and 3). Importantly, the subgroup differences could not be attributed to chronological age, $F(1, 39) = .41, p = .524, \eta_p^2 = .01$, In Figure 1 it can be seen that Subgroup 1 (n = 10) contained the ‘high-performing’ participants, Subgroup 2 (n = 15) the ‘intermediate-performing’ participants, and Subgroup 3 (n = 18) the ‘low-functioning’ participants.

![Figure 1. Cognitive profiles of high- (n = 10), intermediate- (n = 15) and low-performing (n = 18) FXS males. # represents no significant difference of subtest performance between intermediate- and low-performing FXS males.](image)

### 2.3.2.3 Non-verbal mental age reference analysis

The ANOVA showed a main effect of Subtests, $F(9, 351) = 6.95, p < .0001, \eta_p^2 = .15$, indicating a profile of strengths and weaknesses relative to the NVMA reference measure. For all Subgroups, relative strengths were observed for Visuo-Perceptual Recognition and VMA, whereas a relative weakness was found for Abstract Drawing (all p's < .01). The ANOVA yielded a significant interaction effect between Subgroup and Subtests, $F(168, 351) = 4.41, p < .0001, \eta_p^2 = .18$, indicating that profiles differed between subgroups. In Figure 1
it can be seen that for the low-performing subgroup a relative strength was observed for Concrete Object Assembly. For the high-performing subgroup, a relative strength was observed for Concrete Reasoning Abilities, whereas weaknesses were observed for Abstract Sorting and Abstract Drawing (all $p$’s < .01). Strengths and weaknesses in non-verbal (reasoning and performal) abilities relative to the NVMA reference are presented in Table 2 for each subgroup.

Table 2. Strengths and weaknesses in non-verbal abilities for high-, intermediate-, and low-performing FXS males, relative to NVMA and VMA.

<table>
<thead>
<tr>
<th>Measures</th>
<th>Subgroup</th>
<th>df</th>
<th>F ratio</th>
<th>Effect size $\eta^2$</th>
<th>Contrast result</th>
<th>Subgroup</th>
<th>df</th>
<th>F ratio</th>
<th>Effect size $\eta^2$</th>
<th>Contrast result</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Reasoning</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concrete reasoning (CR)</td>
<td>HP</td>
<td>2</td>
<td>8.05**</td>
<td>.29</td>
<td>CR &gt; NVMA</td>
<td>ns</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abstract reasoning (AR)</td>
<td>HP</td>
<td>2</td>
<td>7.03**</td>
<td>.27</td>
<td>AR &lt; NVMA</td>
<td>HP</td>
<td>2</td>
<td>9.74***</td>
<td>.33</td>
<td>AR &lt; VMA</td>
</tr>
<tr>
<td>Sorting (S)</td>
<td>All</td>
<td>1</td>
<td>9.91**</td>
<td>.20</td>
<td>S &gt; NVMA</td>
<td>ns</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concept formation (CF)</td>
<td>All</td>
<td>1</td>
<td>15.96***</td>
<td>.29</td>
<td>VR &gt; NVMA</td>
<td>ns</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visual recognition (VR)</td>
<td>All</td>
<td>1</td>
<td>15.96***</td>
<td>.29</td>
<td>VR &gt; NVMA</td>
<td>ns</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Performal</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concrete object assembly (COA)</td>
<td>LP</td>
<td>2</td>
<td>5.87*</td>
<td>.23</td>
<td>COA &gt; NVMA</td>
<td>HP, AP</td>
<td>2</td>
<td>12.13***</td>
<td>.38</td>
<td>COA &lt; VMA</td>
</tr>
<tr>
<td>Abstract object assembly (AOA)</td>
<td>ns</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>HP</td>
<td>2</td>
<td>7.40***</td>
<td>.28</td>
<td>AOA &lt; VMA</td>
</tr>
<tr>
<td>Abstract drawing (AD)</td>
<td>HP</td>
<td>2</td>
<td>13.79**</td>
<td>.41</td>
<td>AD &lt; NVMA</td>
<td>HP</td>
<td>2</td>
<td>9.62***</td>
<td>.33</td>
<td>AD &lt; VMA</td>
</tr>
</tbody>
</table>

Note: *significant at $p$.01, **significant at $p$.001, ***significant at $p$.0001

2.3.2.4 Verbal mental age reference analysis

The ANOVA with VMA as reference measure yielded a main effect of Subtests, $F(8, 312) = 6.81, p < .0001, \eta^2 = .15$, indicating a profile of strengths and weaknesses. For all Subgroups, significant weaknesses were observed for Abstract Object Assembly, $F(1, 39) = 11.89, p < .001, \eta^2 = .23$, Abstract Sorting, $F(1, 39) = 9.73, p < .01, \eta^2 = .19$, and Abstract Drawing, $F(1, 39) = 15.01, p <$
.0001, \( \eta^2_p = .28 \). The analysis yielded also a significant interaction between Subgroups and Subtests, \( F(16, 312) = 4.41, p < .0001, \eta^2_p = .18 \). Subsequent analyses indicated that weaknesses in Concrete Object Assembly and Concept Formation (all \( p \)'s < .0001) were only evident for high- and intermediate-functioning participants. Table 2 presents the strengths and weaknesses in non-verbal (reasoning and performal) abilities relative to the VMA reference measure for each subgroup.

In sum, the current pattern of findings indicate that both high-, intermediate- and average-functioning FXS males have cognitive profiles that are characterized by strengths in concrete reasoning, and concrete performal abilities. Weaknesses are observed for abstract reasoning, and abstract performal abilities. Interestingly, the subgroup profiling indicates that these strengths and weaknesses are more pronounced for high-functioning FXS males. Thus, not only their strengths are more pronounced, but also their weaknesses are more prominent relative to the lower functioning subgroups. Finally, separate analyses with NVMA and VMA as reference measures, demonstrated the importance of including an additional reference measure in order to avoid bias in interpreting a cognitive ability as strength vs. weakness.

2.3.3 Verbal abilities

Performance on the verbal fluency and verbal reasoning subtests yielded a considerable number of floor effects, hence valid performance for all verbal Subtests could only be established for 18 participants. Repeated measures ANOVAs were performed on the following verbal subtests as within-subjects variables: Receptive (VMA) and Expressive Vocabulary, Sentence Production, Verbal Fluency and Verbal Reasoning, again using the VMA and NVMA references as simple contrasts to determine strengths and weaknesses in verbal abilities. Results are presented in Figure 2.

**VMA reference.** The ANOVA with Subtests (4) as within-subject variables and the VMA reference as simple contrast yielded a significant main effect of Subtests, \( F(4, 68) = 21.06, p < .0001, \eta^2_p = .55 \), indicating a profile of strengths.
and weaknesses. Relative to the VMA reference, within-subject contrast analysis revealed significant weaknesses for Sentence Production, $F(1, 17) = 43.09$, $p < .0001$, $\eta^2_p = .72$, Verbal Fluency, $F(1, 17) = 8.39$, $p < .01$, $\eta^2_p = .33$, and Verbal Reasoning, $F(1, 17) = 118.96$, $p < .0001$, $\eta^2_p = .86$. The ANOVA with the VMA reference yielded no significant strengths.

![Figure 2. Strengths and weaknesses in verbal abilities of FXS males. Strengths in verbal abilities show for vocabulary capacity, whereas weaknesses show for complex verbal abilities. Verbal subtests (colored in black) are contrasted to NVMA and VMA (colored in gray). Error bars represent standard error from the mean (SEM). $n = 18. ^*p < .01; ^**p < .0001$, contrasted to NVMA #p < .01; ###p < .0001, contrasted to VMA.](image)

*NVMA reference.* Additionally, a separate ANOVA was performed with Subtests (5) as within-subject variables and the NVMA reference as simple contrast. VMA was also included in the analysis as a fifth within-subject variable. Results yielded a significant main effect of Subtests, $F(5, 85) = 15.96$, $p < .0001$, $\eta^2_p = .60$. Relative to NVMA, significant strengths were found for Receptive Vocabulary (VMA), $F(1, 17) = 87.33$, $p < .0001$, $\eta^2_p = .84$, Expressive Vocabulary, $F(1, 17) = 244.38$, $p < .0001$, $\eta^2_p = .94$, and Sentence Production, $F(1, 17) = 11.20$, $p = .004$, $\eta^2_p = .40$. The ANOVA with the NVMA reference yielded no significant weaknesses.
Current findings revealed a clear pattern of strengths and weaknesses in the verbal abilities of our FXS participants. Relative to the VMA reference, however, they showed considerable deficits on Sentence Production, Verbal Fluency and Verbal Reasoning. These apparent weaknesses were less clear when NVMA was used as a reference.

![Figure 3](image)

**Figure 3.** Strengths and weaknesses in memory performance in FXS males. A significant weakness shows for verbal STM relative to both NVMA and VMA reference measures. Memory subtests (colored in black) are contrasted to NVMA and VM (colored in gray). Error bars represent standard error from the mean (SEM) n = 16. **p** < .0001, contrasted to NVMA. ### **p** < .0001, contrasted to VMA.

### 2.3.4 Memory performance

Performance on the memory subtests was examined in 16 participants who did not show floor performance on any of these tests. The following subtests were included as within-subjects variables in the ANOVA: Verbal STM, Visuo-Spatial STM and Associative Learning. Figure 3 presents MA performance on the memory Subtests, referenced to NVMA and VMA.

**NVMA reference.** An ANOVA was performed with Subtests (3) as within-subjects variables and the NVMA reference as simple contrast. Results yielded a significant main effect for Subtests, $F(3, 45) = 21.72, p < .0001$, $\eta^2_p = .59$. A significant strength was found for Associative Learning, $F(1, 15) = 21.35,$
2. Strengths and weaknesses in cognitive abilities

$p < .0001, \eta^2_p = .59$, and a significant weakness for Verbal STM, $F(1,15) = 23.12, p < .0001, \eta^2_p = .61$.

**VMA reference.** Additionally, a separate ANOVA was performed with Subtests (3) as within-subjects variables and VMA as simple contrast. Again, results yielded a significant main effect for Subtests, $F(3, 45) = 32.62, p < .0001, \eta^2_p = .69$. A significant weakness was demonstrated for Verbal STM, $F(1, 15) = 89.74, p < .0001, \eta^2_p = .86$. Furthermore, a significant weakness was found for Visuo-Spatial STM, $F(1, 15) = 35.31, p < .0001, \eta^2_p = .70$. To examine the robustness of NVMA and VMA as reference measures, a paired samples t-test was performed to examine whether NVMA and VMA also differed in this specific sub-sample of FXS males. Results indicated a significant difference between NVMA and VMA, $t (15) = -7.55, p < .0001$. Also in this sample of FXS males, VMA is a significant strength relative to NVMA.

In sum, STM for auditory sequential information processing shows as a significant weakness within FXS memory performance when compared to both NVMA and VMA, whereas Visuo-Spatial STM only shows as a weakness when compared to VMA. Interestingly, associative learning is not a weaknesses when compared to VMA, implicating a relative strength in long-term memory performance confined to the rehearsal of ecologically relevant information.

2.3.5 **Executive function**

Performance on the EF subtests was examined in 15 participants who did not show floor effects on any of these tests. The Cognitive Flexibility and Planning subtests were both included as within-subjects variables in the ANOVA. Figure 4 presents MA performance on these EF subtests, referenced to NVMA and VMA.

**NVMA reference.** An ANOVA was performed with Subtests (2) as within-subjects variables and NVMA as simple contrast. Results yielded no significant main effect for Subtests, $F(2, 28) = 3.14, p = .06, \eta^2_p = .18$, referenced to NVMA. This finding suggests that cognitive flexibility and spatial planning abilities do not demonstrate as significant strengths or weaknesses relative to...
the NVMA reference measure. However, contrasted to NVMA, cognitive flexibility just failed to reach significance ($p = .013$).

**VMA reference.** An additional ANOVA was performed with Subtests (2) as within-subjects variables and the VMA reference as simple contrast. The analysis with VMA as reference yielded a significant main effect for Subtests, $F(2, 28) = 12.16, p < .0001, \eta^2_p = .47$. Both Cognitive Flexibility, $F(1, 14) = 19.08, p < .01, \eta^2_p = .58$, and Spatial Planning, $F(1, 14) = 16.51, p < .01, \eta^2_p = .54$, showed as significant weaknesses relative to VMA.

![Figure 4](image.png)

**Figure 4.** Strengths and weaknesses in aspects of executive functioning. Cognitive flexibility and planning abilities only show as weaknesses when contrasted to the VMA reference executive function subtests (colored in black) are contrasted to NVMA and VM (colored in gray). Error bars represent standard error from the mean (SEM) $n = 15$. ***$p < .0001$, contrasted to NVMA. $#p < .01$, contrasted to VMA.

Again, to examine the robustness of NVMA and VMA as reference measures, a paired samples t-test was performed to examine whether NVMA and VMA also differed in this specific sub-sample of FXS males. Results again yielded a significant difference between NVMA and VMA, $t (14) = -7.87, p < .0001$. Also, in this sample of FXS males, VMA can be considered as a significant strength relative to NVMA.

In sum, the analyses on the EF measures with the NVMA and VMA reference contrast further demonstrated the importance of including an additional reference measure to avoid possible interpretation bias in
interpreting strengths and weaknesses in cognitive abilities. That is, also as resulted from the EF analysis, referencing to NMVA and VMA has a differential impact on interpreting a cognitive ability as strength or weakness.

2.4 Discussion
The current study aimed at determining the specific profile of relative strengths and weaknesses of cognitive functioning in a large sample of full mutation FXS males. Our primary aim was to investigate whether subgroups could be identified in our sample of FXS males and to examine whether these subgroups would show distinguishable cognitive profiles. By means of an extensive neuropsychological assessment, we found that FXS is associated with a disharmonic cognitive profile, characterized by domain-specific strengths and weaknesses. In addition, we identified three FXS subgroups that differed in the level of cognitive performance and showed subtle, but significant differences in terms of their strengths and weaknesses profiles. The strengths in cognitive performance are related to concrete (i.e., meaningful) item content, whereas weaknesses refer to abstract item content. Subsequently, subgroup profiling in the current study leads to the notion of a robust cognitive profile in FXS males, regardless of the level of performance.

We first consider the observation of floor performance on the administrated subtests, which could provide the context for interpreting the strengths and weaknesses in FXS cognitive performance. Floor performance is likely to result from the MA range of a particular subtest or test battery. Indeed, performance at floor level was observed primarily for subtests with a relatively small MA range (e.g., 4-to-12 years), whereas subtests yielding above floor performance had larger MA ranges (e.g., 2½-to-17 years). Discarding MA range, the patterning of floor performance is relevant to the cognitive profiling of FXS. To a large extent, floor performance was related to subtests assessing complex verbal abilities (e.g., verbal fluency and verbal reasoning), STM, working memory, and aspects of executive functions. In contrast, subtests yielding above floor performance were related to non-verbal reasoning, performal abilities, visuo-perceptual recognition, and receptive vocabulary. Interestingly,
this pattern of findings is consistent with the cognitive profile obtained from above floor performance. Thus, FXS participants performed particularly poor on subtests used to assess verbal STM, visual STM, verbal fluency and verbal reasoning. These apparent weaknesses are in line with previous studies reporting disproportionate deficits in verbal and visual STM (Munir et al., 2000a) and verbal fluency and verbal reasoning (Abbeduto et al., 2007).

A major aim of the present study was cognitive sub-typing of our sample of FXS males. Model-based cluster analysis identified three subgroups corresponding to high-, intermediate-, and low-functioning individuals. These subgroups were both similar and different. They were similar in that all three subgroups could be characterized by a cognitive profile showing a pronounced dissociation between concrete versus abstract non-verbal reasoning and performal abilities. More specifically, the cognitive profile across subgroups showed significant strengths in visuo-perceptual recognition and concrete sorting abilities relative to the NVMA reference. This pattern of findings is in line with previous studies reporting relative intact visuo-perceptual abilities and processing of meaningful information (Cornish et al., 1999; Dykens et al., 1987; Maes et al., 1994). However, the subgroups were different in that the dissociation between concrete versus abstract non-verbal abilities is more pronounced for the higher-functioning FXS males. The latter finding is consistent with Spearman’s’ (Spearman, 1927) notion of ‘diminished returns’. Spearman (see Tucker-Drob, 2009) suggested that, at low ability levels, a scarcity of domain general resources constrains multiple modes of cognitive functioning but, at higher ability levels, cognitive functioning is constrained by domain-specific resources. Indeed, he observed that the mean correlation between ability tests was higher for lower-functioning children compared to typically functioning children. More recently, Anderson (1992, 2001) submitted the hypothesis that independent cognitive modules contribute differentially to various demands of intellectual functioning but, as the functioning of each of these modules is constrained by a single basic processing mechanism, the performance across different domains is correlated. Cognitive modules are less constrained in individuals having a more efficient basic processing mechanism.
at their disposal and, thus, in those individuals performance levels across
cognitive domains become less correlated. Interestingly, Anderson (1992)
assumed that basic processing speed would qualify as the limiting basic
processing speed. Within this context, the higher peaks and deeper troughs in
the cognitive profile of the relatively higher functioning subgroup of FXS
participants in the current study would be interpreted to suggest that their
basic processing mechanism is functioning more efficiently (i.e., faster). This
interpretation seems to mesh rather nicely with neurobiological evidence
suggesting that various genetically related types of mental retardation arise
from abnormal neural network formation during early development, which
results in abnormal neural connectivity and, thus, inefficient information
processing (Ramakers, 2002; Van Galen & Ramakers, 2005; Vanderklish &
Edelman, 2005). Obviously, more work is needed to systematically assess our
speculative account of the subtle differences in cognitive profiles between the
FXS subgroups.

The current study contributes to the recent literature indicating that
FXS males perform particularly poor on tasks with a high demand on executive
control (see Hooper et al., 2008, for a review), FXS males performed poorly on
verbal fluency and verbal reasoning tasks. These findings are in accord with
previous studies reporting deficits in verbal fluency and verbal reasoning
abilities (Abbeduto et al., 2007; Abbeduto et al., 2003; Roberts, Mirrett, &
Burchinal, 2001). Verbal fluency is generally referred to as the ability to
generate novel verbal responses, and the ability to switch between semantic or
phonemic categories (Turner, 1999). Furthermore, it has been argued that
verbal fluency tasks require the ability to successful inhibit inappropriate
verbal responses and to update verbal responses (Henry & Crawford, 2004). In
this regard, weaknesses in verbal fluency observed in the present study are
interpreted in terms of deficits in executive control processes involved in
regulating and generating accurate verbal responses. Similarly, impairments in
verbal reasoning have been argued to depend more on higher-order effortful
selection and organization of ideas, rather than automatized, easy selective
processes (Van der Sluis, De Jong, & Van der Leij, 2007).
Second, the current pattern of findings point to serious deficits in STM and working memory processes in our sample of FXS participants. This finding is consistent with previous observations that performance on working memory tasks is particularly weak in FXS males (Munir et al., 2000a). Tasks in the current study required the ability to update information with new incoming information, as well as the ability to employ an effective, self-ordered search strategy to keep track of items previously inspected. Updating and self-ordered search are frequently postulated to depend on executive control (Baddeley, 1996; Miyake et al., 2000; Tubau, Hommel, & Lopez-Moliner, 2007). The current findings revealed that associative learning ability for concrete information was relatively spared in our sample of FXS males. The associative learning task used in the current study required successful encoding and retrieval of familiar verbal labels which corresponded to concrete visual stimuli (i.e., pictures of cats and butterflies). Recently, it has been suggested that encoding processes recruited during associative learning can be considered as distinct cognitive processes from those recruited during working memory tasks (Kaufman, DeYoung, Gray, Brown, & Mackintosh, 2009). In addition, it has been suggested that associative learning and working memory rely on different neural substrates (Ranganath, 2006). Subsequently, it could be argued that associative learning imposes less demand on executive control than working memory processes.

Third, assuming that executive control is deficient in FXS males, our participants should perform poorly on executive function tasks. The current test battery included two aspects of executive function, Cognitive Flexibility and Spatial Planning. Consistent with previous studies examining executive functioning in FXS males (Hooper et al., 2008; Kogan et al., 2009; Loesch et al., 2003; Munir et al., 2000b; Woodcock et al., 2009) the current results showed poor performance on both tests, but only when referenced to VMA. When NVMA was taken as a reference, performance on Cognitive Flexibility and Spatial Planning was not particularly poor. It should be noted, however, that the IED test used for examining cognitive flexibility consists of two parts requiring intra- vs. extra-dimensional shifts, respectively. Intra-dimensional
shifting requires visuo-perceptual abilities (e.g., object recognition, stimulus discrimination) that have been observed to be relatively spared in the current sample of FXS males. In contrast, extra-dimensional shifts have been argued to involve shifting between contextual or cognitive sets (Geurts, Corbett, & Solomon, 2009; Ravizza & Carter, 2008) and, thus, impose a higher demand on executive control (Miyake et al., 2000). Only few participants were able to complete this part of the IED task, which is consistent with the alleged executive control deficit in FXS males. In regard to the Spatial Planning task, it should be noted that only 37% of the participants were able to complete this task. This proportion is similar to the proportions that Hooper et al. (2008) observed for FXS boys performing on planning tasks. The failure of FXS individuals to complete planning tasks is interpreted to suggest deficient executive control (see also Kogan et al., 2009; Loesch et al., 2003; Munir et al., 2000b).

Finally, the observation that performance on the executive function tasks is poor when performance on these tasks is referenced to VMA but not NVMA points to the importance of selecting the appropriate reference when examining strengths and weaknesses in FXS individuals. The current study indicates that the reference issue goes beyond the domain of executive functioning. That is, taking VMA as a reference, performance on tasks requiring non-verbal reasoning, performat abilities, working memory or executive function was particularly weak in our sample of FXS participants. Taking NVMA as a reference, the current findings showed relative strengths for expressive vocabulary and simple grammatical abilities. Contrasted to VMA, however, these abilities showed up as weaknesses. In this regard, the use of VMA as a reference may underestimate the “true” level of functioning of FXS individuals, whereas the use of NVMA may overestimate their abilities. Obviously, when studying strengths and weaknesses in cognitive functioning, errors related to over- and underestimation biases are prevalent (cf. Burack, Larocci, Bowler, & Mottron, 2002; Mottron, 2004). Given the absence of a golden standard, it is recommended here to use both VMA and NVMA as a safeguard against estimation biases.
In conclusion, the present study contributes to the existing FXS literature by reporting four main findings: (1) the cognitive profiling of FXS resulted in three distinct subgroups, which differed in terms of performance level; (2) the cognitive profiles of these subgroups are both similar and subtly different. Strengths refer to concrete information processing and weaknesses to abstract information processing. Both strengths and weaknesses were somewhat more pronounced for the subgroup functioning at a relatively higher level; (3) to a large extent, deficits in cognitive abilities are dependent on executive control processes. This finding contributes to the recent literature suggesting a fundamental deficiency of higher-level cognitive processes; (4) the present study illustrated the importance of choosing an appropriate reference in order to determine strengths and weaknesses in cognitive performance. Future studies should aim at teasing apart these higher-level executive control processes from lower-level cognitive processes, and subsequently relate them to underlying neural mechanisms. Such studies should take advantage of electrophysiological and brain-imaging techniques. In combination with neurobiological investigations on the determinants of abnormal FXS brain development, the specific cognitive profile associated with FXS could then be further defined in terms of an endophenotype.
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 Barcëlo 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 IED1. 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 &
Profiling cognition in fragile X syndrome

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
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.

<table>
<thead>
<tr>
<th></th>
<th>FXS</th>
<th>DS</th>
<th>MA</th>
<th>CA</th>
<th>Significant difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participants (n)</td>
<td>27</td>
<td>20</td>
<td>40</td>
<td>31</td>
<td>n.s.</td>
</tr>
<tr>
<td>Age in years</td>
<td>27.81 (7.08)</td>
<td>22.42 (3.35)</td>
<td>5.75 (1.15)</td>
<td>27.26 (11.32)</td>
<td>MA* &lt; FXS, DS, CA</td>
</tr>
<tr>
<td>Gender (Male/Female)</td>
<td>27/0</td>
<td>10/10</td>
<td>22/18</td>
<td>17/14</td>
<td>FXS*</td>
</tr>
<tr>
<td>SON-R Mental age</td>
<td>5.54 (1.17)</td>
<td>5.66 (1.08)</td>
<td>-</td>
<td>-</td>
<td>n.s.</td>
</tr>
<tr>
<td>Raven Percentile</td>
<td>-</td>
<td>-</td>
<td>77.63 (17.2)</td>
<td>69.29 (21.1)</td>
<td>n.s.</td>
</tr>
<tr>
<td>IED stages completed</td>
<td>6.85 (1.51)</td>
<td>7.80 (0.95)</td>
<td>7.53 (0.78)</td>
<td>8.74 (0.68)</td>
<td>CA &gt; FXS, DS, MA****</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>DS &gt; FXS*</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Stage</th>
<th>FXS</th>
<th>MA</th>
<th>DS</th>
<th>CA</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M (SEM)</td>
<td>M (SEM)</td>
<td>M (SEM)</td>
<td>M (SEM)</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trials (n)</td>
<td>10.37 (1.16)</td>
<td>10.15 (0.93)</td>
<td>8.65 (1.02)</td>
<td>6.65 (0.13)</td>
<td>FXS &gt; CA</td>
</tr>
<tr>
<td>errors (n)</td>
<td>1.70 (0.62)</td>
<td>1.63 (0.47)</td>
<td>0.76 (0.37)</td>
<td>0.06 (0.04)</td>
<td>FXS &gt; CA</td>
</tr>
<tr>
<td>Participants (n)</td>
<td>27</td>
<td>40</td>
<td>20</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>SD-R</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trials (n)</td>
<td>12.04 (1.13)</td>
<td>11.15 (0.82)</td>
<td>9.80 (1.42)</td>
<td>7.77 (0.32)</td>
<td>FXS &gt; DS, CA</td>
</tr>
<tr>
<td>errors (n)</td>
<td>4.19 (0.66)</td>
<td>2.38 (0.28)</td>
<td>2.80 (1.11)</td>
<td>1.26 (0.12)</td>
<td>FXS &gt; MA, DS, CA</td>
</tr>
<tr>
<td>Participants (n)</td>
<td>27</td>
<td>40</td>
<td>20</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>CD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trials (n)</td>
<td>13.52 (2.14)</td>
<td>9.03 (0.61)</td>
<td>7.10 (0.42)</td>
<td>7.74 (0.55)</td>
<td>FXS &gt; DS, CA</td>
</tr>
<tr>
<td>errors (n)</td>
<td>3.00 (1.00)</td>
<td>1.20 (0.24)</td>
<td>0.65 (0.25)</td>
<td>0.71 (0.21)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Participants (n)</td>
<td>27</td>
<td>40</td>
<td>20</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>CD-I</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trials (n)</td>
<td>10.19 (1.32)</td>
<td>8.23 (1.00)</td>
<td>6.15 (0.11)</td>
<td>6.06 (0.04)</td>
<td>FXS &gt; DS, CA</td>
</tr>
<tr>
<td>errors (n)</td>
<td>1.73 (0.57)</td>
<td>1.00 (0.48)</td>
<td>0.20 (0.12)</td>
<td>0.13 (0.08)</td>
<td>FXS &gt; DS, CA</td>
</tr>
<tr>
<td>Participants (n)</td>
<td>27</td>
<td>40</td>
<td>20</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>CD-R</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trials (n)</td>
<td>16.50 (2.49)</td>
<td>11.30 (1.54)</td>
<td>11.15 (1.97)</td>
<td>7.42 (0.23)</td>
<td>FXS &gt; MA, DS, CA</td>
</tr>
<tr>
<td>errors (n)</td>
<td>5.69 (1.17)</td>
<td>2.50 (0.63)</td>
<td>3.00 (1.97)</td>
<td>1.10 (0.10)</td>
<td>FXS &gt; MA, DS, CA</td>
</tr>
<tr>
<td>Participants (n)</td>
<td>27</td>
<td>40</td>
<td>20</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>ID</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trials (n)</td>
<td>9.13 (1.43)</td>
<td>8.68 (0.63)</td>
<td>9.40 (1.11)</td>
<td>6.39 (0.12)</td>
<td>FXS &gt; CA</td>
</tr>
<tr>
<td>errors (n)</td>
<td>1.43 (0.26)</td>
<td>1.33 (0.21)</td>
<td>1.30 (0.40)</td>
<td>0.35 (0.10)</td>
<td>FXS &gt; CA</td>
</tr>
<tr>
<td>Participants (n)</td>
<td>23</td>
<td>40</td>
<td>20</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>ID-R</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trials (n)</td>
<td>13.55 (2.52)</td>
<td>10.45 (0.92)</td>
<td>11.85 (1.84)</td>
<td>7.23 (0.26)</td>
<td>FXS &gt; CA</td>
</tr>
<tr>
<td>errors (n)</td>
<td>4.55 (1.32)</td>
<td>2.13 (0.28)</td>
<td>3.30 (0.90)</td>
<td>17.74 (2.66)</td>
<td>FXS &gt; CA</td>
</tr>
<tr>
<td>Participants (n)</td>
<td>22</td>
<td>40</td>
<td>20</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>ED</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trials (n)</td>
<td>39.71 (3.38)</td>
<td>40.60 (2.35)</td>
<td>39.25 (3.61)</td>
<td>17.74 (2.66)</td>
<td>FXS &gt; CA</td>
</tr>
<tr>
<td>errors (n)</td>
<td>20.57 (2.12)</td>
<td>26.07 (2.99)</td>
<td>20.05 (2.31)</td>
<td>5.80 (1.29)</td>
<td>FXS &gt; CA</td>
</tr>
<tr>
<td>Participants (n)</td>
<td>21</td>
<td>14</td>
<td>9</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>ED-R</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trials (n)</td>
<td>27.86 (2.59)</td>
<td>26.07 (2.99)</td>
<td>15.44 (2.01)</td>
<td>6.22 (0.16)</td>
<td>FXS &gt; DS, CA</td>
</tr>
<tr>
<td>errors (n)</td>
<td>22.14 (2.59)</td>
<td>19.07 (2.77)</td>
<td>12.89 (3.28)</td>
<td>1.22 (0.14)</td>
<td>FXS &gt; CA</td>
</tr>
<tr>
<td>Participants (n)</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>27</td>
<td></td>
</tr>
</tbody>
</table>

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.
3. Attentional set-shifting in fragile X syndrome

**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.

![Graphs showing the 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.]

**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. The ANOVA yielded main effects for Error type, $F(2, 216) = 83.22, \ p < .0001, \ \eta^2 = .44$, and Stage, $F(7, 756) = 38.04, \ p < .0001$. Errors committed on the first trial of a reversal stage and were similar between groups per IED stage (all $p$'s < .05).

---

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 Bonferroni 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 $\Lambda = .17, \chi^2(72) = 168.80, p < .0001$. 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.

![Discriminant Functions](image)

**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).

<table>
<thead>
<tr>
<th></th>
<th>FXS</th>
<th>MA</th>
<th>DS</th>
<th>CA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronological Age</td>
<td>-0.09</td>
<td>0.06</td>
<td>0.04</td>
<td>0.10</td>
</tr>
<tr>
<td>Intellectual Level</td>
<td>0.49**</td>
<td>0.04</td>
<td>0.08</td>
<td>0.24</td>
</tr>
</tbody>
</table>

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;
Profiling cognition in fragile X syndrome

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; Hoefl et al., 2010; Hoefl et al., 2007; Hoefl 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.
Chapter 4

Auditory change detection in fragile X syndrome:

A brain potential study

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Abstract

Objective: The present study investigated involuntary change detection in a two-tone pre-attentive auditory discrimination paradigm in order to better understand the information processing mechanisms underlying attention deficits in FXS males.

Method: Sixteen males with the FXS full mutation and 20 age-matched control participants (mean age 29 years) were presented with series of auditory stimuli consisting of standard and deviant tones while watching a silent movie.

Results: Brain potentials recorded to the tones showed that N1 and P2, sensory evoked potentials, were significantly enhanced in FXS compared to age-matched control participants. Both mismatch negativity and P3a generation, reflecting automatic change detection and the involuntary switch of attention, respectively, were significantly attenuated in FXS males.

Conclusions: The current study demonstrates that auditory stimulus discrimination in the FXS brain is already compromised during the pre-attentive stages of information processing. Furthermore, the apparent deficit in pre-attentive information processing deficiencies in FXS coincides with failure in the involuntary engagement of attentional resources

Significance: The stimulus-driven information processing deficiencies in FXS might compromise information processing in several domains and, thus, present a key-deficit in FXS neurocognition.
4.1 Introduction

Fragile X Syndrome (FXS) is the most common inherited type of intellectual disability, with a prevalence of approximately 1 to 4000 in males and 1 to 8000 in females (O'Donnell & Warren, 2002). Males with FXS typically show a global reduction in cognitive performance with a distinct profile of disproportionate weaknesses in several cognitive abilities (Hagerman & Hagerman, 2002; Hodapp & Dykens, 2001; Van der Molen et al., 2010). Additionally, FXS males show striking attentional deficits, including an impairment in inhibiting prepotent responses (Cornish, Munir, & Cross, 2001; Scerif, Cornish, Wilding, Driver, & Karmiloff-Smith, 2007; Wilding, Cornish, & Munir, 2002) reduced sustained, focused and divided attention, and problems with switching attention from one concept to another (Cornish et al., 2001; Munir, Cornish, & Wilding, 2000). To date, however, the basic mechanisms underlying these attentional deficits in the FXS brain remain poorly understood.

To understand attentional dysfunction in FXS at the behavioral level it is important to appreciate the basic neurobiological and neuronal developmental processes that characterize FXS. Males with the FXS full mutation exhibit an abnormal expansion of trinucleotide (CGG) repeats in the 5’-untranslated promoter region of the Fragile X Mental Retardation 1 (FMR1), which causes transcriptional silencing of the FMR1 gene (Oostra & Chiurazzi, 2001; Verkerk et al., 1991). This FMR1 gene-silencing results in reduced or absent expression of the FMR1 protein (FMRP) (Pieretti et al., 1991), which is involved in protein-synthesis dependent synaptic plasticity and dendritic refinement (Greenough et al., 2001; Irwin et al., 2001), key neuronal processes in early brain development. Additionally, these FMRP dependent neuronal processes are regulated by a subset of metabotropic glutamate receptors (Huber, 2007; Huber, Gallagher, Warren, & Bear, 2002; Pfeiffer & Huber, 2007), which are important regulators of excitatory synaptic inputs in the brain. In FXS, both excitatory (glutamatergic) and inhibitory (GABAergic) neurotransmitter systems are shown to be altered due to FMR1 silencing (Bear, Huber, & Warren, 2004; D’Hulst & Kooy, 2007) and have been linked to altered
circuit plasticity and neuronal hyperexcitability in the FXS brain (Gibson, Bartley, Hays, & Huber, 2008).

Neuronal hyperexcitability in FXS is indicated by epileptic seizures and/or hypersensitivity to sensory stimulation, as reflected by augmented sensory evoked potentials (Ferri et al., 1994; Musumeci et al., 1991; Musumeci et al., 1999). Although reported for all sensory modalities (Hagerman & Hagerman, 2002; Miller et al., 1999), hypersensitivity occurs most notably for auditory stimulation in FXS males (Castrén, Paakkonen, Tarkka, Ryynanen, & Partanen, 2003; Frankland et al., 2004; Hesl et al., 2009; Rojas et al., 2001) as well as in the FMR1 knockout mouse. Additionally, a defect in prepulse inhibition, a sensorimotor index of sensory gating, has been demonstrated in both FXS humans (Frankland et al., 2004; Hesl et al., 2009) and the FMR1 knockout mouse (Chen & Toth, 2001). Together, these findings implicate deficiencies in basic information processing, which could contribute to the cognitive deficits in FXS males observed at the behavioral level.

Hypersensitivity to sensory stimuli may have adverse effects on subsequent information processing, thereby compromising attention and cognitive function in a bottom-up fashion. As cognition and behavior arise from complex transformations of sensory input (Mesulam, 1998), it could be hypothesized that, due to altered neuronal excitability (Gibson et al., 2008), inefficient sensory gating processes (Frankland et al., 2004; Hesl et al., 2009) obstruct the sensory input necessary for directing attention to changes in the environment. Change detection within the environment is considered to be an essential process for adaptive behavior, as acting upon a novel event depends on the demands of the current situation (Posner & Petersen, 1990; Sokolov, 1963). In order to better understand potential deficits in basic information processing, the present study investigated auditory change detection processes in FXS males by means of event-related brain potentials (ERPs).

Few studies have addressed the psychophysiological aspects associated with stimulus representation in the FXS brain. Castrén and co-workers (2003) recorded ERPs in a sample of four boys and one adult male with FXS using a two-tone passive auditory oddball paradigm, from which only
ERPs to standard stimuli were reported. The authors reported augmented N1 responses to standard stimuli, as well as a lack of habituation of the N1 response to stimulus repetition in the FXS group. N1 augmentation in FXS males was corroborated with a single-tone paradigm using magnetoencephalography by Rojas et al. (2001), and is suggested to indicate increased sensory sensitivity for auditory stimuli in FXS, as well as an increased number of active cells generating the N1 in FXS (Castrén et al., 2003). Subsequently, N1 dishabituation to auditory stimulus repetition could interfere with establishing of a memory template created by the repetitive aspects of the standard stimulus (Näätänen, 2008; Näätänen, Jacobsen, & Winkler, 2005; Näätänen, Paavilainen, Rinne, & Alho, 2007; Näätänen & Winkler, 1999). This implicates abnormal processing of auditory sensory information, which could negatively influence stimulus discrimination and classification processes. To date, however, information-processing characteristics related to automatic change detection in the FXS brain remain to be investigated.

In the present study we used a passive auditory oddball paradigm to investigate whether pre-attentive change detection would be impaired in FXS males in comparison to a chronologically age-matched control group. The following ERP components were investigated: (1) The N1 and P2, peaking at respectively 100 and 200 ms after stimulus onset and are both associated with sensory aspects of stimulus representation in the brain (Näätänen & Picton, 1987). (2) The mismatch negativity (MMN), an index of early sensory change detection and sensory memory (Näätänen, Kujala, & Winkler, 2011; Näätänen et al., 2007; Näätänen, Paavilainen, Tiitinen, Jiang, & Alho, 1993). The MMN can be best seen in the deviant minus standard ERP difference wave and peaks between 100-200 ms after stimulus onset (Escera, Alho, Winkler, & Näätänen, 1998; Escera, 2007) (3) The N2b and P3a components peaking approximately between 200-350 ms after stimulus onset, which are associated with the involuntary triggering of attention to changes in the environment (Näätänen et al., 2007; Polich, 2007).

We hypothesized that, in contrast to controls, FXS males would show augmented N1 responses to standard stimuli (Castrén et al., 2003; Rojas et al.,
2001). Consistent with the reported habituation deficit in FXS (Castrén et al., 2003), we hypothesized that N1 responses to deviant stimuli would not differ from those elicited by standard stimuli. In addition, we expected that deviance detection would be impaired due to the augmented sensory components of the auditory ERP. Therefore, the MMN was expected to be smaller in FXS males than in controls. Finally, as the MMN is a marker of automatic deviancy detection (Escera, 2007; Näätänen et al., 2011), we expected that reduced MMN would also impair P3a generation in FXS. Therefore, we examined whether smaller MMN amplitudes in FXS were related to smaller P3a amplitudes

4.2 Methods and Materials

4.2.1 Participants

Sixteen male participants diagnosed with the FXS full mutation (mean age = 29.6 years, range = 18-42 years) and 20 healthy age-matched male control participants (mean age = 29.2 years, range 19-47 years) participated in this study. Non-verbal intelligence was assessed using the Raven Standard Progressive Matrices (Raven & Court, 1998). Raw scores were significantly lower in FXS males (mean = 19.88, SD = 8.15) than in control participants (mean = 55.65, SD= 3.76). Average Raven-IQ of the control participants was 121.5 (SD = 25.79), t(34) = 17.49, p < .0001. IQ of the FXS participants was equivalent to an average mental age of 7.73 years (SD = 1.59).

FXS participants were recruited with the help of the Dutch Fragile X Parent Network. Control participants were recruited from or within the proximity of the university, and received course-credit or a monetary compensation. None of the participants were on medication during the experiment. All participants reported intact hearing and had normal or corrected-to-normal vision. Signed informed consent was obtained prior to the experiment from control participants. For the FXS participants, the pertinent information was obtained from parents or primary caregivers. The protocol for this study was reviewed and approved by the ethical review committee of the University of Amsterdam.
4.2.2 Stimuli and design
Stimuli were generated using Tone Generator software of NCH (http://nch.com.au). Stimuli were 75 ms 1000 Hz and 1500 Hz sinusoidal tones with 5 ms rise and fall times. Stimuli were presented through stereo headphones (Sennheiser, HD-201) at 80dB SPL. The inter-stimulus-interval was 1000 ms. For half of the participants, the 1000 Hz tone was standard and the 1500 Hz tone was deviant (and vice versa for the other half). Standard and deviant tones were presented pseudo randomly with the restriction that deviant tones were separated by at least two standard tones. The probability of standard and deviant tones was 90% and 10%, respectively. A single sequence of 1000 stimuli was presented to the participants.

Participants were seated at approximately 70 cm from a 17-inch laptop screen (Dell Latitude D530). All participants watched a silent movie (Shrek 2: Adamson, 2004) and were told that the auditory stimuli were irrelevant. Participants were asked to avoid movements and to minimize eye movements and blinks. The participant was visually observed at all times to minimize movement artifacts. The duration of the task was approximately 18 minutes. The passive oddball experiment was carried out during a single session. EEG recordings from the FXS participants and half of the number of control participants were performed at home locations. Electronic devices not used for EEG recordings were turned off. The out-of-lab recordings were performed in a quiet room with dimmed lights. The results of out-of-lab recordings of control participants did not differ from those obtained in the lab (all p's > .10).

4.2.3 EEG recording
EEG was recorded using an EasyCap electrode cap with 28 Ag/AgCl sintered ring electrodes using the 10/20 system placement. Recorded electrode positions included: Fp1, Fp2, F7, F3, Fz, F4, F8, FC1, FCz, FC2, FC6, T7, C3, Cz, C4, T8, TP9, CP1, CP2, TP10, P7, P3, Pz, P4, P8, O1, Oz, and O2. Electrodes placed at the left and right mastoids were used for linked reference. The ground was placed at FT9. Horizontal eye movements (HEOG) were recorded using bipolar electrodes placed at the outer canthi of the eyes. Vertical eye movements
(VEOG) were placed just above and under the left eye. Electrode impedances were kept below 10 kΩ. Signals were recorded with a BrainAmp DC amplifier (Brain Products) using Brain Vision Recorder software, at a sampling rate of 500 Hz and an online filter between 0.3 and 70 Hz.

Continuous EEG was bandpass filtered offline at 1-20 Hz (Duncan et al., 2009) using Brain Vision Analyzer software (Version 1.05, © Brainproducts), and Matlab (Version 7.7.0, © Mathworks). Ocular artifacts were removed from the EEG (Gratton, Coles, & Donchin, 1989). Subsequently, 650 ms epochs were created time-locked to the onset of the stimulus, including a 50 ms pre-stimulus interval (Duncan et al., 2009). Epochs were removed when exceeding a threshold of ± 50 μV at any electrode site or with a voltage step exceeding 50μV per sampling point. This resulted in a total of 895/99 (standard/deviant) and 892/99 (standard/deviant) artifact free trials in controls and FXS respectively, \( R(1, 34) = 1.51, p > .05 \). Subsequently, ERPs were baseline corrected using the 50 ms pre-stimulus interval and averaged for standards and deviants (Duncan et al., 2009).

Peak amplitude and latency of the N1, P2, MMN, N2b, and P3a components were determined at Fz, FCz, Cz, Pz, and Oz. The N1 was quantified as the most negative peak within the 80-140 ms latency range following a tone (Cowan, Winkler, Teder, & Näätänen, 1993; Näätänen et al., 2005). The time at which this negative peak occurred was taken as the N1 latency. Peak amplitude and latency of the P2 were determined within the 120-200 ms latency range (Näätänen & Picton, 1987). The MMN was determined within the 100-200 ms latency range of the ERP difference waves, obtained by subtracting the ERP waveforms to standards from those to deviants (Duncan et al., 2009; Näätänen et al., 2007). The time at which the most negative peak occurred after the N1 was taken as the MMN latency. The N2b was defined as the most negative peak occurring after the P2 and before the P3a, within the 200-350 latency range (Näätänen & Picton, 1987). Finally, the P3a was defined as the most positive peak within the 220-400 interval (Escera et al., 1998).
4.2.4 Statistical Analysis
Repeated measures Analyses of Variance (ANOVA) were performed with Tone (two levels: Standard, Deviant), Laterality (three levels: Left, Midline, Right), and Site (five levels: Fz, FCz, Cz, Pz, Oz), as within-subjects factors and Group (FXS and Control) as between-subjects factor. MMN peak data were submitted to repeated measures ANOVAs with Site as within-subjects factors and Group as between-subjects factor. To test whether the magnitude of the MMN could successfully predict the magnitude of the P3a component, linear regression analyses were performed with peak MMN amplitude as predictor variable (independent) and peak P3a amplitude as dependent variable. Analyses were performed using Statistical Package for Social Sciences (SPSS Inc, 2008). Greenhouse-Geisser correction was applied, where appropriate, and alpha was set at .05. Additional post-hoc significance testing was performed using Bonferroni correction.

4.3 Results
4.3.1 N1 amplitude
Figure 1 depicts the grand averaged ERP waveforms for both groups elicited by standard and deviant tones, as well as the deviant minus standard ERP difference wave, recorded at the left and right lateral and the midline electrode sites. The ANOVA yielded significant main effects for Tone, $F(1, 34) = 19.71, p < .0001, \eta^2 = .37$, Site, $F(4, 136) = 126.31, p < .0001, \eta^2 = .79$, and Group, $F(1, 34) = 8.73, p < .01, \eta^2 = .20$. The significant Tone by Group interaction, $F(1, 34) = 4.45, p < .05$, $\eta^2 = .12$, showed that N1 amplitudes to standard tones were significantly larger in FXS males than in controls (mean difference = -2.09 $\mu$V). As can be seen in Figure 2, N1 amplitudes in controls were significantly larger for deviant tones than for standard tones (mean difference = -1.45 $\mu$V), whereas in FXS males the difference between tones (mean difference = -0.52 $\mu$V) failed to reach significance ($p > .05$). The significant Site by Group interaction, $F(4, 136) = 10.31, p < .0001, \eta^2 = .23$, showed that N1 amplitudes were significantly larger at the frontocentral sites (Fz, FCz, Cz) in FXS males relative to controls ($p's < .05$). N1 peaked at FCz in both groups.
Figure 1. Grand average ERP waveforms to standard and deviant tones and the difference waveforms are displayed of control (A) and FXS participants (B) are presented for the midline (Fz, FCz, Cz, Pz), lateral (F3, F4, C3, C4), electrode Sites. ERPs at Cz are enlarged for control (C) and FXS participants (D). Negative is plotted upward.
4.3.2  

**N1 latency**

No significant N1 latency differences were found at FCz (all $p$’s > .05).

![Figure 2](image)

**Figure 2.** Peak N1 amplitude (A) and latency (B) at FCz with corresponding voltage maps (C) for standards and deviants tones are presented for FXS males and control participants. Error bars represent standard error of the mean. Asterisks (*) represent significant within-group differences at $p < .05$.

4.3.2  

**P2 Amplitude**

The ANOVA yielded significant main effects for Site, $F(4, 136) = 22.28, p < .0001, \eta^2 = .40$, and Group, $F(1, 34) = 30.09, p < .0001, \eta^2 = .47$, and a significant interaction between these effects, $F(4, 136) = 9.08, p < .01, \eta^2 = .21$. At all sites, P2 amplitudes were significantly larger in FXS than in control participants to both standard (mean difference = 1.97 $\mu$V) and deviant stimuli (mean difference = 3.42 $\mu$V). As shown in Figure 1, P2 amplitudes were largest at Cz and smallest at Oz. This difference was significant in FXS males (mean difference = 2.45 $\mu$V) but not in controls (mean difference = 0.61 $\mu$V). As can be seen in Figure 3, the significant Tone by Group interaction $F(1, 34) = 10.16, p < .01, \eta^2 = .23$, revealed that deviant stimuli elicited smaller P2 amplitudes than
standards in controls ($p < .05$). In FXS males, however, P2 amplitude did not discriminate between tones ($p = .73$).

**Figure 3.** Peak P2 amplitude (A) and latency (B) at Cz with corresponding voltage maps (C) for standards and deviants tones are presented for FXS males and control participants. Error bars represent standard error of the mean. Asterisks (*) and hash symbols (#) represent significant within- and between-group differences at $p < .05$, respectively.

### 4.3.3 P2 Latency

P2 latency at Cz is depicted in Figure 3 for both groups. The ANOVA on P2 latency at Cz failed to show significant effects (all $F$ values $< 1.00$).

### 4.3.5 MMN Amplitude

The ANOVA on MMN amplitude yielded main effects of Site, $F(4, 136) = 18.28, p < .0001$, $\eta^2 = .35$, and Group, $F(1, 34) = 9.20, p < .01$, $\eta^2 = .21$, which were included in the significant Site by Group interaction, $F(4, 136) = 9.28, p = .01$, $\eta^2 = .21$. MMN amplitudes were larger in controls than in FXS males and this difference reached significance ($p$'s $< .05$) at Cz (mean difference = -1.40 $\mu$V), Pz (mean difference = -1.15 $\mu$V), and Oz (mean difference = -1.64 $\mu$V). MMN amplitude was largest at Cz in controls (mean = -2.95 $\mu$V) and at Fz in FXS
males (mean = -1.77 μV). Peak MMN amplitude for both groups is presented in Figure 4.

Figure 4. Difference waveforms (A) are presented for those electrode sites showing maximum MMN amplitude (Cz for controls and Fz for FXS males, negative is plotted upward). Corresponding voltage maps (B) are presented for MMN peak latencies.

4.3.6 MMN Latency

MMN latency for the sites showing peak MMN amplitudes is depicted in Figure 4. Mean MMN latencies were somewhat longer in controls (mean = 158.56 ms) than in FXS males (mean = 148.18), but this difference failed to reach significance (p > .05).

4.3.7 N2b amplitude

Peak N2b amplitude in both groups is presented in Figure 5. The ANOVA showed a main effect of Site, F(4, 136) = 4.09, p < .05, η² = .11, which was included in the significant Site by Group interaction, F(4, 136) = 4.19, p = .02, η² = .11. N2b amplitudes peaked at Oz in controls (mean = -0.61 μV) and at FCz in FXS (mean = -1.63 μV). Subsequent analysis indicated that N2b peak amplitude was larger in FXS males than in controls for both standard and deviant stimuli (p’s < .05). Further, N2b peak amplitude was larger to deviants than standard
tones in controls ($p < .05$), but this difference failed to reach significance in FXS males ($p = .07$).

![Figure 5](image)

**Figure 5.** Peak N2b amplitude and latency is presented at Oz (controls) and FCz (FXS) with corresponding voltage maps (C) for FXS males and control participants. Error bars represent standard error of the mean. Asterisks (*) and hash symbols (#) represent significant within- and between-group differences at $p < .05$, respectively.

### 4.3.8 N2b latency

N2b latency at the sites showing peak amplitude are depicted in Figure 5. The ANOVA yielded main effects for Tone, $F(1, 34) = 15.58, p < .0001, \eta^2 = .31$, and Group $F(1, 34) = 14.64, p = .001, \eta^2 = .30$. In both groups, N2b latencies were significantly shorter for deviant than for standard tones ($p$'s < .05). Further, N2b latencies were significantly longer in FXS males than in controls to both standard (mean difference = 42.35 ms) and deviant (mean difference = 22.75 ms) tones ($p$'s < .05).

### 4.3.9 P3a amplitude

Peak P3a amplitude is presented in Figure 6. Results showed a main effect of Tone, $F(1, 34) = 67.57, p < .0001, \eta^2 = .67$, which revealed that deviant tones
elicited larger P3a amplitudes than standard tones in both controls (mean difference = 2.21 μV) and FXS males (mean difference = 2.12 μV). In addition, a main effect was observed for Site, $F(4, 136) = 19.08, p < .0001, \eta^2 = .36$, which was included in a significant Group by Laterality by Site interaction, $F(8, 272) = 5.11, p = .001, \eta^2 = .13$. In both groups, P3a amplitudes peaked at the central electrode leads ($p$'s < .05). However, in controls this maximum was observed at the midline (Cz; Mean = 3.38 μV), whereas P3a amplitude in FXS males peaked at the left lateralized central site (C3; Mean = 2.02 μV). As can be seen in Figure 6, subsequent analyses indicated that peak P3a amplitudes were significantly larger in controls than in FXS males to both standard (mean difference = 1.03 μV) and deviant (mean difference = 1.70 μV) stimuli (both $p$'s < .05).

4.3.10 P3a latency

The ANOVA performed on P3a latencies yielded a significant main effect of Group, $F(1, 34) = 60.59, p < .0001, \eta^2 = .64$. As can be seen in Figure 6, P3a

![Figure 6](image-url)
latencies were significantly longer in FXS males than in controls to both standard (mean difference = 44.63 ms) and deviant (mean difference = 58.38 ms) stimuli (both $p$'s < .001).

![Graph showing correlation between MMN and P3a amplitude](image)

**Figure 7.** Correlation between the MMN and P3a amplitude in controls and fragile X syndrome males

### 4.3.11 Relation between sensory change detection and involuntary attentional processes

Linear regression analyses were performed to test whether the magnitude of sensory change detection (i.e., MMN amplitude) would affect the magnitude of involuntary triggering of attention (i.e., P3a amplitude to deviant stimuli)\(^1\). The analysis failed to yield a significant association between MMN amplitude and P3a amplitude in both FXS males, $R^2 = .15$, $F(1, 14) = 2.38$, $p > .05$, and controls, $R^2 = .08$, $F(1, 18) = 1.52$, $p > .05$.

### 4.4 Discussion

The present study aimed at bridging neurobiological and behavioral studies of attention deficits in FXS males by examining ERPs associated with pre-attentive

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\(^1\) As MMN peak latency has also been argued to be an accurate measure of the magnitude of deviance detection (Horvath et al., 2008), similar analyses were performed with MMN peak latency as predictor of P3a activity. However, these analyses also failed to yield significant results (all $p$'s > .05).
change detection. Major results included enhancement of both N1 and P2 components of the auditory ERP in FXS males relative to age-matched control participants. The MMN component, believed to index early sensory memory, was significantly reduced in FXS males relative to controls. In addition, the N2b was augmented in FXS to both standard and deviant stimuli, whereas the P3a was significantly attenuated in FXS males. Together, these findings provide novel evidence showing deficiencies in the pre-attentive change detection mechanisms, which may compromise attentional functioning in FXS in a bottom-up fashion.

N1 enhancement in response to standard tones in FXS males is in line with previous findings of Castrén et al. (2003). In addition, we present the novel finding of P2 augmentation in FXS males relative to controls, providing further evidence of hypersensitivity to auditory stimulation in FXS (Castrén et al., 2003; Frankland et al., 2004; Hssl et al., 2009; Rojas et al., 2001). Interestingly, our findings show that this increased sensitivity is not restricted to the repetitive aspects of auditory stimulation. FXS males were hypersensitive to both (repetitive) standard stimuli and deviant stimuli. Moreover, in FXS males, N1 and P2 amplitudes to deviant stimuli were similar to amplitudes elicited to standard stimuli. This discrimination failure could result from decreased or even absent habituation to stimulus repetition within the auditory modality (May et al., 1999; Näätänen & Picton, 1987; Sable, Low, Maclin, Fabiani, & Gratton, 2004). A habituation failure in FXS males has in fact been demonstrated by Castrén et al. (2003), who found that N1 amplitudes of second and fourth tones in a train of standard stimuli failed to show an amplitude reduction relative to N1 amplitude to the first tone.

In controls, habituation is typically observed as the decrement of N1 amplitude associated with stimulus repetition (May & Tiitinen, 2010). This adaptation is confined to the neurons responding to the acoustic aspects (i.e., frequency) specific to the standard stimulus. In contrast, deviant stimuli activate a different set of neurons, which do not habituate and therefore produce larger N1 amplitudes. Recent neurobiological studies demonstrated a significant impairment of local feedback inhibition onto excitatory neurons in
FXS knock-out mice, which was suggested to cause neuronal network hyperexcitability in FXS individuals (Gibson et al., 2008). As the process of neural adaptation is believed to depend on the functional balance between excitatory and inhibitory circuits (May & Tiitinen, 2010), the observed N1 augmentation might result from the imbalance in the glutamatergic and GABAergic neurotransmitter systems in FXS (Bear et al., 2004; Huber, 2007).

The N1 and P2 enhancement implicates network dependent abnormalities during the early stages of auditory information processing in FXS. Importantly, these early sensory discrimination deficits could have repercussions for the efficient build-up of a sensory memory template - a necessity for pre-attentive change detection indexed by the MMN (Näätänen, 2008; Näätänen et al., 2011; Näätänen et al., 2007). This notion is supported in our study by an attenuated MMN component in FXS males. The MMN generator process is postulated to be of key importance to trigger attention to the novel event (Escera et al., 1998; Escera, 2007; Näätänen et al., 2011; Polich, 2007). Putatively, this hypersensitive feature detection system (N1, P2) in FXS males hinders the MMN generator process, thereby compromising the conscious perception of deviant or important information, as indexed by the subsequent N2b and P3a components (Näätänen et al., 2011).

This suggestion is consistent with the observation that factors affecting MMN activity impact on the amplitude and latency of the P3a component (for a review, see Näätänen et al., 2011). However, a direct association between MMN and P3a amplitude (or latency) was not observed in the current study, which could be interpreted to suggest that MMN activity is not a prerequisite for P3a generation. This notion is in line with previous findings (Escera, 2007; Sabri, Liebenthal, Waldron, Medler, & Binder, 2006) and can possibly be explained by differences in the neural mechanisms generating the MMN and P3a. That is, the MMN has been predominantly linked to generators in the auditory and frontal cortices, reflecting bottom-up, stimulus-driven information processing, whereas the P3a has been linked to frontocentral neural mechanisms, reflective of top-down attentional information processing (Escera et al., 1998; Escera, 2007; Sabri et al., 2006). Besides distinct underlying mechanisms, it has been
demonstrated that MMN and P3a are differently affected by changes in stimulus properties (e.g., intensity, duration, or frequency differences between standard and deviant stimuli) and/or contextual demands (e.g., probability of deviant stimuli or relevance of stimulus for current task) (Katayama & Polich, 1998; Sussman, 2007; Winkler, 2007). Together, delineating the endogenous and exogenous factors affecting MMN and P3a generation in both typical and atypical development is an important task for future studies. These studies may also address the consequences of the observed hypersensitive feature detection system for deviance-detection in both passive and active attentional information processing in FXS.

Importantly, our current results provide clear evidence of aberrant auditory information processing in both sensory (N1, P2, MMN) and top-down (N2b and P3a) stages of information processing in FXS. Alongside early sensory information processing deficiencies, our findings showed that N2b amplitude failed to significantly discriminate between deviant vs. standard tones, and that P3a amplitude to both tones was significantly reduced in FXS males compared to control participants. Moreover, N2b and P3a latencies tended to be longer in FXS males compared to controls, regardless of stimulus type.

P3a generation is frequently coined as the involuntary ‘trigger’ of attention (Escera et al., 1998) and can be observed in the ERP when a deviant stimulus is sufficiently distinctive from the standard stimulus (Polich, 2007). Although deviant stimuli elicited larger P3a amplitudes than standard stimuli in both groups, our current findings show that the P3a generating system is significantly compromised in FXS, as reflected by smaller amplitudes and latencies in response to both tones. Similar findings have been reported for the task-related P3b component in FXS, which amplitude was significantly reduced upon the presentation of a ‘target’ tone (St Clair, Blackwood, Oliver, & Dickens, 1987; Van der Molen et al., in press). Our current results complement these findings by showing lateralization differences between controls and FXS males in P3a generation. That is, in contrast to the peak amplitudes observed at the central midline in controls, P3a generation in FXS males reached peak amplitudes at the left central electrode leads. These lateralization differences
correspond to prior neuroimaging studies demonstrating similar left lateralized brain activity during working memory tasks in adult FXS males (Hoeft et al., 2007), which was interpreted to suggest compensatory brain activity to assist in task performance. Putatively, the current P3a lateralization differences may reflect similar compensatory brain activity necessary for the recruitment of attentional resources. Although FMR1 expression is argued to selectively alter neural circuitries (Haas et al., 2009), further investigations are warranted to test this assumption. Together, the observed P3a findings in FXS are likely to reflect a deficiency in processes required for directing attention towards an unexpected or important event. The current P3a results suggest that this deficit is not limited to task-related attention, but also pertains to passively (or involuntary) directed attention, in the absence of task demands.

In conclusion, impaired auditory change detection in FXS males may arise from deficiencies in the earliest stages of auditory stimulus processing (N1, P2), which subsequently affects sensory stimulus discrimination and the generation of a sensory memory template, necessary for discriminating between stimuli. Impaired deviance detection could alter the evaluation of relevant information and thereby hamper decision-making processes. The current findings shed new light on the central attention problem observed in FXS, which is generally considered to reflect deficient executive control (i.e., top-down control of attention). Although the latter is likely to contribute to attention deficiency in FXS, bottom-up impairment of sensory information processing appears to be another important cause of the attention problem in FXS.
Chapter 5

Auditory and visual cortical activity during selective attention in fragile X syndrome:

A cascade of processing deficiencies

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Abstract

Objective: This study examined whether attention deficits in fragile X syndrome (FXS) can be traced back to abnormalities in basic information processing.

Method: Sixteen males with FXS and 22 age-matched control participants (mean age 29 years) performed a standard oddball task to examine selective attention in both auditory and visual modalities. Five FXS males were excluded from analysis because they performed below chance level on the auditory task. ERPs were recorded to investigate the N1, P2, N2b, and P3b components.

Results: N1 and N2b components were significantly enhanced in FXS males to both auditory and visual stimuli. Interestingly, in FXS males, the P3b to auditory stimuli was significantly reduced relative to visual stimuli. These modality differences in information processing corresponded to behavioral results, showing more errors on the auditory than on the visual task.

Conclusions: The current findings suggest that attentional impairments in FXS at the behavioral level can be traced back to abnormalities in event-related cortical activity. These information-processing abnormalities in FXS may hinder the allocation of attentional resources needed for optimal processing at higher levels.

Significance: These findings demonstrate that auditory information processing in FXS males is critically impaired relative to visual information processing.
5.1 Introduction

Fragile X syndrome (FXS) is the most common inherited cause of intellectual disability with a prevalence of 1:4000 males and 1:8000 females (Turner, Webb, Wake, & Robinson, 1996). FXS is caused by silencing of the fragile X mental retardation 1 (FMR1) gene, resulting in reduction or absence of the FMRI protein (FMRP) (Pieretti et al., 1991; Verkerk et al., 1991). FMRP plays an important role in early brain development by regulating the translation of proteins important for synaptic development and dendritic refinement (Pfeiffer & Huber, 2007). In full mutation FXS males, absence of FMRP is linked to a global reduction in cognitive performance (Cornish, Sudhalter, & Turk, 2004; Maes, Fryns, Van Wallegem, & Van den Berghe, 1994; Van der Molen et al., 2010) and behavioral problems (Backes et al., 2000; Dykens, Hodapp, & Leckman, 1987; Hagerman & Hagerman, 2002; Reiss & Freund, 1992) with deficits most notably in the attentional domain (Munir, Cornish, & Wilding, 2000; Scerif, Cornish, Wilding, Driver, & Karmiloff-Smith, 2007; Wilding, Cornish, & Munir, 2002). However, few human studies addressed the question whether these attentional deficits can be traced back to impairments at lower levels of information processing in FXS.

Lower-level information processing has been overlooked as critical factor in contributing to impairments in higher-level cognitive and behavioral deficits (Belmonte & Bourgeron, 2006; Bertone, Hanck, Kogan, Chaudhuri, & Cornish, 2010a, 2010b). There is evidence to suggest, however, that sensitivity to sensory stimuli is enhanced in FXS, in particular in the auditory modality (Castrén, Paakkonen, Tarkka, Ryynanen, & Partanen, 2003; Chen & Toth, 2001; Hessl et al., 2009; Moon et al., 2006). For example, abnormally large sensory evoked brain potentials have been reported to simple auditory stimuli in FXS humans (Castrén et al., 2003; Frankland et al., 2004; Hessl et al., 2009; Rojas et al., 2001), as well as in the FMR1 knockout mouse (Chen & Toth, 2001; Moon et al., 2006). Moreover, recent findings have demonstrated early information processing abnormalities during passive auditory discrimination and involuntary attentional processes. That is, both the mismatch negativity (MMN) and P3a generation were significantly altered during a passive auditory change
detection task (Van der Molen et al., 2011). As the MMN and P3a are associated with sensory change detection (Nääätänen, Paavilainen, Rinne, & Alho, 2007) and the involuntary triggering of attention (Escera, Alho, Winkler, & Nääätänen, 1998; Escera, 2007), respectively, it could be argued that the observed attentional deficits at the behavioral level can be traced back to early information processing deficiencies in FXS. This notion is supported by recent findings of Hessl and co-workers (2009) who demonstrated deficient auditory prepulse inhibition in FXS, implicating that information processing shows early perceptual abnormalities, probably impacting on higher-level information processing. To date, it is unclear whether similar information processing abnormalities can be demonstrated in the visual modality.

The primary goal of the present study was to perform an event-related potential (ERP) analysis of the alleged information processing deficits in FXS. To this end, ERPs were investigated during an active two-stimulus auditory and visual discrimination task. ERPs provide a suitable window on stimulus processing in the brain, reflecting both sensory (i.e., bottom-up) and higher-level (i.e., top-down) information processing with high temporal accuracy. Thus, the N1 and P2 components of the ERP are associated with the pre-attentive detection of a stimulus, reflecting sensory processing (Crowley & Colrain, 2004; Nääätänen & Picton, 1987), whereas the later occurring N2b is argued to be an electrocortical marker of attentive deviancy detection (Escera et al., 1998; Folstein & Van Petten, 2008). Finally, the P3b is a reflection of attention-driven stimulus evaluation and decision-making processes (Escera et al., 1998; Folstein & Van Petten, 2008; Nieuwenhuis, Aston-Jones, & Cohen, 2005; Nieuwenhuis, De Geus, & Aston-Jones, 2010; Polich, 2007), regulated by stimulus-evoked neuromodulatory mechanisms (e.g., acetylcholine and norepinephrine), which modulate the encoding of rare and potentially important events (Escera et al., 1998; Folstein & Van Petten, 2008; Nieuwenhuis et al., 2005; Nieuwenhuis et al., 2010; Ranganath & Rainer, 2003).

For the first time in FXS, ERPs will be recorded during an oddball paradigm for both auditory and visual modalities. Our ERP analysis should reveal whether FXS abnormalities in early stimulus processing is typical for the
auditory modality or can be observed for both the auditory and visual modalities. In addition, the ERP analysis should reveal whether early sensory deficits, as reflected in the N1 and P2 are associated with impairments at higher-level processing as indexed by the N2b and P3b components of the ERP. Finally, we asked whether ERP deficits in FXS males would be related to their task performance.

5.2 Method
5.2.1 Participants
Sixteen male participants diagnosed with the FXS full mutation (age range 18-42 years, mean age 29.6 years) and 22 healthy male controls (age range 19-47 years, mean age 29.2 years) participated in this study. FXS participants were recruited with the help of the Dutch Fragile X Parent Network. Prior DNA testing confirmed the diagnosis of the FXS full mutation. Controls were university students or college graduates recruited from or within the proximity of the university, and were rewarded either with course-credits or a monetary compensation for their participation. None of the participants were on medication during the experiment. All participants reported intact hearing and had normal or corrected-to-normal vision. This information was gathered from primary caregivers of the FXS participants. Non-verbal intelligence was assessed with the Raven Standard Progressive Matrices (Raven & Court, 1998). Raw scores were significantly lower in FXS (mean = 19.9, SD = 8.2) than in controls (mean = 55.7, SD = 3.8). Average non-verbal IQ of the controls was 121.5 (SD = 25.8). IQ in FXS could not be calculated, but performance was equivalent to an average mental age of 7 years and 7 months (SD = 1.6). Verbal mental age in FXS was assessed with the Dutch version of the Peabody Picture Vocabulary Test, third edition (Schlichting, 2004), which resulted in an average verbal mental age of 9.1 years (SD = 2.7). Participants were all naïve about the hypotheses of the experiment. Signed informed consent was obtained prior to the experiment from controls and from primary caregivers of FXS participants. The protocol for this study was reviewed and approved by the ethical review committee of the university, and complied with relevant laws and guidelines.
5.2.2 Stimuli and design

Both acoustic and visual stimuli were presented using Presentation software (Neurobehavioral Systems, Albany, CA). Acoustic stimuli were 1000 Hz and 1500 Hz sinusoidal tones with a duration of 100 ms, including 5 ms rise and fall times. The acoustic stimuli were generated using Tone Generator software of NCH (http://nch.com.au), and presented through padded stereo headphones (Sennheiser, HD-201) at 80dB SPL. Visual stimuli consisted of blue and yellow colored smiley faces (9.34 cd/m², width 3.66°, height 3.68°), and were viewed from a distance of 70 cm and presented against a black background (2.19 cd/m²) in the center of a 17-inch laptop screen (Dell Latitude D530).

At the start of each trial participants fixated on a white cross (width/height 0.51°) that was presented in the center of the screen. After a fixed period of 500 ms, a target (deviant) or a non-target stimulus (standard) was presented for 100 ms (similar in both auditory and visual tasks). In the visual task, standards and deviants replaced the fixation cross, whereas in the auditory task, the fixation cross was present during the entire trial. Trials ended with a 1400 ms period during which responses on deviants (hits) and standards (false alarms) were registered within a 100-to-1200 ms time-window after stimulus offset. Each trial had a fixed duration of 2000 ms. Figure 1 depicts a schematic of a trial of the auditory and visual task.

Which auditory stimulus (1000 Hz or 1500 Hz) or visual stimulus (yellow or blue smiley face) was designated as the deviant stimulus was counterbalanced across participants. Standard and deviant stimuli were presented pseudo randomly with the restriction that at least two standard stimuli intervened between deviants. Standard stimuli were presented 80% of the time and deviant stimuli were presented 20% of the time. Both the auditory and visual oddball tasks included a total of 240 standard and 60 deviant stimuli, and were presented in three blocks, each containing 80 standards and 20 deviants. Each block lasted 5 min, amounting to a 30-min duration of the experiment. Between blocks, a short 2-min resting period was included. A 5-min resting period was included between tasks. The order in which type of
oddball task (auditory/visual) was performed first was counterbalanced across participants. Preliminary analyses did not show order effects (all \( p's > .10 \)).

For both the auditory and visual tasks, participants were instructed to look at the fixation cross during the experiment. Participants were instructed to respond as quickly and accurately as possible to the onset of a deviant stimulus, by pressing the spacebar on the laptop. Participants were asked to refrain from responding at the onset of the standard stimulus, and to minimize eye movements and blinks. Prior to testing, participants were presented with a passive auditory oddball task with a duration of 18 min. During this task, participants were instructed to ignore the auditory stimuli and watch a silent movie. A 15-minute break was included before participants started with the active oddball tasks. The data of the passive task are reported elsewhere. EEG recordings from the FXS males and half of the control participants were performed at home locations. Electronic devices not used for EEG recordings were turned off. The out-of-lab recordings were performed in a quiet room with dimmed lights. The results of out-of-lab recordings did not differ from those obtained in the lab (all \( p's > .10 \)).

Figure 1. Schematic of a trial from the auditory and visual oddball task. Participants were required to respond to the target stimulus (blue or yellow smiley face; 1000 Hz or 1500 Hz tone). Responses (correct/incorrect) were registered within the 100-to-1200 ms response window after stimulus onset. In the above example, yellow smiley faces (visual task) and 1000 Hz tones were used as deviant stimuli.
5.2.3 **EEG recording and ERP analysis**

EEG was recorded using an EasyCap electrode cap with 28 Ag/AgCl sintered ring electrodes based on the 10/20 system from the following electrode positions: Fp1, Fp2, F7, F3, Fz, F4, F8, FC1, FCz, FC2, FC6, T7, C3, Cz, C4, T8, TP9, CP1, CP2, TP10, P7, P3, Pz, P4, P8, O1, O2, and O2. Left and right mastoids served as linked reference, the ground was placed at FT9. Horizontal eye movements (HEOG) were recorded using bipolar electrodes placed at the outer canthi of the eyes. Vertical eye movements (VEOG) were recorded using bipolar electrodes placed just above and under the left eye. Electrode impedances were kept below 10 kΩ. Signals were recorded with a BrainAmp DC amplifier (Brain Products) using Brain Vision Recorder software, at a sampling rate of 500 Hz and an online filter between 0.3 and 70 Hz.

EEG data were offline analyzed using Brain Vision Analyzer software (Version 1.05, © Brainproducts), and Matlab (Version 7.7.0, © The Mathworks). Continuous EEG was bandpass filtered at 0.1-20 Hz. Ocular artifacts were removed from the EEG using the simultaneous multiple regression method of Gratton, Coles, and Donchin (1989) as implemented in Brain Vision Analyzer. Subsequently, 900 ms epochs were created time-locked to the onset of standard or deviant stimuli, including a 100 ms pre-stimulus interval. Epochs with signals exceeding a threshold of ± 100 μV or with a voltage step exceeding 100 μV per sampling point at any electrode site were omitted from the analysis. For the auditory task, this resulted in a loss of 2% of all trials in both control and FXS participants. In the visual task, the loss was 11% and 13% in controls and FXS participants, respectively. For both groups, however, a sufficient number of artifact-free trials (> 30) remained for analysis (Cohen & Polich, 1997). Ratios for standard/deviant trials were 236/58 for controls and 234/59 for FXS within the auditory task, and 216/48 for controls and 212/48 for FXS within the visual task. ERPs were baseline corrected using the 100 ms pre-stimulus interval and averaged for standards and deviants separately. Peak amplitude and latency of the N1, P2, N2b and P3b components were determined at the F3, Fz, F4, FC1, FCz, FC2, C3, Cz, C4, P3, Pz, P4, O1, Oz, and O2 channel locations.
Peak amplitudes of the ERP components were defined, relative to the pre-stimulus baseline, by the largest voltage deflection within a pre-determined latency window of the grand-averaged ERP waveform. For each participant, the peaks of the ERP components were visually inspected after automatic peak detection. The time at which the ERP components reached peak amplitude was taken as peak latency. The time-windows in which peaks of the ERP components were derived from ERP guidelines (Duncan et al., 2009; Picton et al., 2000) and were as follows: N1 = 80-120 ms, P2 = 120-200 ms, N2b = 200-350 ms, and P3b = 280-400 ms, relative to stimulus onset.

5.2.4 Statistical analysis
Analyses were carried out in five successive steps: (1) Task performance analyses included the proportion of correct responses (hit rate), proportion of responses to standard stimuli (false alarms) and mean reaction time (RT) to deviant stimuli. Repeated measures analyses of variance (ANOVA) were separately performed for these variables, with Modality (two levels: auditory, visual) as within-subjects factors and Group (two levels: FXS, Control) as between-subjects factor; (2) Repeated measures ANOVAs were carried out separately for the peak amplitudes of the ERP components (N1, P2, N2b, P3b) with Stimulus (two levels: standard, deviant), Laterality (three levels: left, midline, right) and Site (five levels: frontal, frontocentral, central, parietal, occipital) as within-subjects factors, and Group (two levels: FXS, controls) as between-subjects factors. Subsequent comparisons were carried out only for those electrode sites showing maximum ERP peak amplitude, to determine group and stimulus differences; (3) For the ERP latency data, additional repeated measures ANOVAs were carried out for the site(s) that showed maximum peak amplitude; (4) To assess modality (auditory vs. visual) differences in the magnitude of the ERP components, difference scores were calculated by subtracting the peak amplitude/latency for standard stimuli from peak amplitude/latency for deviant stimuli. This resulted in N1, P2, N2b, P3b difference scores for both amplitude and latency in the auditory and visual modality. Repeated measures ANOVAs were performed separately for the
amplitude and latency of the ERP component difference scores, with Modality (2 levels: auditory, visual) as within-subjects factor and Group (2 levels: FXS, controls) as between-subjects factor; (5) Finally, hierarchical linear regression analyses were performed, separately, on the auditory and visual oddball performance indices (TRT, hit rate, and false alarms) to investigate the predictive value of the ERP components (N1, P2, N2b, P3b) vis-à-vis oddball task performance. The N1, P2, N2b, P3b amplitude difference scores were used as predictors and entered hierarchically (P3b, N2b, P2, N1) into linear regression analyses, with the oddball performance indices as dependent variables. This resulted in three separate regression analyses per group (FXS, controls) per modality (auditory, visual). All analyses were performed using Statistical Package for Social Science (SPSS Inc, 2008). Greenhouse-Geisser correction was applied where appropriate, and non-adjusted degrees of freedom are reported for transparency. Alpha was set at .05 and additional post-hoc significance testing was performed using a Bonferroni correction. Only significant main effects or interactions are reported.

5.3 Results

5.3.1 Performance data

Five FXS participants (mean age in years = 38.2, SD = 5.7) were excluded from analyses based on their performance below chance level (19% hit rate) on the auditory oddball task (see Table 1). These five participants were significantly (p’s < .05) older (mean age difference = 10.2 years) and had lower Raven SPM raw scores (mean difference = 9.4) compared to the FXS participants performing above chance level (55% hit rate). Their exclusion resulted in a reduced sample of 11 FXS participants. Table 1 shows chronological age, Raven raw scores, and performance measures both for control and FXS participants. For the latter group verbal mental age is presented also. The groups differed in hit rate, \( F(1, 31) = 17.89, p < .0001, \eta^2 = .37 \), false
### Table 1. Participant characteristics and performance measures on the auditory and visual oddball tasks in controls and FXS.

<table>
<thead>
<tr>
<th>Performance measure</th>
<th>Controls (n = 22)</th>
<th>FXS Good Performers (n = 11)</th>
<th>FXS Poor Performers (n = 5)</th>
<th>Group differences</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Auditory oddball paradigm</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Target RT (ms)</td>
<td>347 (13.1)</td>
<td>465 (26.0)</td>
<td>715 (46.9)</td>
<td>Controls &gt; FXS GP &gt; FXS PP*</td>
</tr>
<tr>
<td>Hit rate (%)</td>
<td>99.6 (0.2)</td>
<td>89.1 (2.8)</td>
<td>187 (4.1)</td>
<td>Controls &gt; FXS GP &gt; FXS PP*</td>
</tr>
<tr>
<td>False alarms (%)</td>
<td>0.3 (0.1)</td>
<td><strong>7.2 (1.6)</strong></td>
<td>133 (2.4)</td>
<td>Controls &gt; FXS GP, PP*</td>
</tr>
<tr>
<td><strong>Visual oddball paradigm</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Target RT (ms)</td>
<td>342 (9.5)</td>
<td>499 (27.7)</td>
<td>695 (42.4)</td>
<td>Controls &gt; FXS GP &gt; FXS PP*</td>
</tr>
<tr>
<td>Hit rate (%)</td>
<td>99.9 (0.1)</td>
<td>92.7 (2.2)</td>
<td>727 (3.3)</td>
<td>Controls &gt; FXS GP &gt; FXS PP*</td>
</tr>
<tr>
<td>False alarms (%)</td>
<td>0.3 (0.1)</td>
<td>5.2 (1.3)</td>
<td>4.0 (2.0)</td>
<td>Controls &gt; FXS GP, PP*</td>
</tr>
<tr>
<td><strong>Participant characteristics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronological age (years)</td>
<td>28.8 (1.7)</td>
<td>26.4 (7.7)</td>
<td>36.6 (4.7)</td>
<td>FXS GP &gt; FXS PP*</td>
</tr>
<tr>
<td>RAVEN SPM (raw scores)</td>
<td>55.6 (0.8)</td>
<td>22.8 (2.4)</td>
<td>13.4 (2.0)</td>
<td>Controls &gt; FXS GP &gt; FXS PP*</td>
</tr>
<tr>
<td>PPVT verbal mental age (years)</td>
<td>-</td>
<td>9.7 (3.0)</td>
<td>7.7 (1.7)</td>
<td>FXS GP &gt; FXS PP*</td>
</tr>
</tbody>
</table>

Note. Abbreviations: GP = Good Performers; PP = Poor Performers. SPM = Standard Progressive Matrices. PPVT = Peabody Picture Vocabulary Test. SEM between parentheses. Bold text indicates significant within group differences between the auditory and visual performance indices. *Significant at $p < .05$.

alarms, $F(1, 31) = 11.43, p < .0001, \eta^2 = .27$, and RT to deviant stimuli, $F(1, 31) = 19.12, p < .0001, \eta^2 = .38$. FXS males were less accurate, showed more false alarms, and were slower on both the visual and auditory tasks than controls (all $p’s < .05$). The ANOVA performed on the false alarm data yielded a significant interaction between Modality and Group, $F(1, 31) = 8.49, p < .01, \eta^2 = .22$. FXS males committed significantly more false alarms on the auditory than on the visual task ($p < .05$). In addition, FXS males were somewhat faster on the auditory than on the visual task, but this Modality by Group interaction just failed to reach significance, $F(1, 31) = 4.36, p > .05, \eta^2 = .12$. Together, these performance data show that selective attention is more compromised in the auditory than in the visual modality in FXS.

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1Similar analyses were carried out for the visual ERP data, and with chronological age and PPVT scores as covariates; however, there was no significant contribution of intellectual level and chronological age to the observed findings.
5.3.2 Auditory ERP data

Eleven FXS and 22 control participants were included for analysis, based on their performance on the auditory task (see above). Preliminary analyses indicated no significant relation between intellectual level (Raven SPM) and the amplitude and latency measures of the ERP components ($p$’s $> .05$). Figure 2 depicts grand averaged ERPs for the frontal, frontocentral, central, parietal, and occipital electrode sites in response to standard and deviant stimuli in both FXS and control participants. Scalp distribution (voltage maps) of the ERP components is plotted for both standard and deviant stimuli for the N1, P2, N2b, and P3b components. Table 2 presents peak amplitude and latency of all ERP components for controls and FXS males.

5.3.2.1 Auditory N1 amplitude.

A significant Stimulus by Laterality by Site by Group interaction, $F(8, 248) = 2.72, p < .05$, $\eta^2 = .08$, revealed that N1 amplitude to standard stimuli peaked at FCz in both groups. N1 amplitude to deviant stimuli, however, peaked at FCz in controls and at FC1 in FXS males. Post-hoc comparisons revealed that peak amplitude was larger for both stimuli in FXS males compared to controls, $F(1, 31) = 10.86, p < .01$, $\eta^2 = .26$.

5.3.2.2 Auditory N1 latency.

The ANOVA on the N1 latency data yielded no significant main or interaction effects.

5.3.2.3 Auditory P2 amplitude.

In both groups, P2 amplitudes showed their maximum at FCz, $F(4, 124) = 21.18, p < .0001$, $\eta^2 = .41$. The main effect of Stimulus, $F(1, 31) = 4.69, p < .05$, $\eta^2 = .13$, $^2$ Analyses were also performed using a sample of 11 chronologically age-matched controls for both auditory and visual ERP, and we performed analyses (for the visual data only) with the total sample of FXS participants (n=16), however, the pattern of results did not differ from those reported here.
Figure 2. ERP waveforms for standard and deviant stimuli within the auditory (A) and visual (C) modalities for controls (left) and FXS males (right). Scalp distributions are presented for the N1, P2, N2b, and P3b components of the ERPs to auditory (B) and visual (D) standard and deviant stimuli.
revealed that, in both groups, deviant stimuli elicited significantly smaller P2 amplitudes than standard stimuli ($p < .05$).

5.3.2.4 Auditory P2 latency.
The ANOVA performed on the P2 latency data for FCz resulted in a significant main effect of Stimulus, $F(1, 31) = 10.92, p < .01$, $\eta^2 = .26$. In both groups, deviant stimuli elicited significantly shorter P2 latencies than standard stimuli ($p < .05$).

5.3.2.5 Auditory N2b amplitude.
The significant Group by Site interaction, $F(4, 124) = 12.14, p < .0001$, $\eta^2 = .28$, revealed that N2b amplitudes peaked at Fz in Controls and at FCz in FXS males. Post-hoc comparisons showed that N2b peak amplitudes were significantly larger in FXS males than in control participants ($p < .05$).

5.3.2.6 Auditory N2 latency.
Significant main effects were found for Stimulus, $F(1, 31) = 18.55, p < .0001$, $\eta^2 = .37$, and Group, $F(1, 31) = 11.92, p < .01$, $\eta^2 = .28$. N2b latencies were significantly longer in FXS males than in control participants (mean difference = 43 ms) and, in both groups, deviant stimuli elicited shorter N2b latencies than standard stimuli ($p's < .05$).

5.3.2.7 Auditory P3b amplitude.
The ANOVA yielded a significant Stimulus by Site by Group interaction, $F(4, 124) = 16.39, p < .0001$, $\eta^2 = .35$. P3b amplitudes peaked at Cz in control participants for both stimuli, and at Oz (standard stimuli) and Pz (deviant stimuli) in FXS males. Post-hoc comparisons revealed that P3b amplitudes were significantly smaller in FXS males relative to controls, for both standard and deviant stimuli ($p's < .05$).
5.3.2.8  **Auditory P3b latency.**

The ANOVA showed significant main effects of Site, $F(1, 31) = 26.07$, $p < .0001$, $\eta^2 = .46$, and Group, $F(1, 31) = 5.00$, $p < .05$, $\eta^2 = .46$, which were included in a significant Site by Group interaction, $F(1, 31) = 5.07$, $p < .05$, $\eta^2 = .46$. In both groups, P3b latencies were significantly longer at Cz than at Oz ($p$'s < .05). P3b latencies were longer in FXS males than in control participants but this difference was only significant at Cz ($p < .05$).

**Table 2.** Peak amplitude and latency of the ERP components for the auditory and visual modalities.

<table>
<thead>
<tr>
<th>Modality</th>
<th>Standard Stimuli</th>
<th>Deviant Stimuli</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls Site</td>
<td>Controls Site</td>
</tr>
<tr>
<td>ERP</td>
<td>Controls Site</td>
<td>Controls Site</td>
</tr>
<tr>
<td><strong>Auditory</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amplitude</td>
<td>N1 FCz</td>
<td>-5.4 (0.6)</td>
</tr>
<tr>
<td>Latency</td>
<td>125 (1.1)</td>
<td>126 (1.4)</td>
</tr>
<tr>
<td>Amplitude</td>
<td>P2 FCz</td>
<td>5.6 (0.8)</td>
</tr>
<tr>
<td>Latency</td>
<td>233 (6.0)</td>
<td>217 (8.5)</td>
</tr>
<tr>
<td>Amplitude</td>
<td>N2b Fz</td>
<td>1.5 (0.6)</td>
</tr>
<tr>
<td>Latency</td>
<td>291 (11.8)</td>
<td>337 (17.1)</td>
</tr>
<tr>
<td>Amplitude</td>
<td>P3b Cz</td>
<td>2.9 (0.6)</td>
</tr>
<tr>
<td>Latency</td>
<td>339 (8.1)</td>
<td>311 (9.2)</td>
</tr>
</tbody>
</table>

| Visual    |                   |                   |             |             |
| Amplitude | N1 Oz             | -1.4 (0.7)        | Oz          | -3.6 (0.8)  | Fcz         | -0.9 (0.8)  | Fcz         | -4.5 (0.7)  |
| Latency   | 98 (6.1)          | 113 (5.1)         | Oz          | 9.0 (1.1)   | Oz          | 7.5 (0.8)   | Oz          | 10.0 (1.2)  |
| Amplitude | P2 Oz             | 6.7 (0.8)         | Oz          | 9.0 (1.1)   | Oz          | 7.5 (0.8)   | Oz          | 10.0 (1.2)  |
| Latency   | 167 (8.6)         | 155 (12.1)        | Oz          | 159 (13.1)  |
| Amplitude | N2b F3            | -0.8 (0.5)        | Fz          | -4.5 (1.0)  | F4          | -0.8 (0.6)  | F4          | -5.7 (1.4)  |
| Latency   | 225 (8.0)         | 286 (11.5)        | Fz          | 270 (11.9)  |
| Amplitude | P3b Cz            | 7.3 (0.7)         | Pz          | 3.9 (1.1)   | Fcz         | 16.8 (0.8)  | Oz          | 9.9 (1.2)   |
| Latency   | 339 (8.3)         | 378 (9.4)         | 349 (5.2)   | 363 (8.2)   |

*Note: Amplitude in microvolts (µV), latency in milliseconds (ms), SEM between parentheses. * Bold text represents group differences significant at $p < .05$. *

5.3.3  **Visual ERP data**

5.3.3.1  **Visual N1 amplitude.**

The ANOVA yielded a significant interaction of Laterality by Site by Group, $F(2, 62) = 7.12$, $p < .01$, $\eta^2 = .19$, which revealed that N1 peak amplitudes were maximal at Oz in control participants and at FCz in FXS males. Post-hoc
analyses showed that N1 amplitudes were significantly larger in FXS males than in controls for both stimuli, but only at FCz ($p < .05$).

5.3.3.2 Visual N1 latency.
The ANOVA yielded a main effect of Site, $F(4, 124) = 4.79$, $p < .05$, $\eta^2 = .13$. For both groups, N1 latencies were significantly longer at Fz than at Oz ($p < .05$).

5.3.3.3 Visual P2 amplitude.
Significant main effects of Site, $F(4, 124) = 25.53$, $p < .0001$, $\eta^2 = .45$, and Laterality, $F(2, 62) = 22.02$, $p < .0001$, $\eta^2 = .42$, indicated that, in both groups, maximum amplitude was at Oz.

5.3.3.4 Visual P2 latency.
All main effects and interactions failed to reach significance.

5.3.3.5 Visual N2b amplitude.
The ANOVA yielded a significant Stimulus by Laterality by Site by Group interaction $F(2, 62) = 5.00$, $p < .05$, $\eta^2 = .14$, which revealed that N2b amplitudes peaked at F3 (standard stimuli) and Fz (deviant stimuli) in control participants, and at F4 in FXS males. Post-hoc comparisons revealed that for both stimuli, N2b amplitudes were significantly larger in FXS males than in control participants (both $p$'s $< .05$).

5.3.3.6 Visual N2b latency.
The main effect of Group, $F(1, 31) = 19.31$, $p < .0001$, $\eta^2 = .38$, was included in a significant Stimulus by Group interaction, $F(1, 31) = 14.10$, $p < .01$, $\eta^2 = .31$. For both stimuli, N2b latencies were longer in FXS males than in control participants ($p < .05$). In control participants, N2b latencies were significantly longer for deviant than for standard stimuli ($p < .05$). In FXS males, N2b latencies were somewhat shorter for deviant than for standard stimuli, but this difference failed to reach significance ($p > .05$).
5.3.3.7 Visual P3b amplitude.
The ANOVA yielded significant interactions of Laterality by Group, $F(2, 62) = 5.58, p < .05, \eta^2 = .15$, and of Stimulus by Site by Group, $F(4, 124) = 10.75, p < .0001, \eta^2 = .28$, which showed that P3b amplitudes for standard and deviant stimuli peaked, respectively, at Cz and Pz in control participants and at FCz and Oz in FXS males. For both stimuli, P3b amplitudes were significantly smaller in FXS males than in control participants ($p's < .05$). In both groups, P3b amplitudes were significantly larger for deviant than for standard stimuli ($p's < .05$).

5.3.3.8 Visual P3b latency.
The ANOVA yielded a main effect of Group $F(1, 31) = 9.06, p < .01, \eta^2 = .23$. P3b latencies were longer in FXS males relative to control participants, but this difference was significant only for standard stimuli ($p < .05$).

5.3.4 Auditory vs. Visual P3b comparison
The ANOVA on the P3b amplitude data yielded a significant main effect of Group, $F(1, 31) = 21.82, p < .0001 \eta^2 = .41$, which was included in a significant Modality by Group interaction, $F(1, 31) = 4.29, p < .05, \eta^2 = .12$. As can be seen in Figure 3, the P3b component was larger in controls than in FXS males, in both auditory and visual modalities. For FXS males, the P3b component was
significantly larger in the visual modality as compared to the auditory modality ($p < .05$).

**Figure 3.** Auditory and visual ERP waveforms to standard and deviant stimuli in controls (A) and FXS (B) depicted for the electrode leads showing maximum P3b peak amplitude (negative is plotted upward). In controls, peak amplitude was found at Cz and Pz, whereas in FXS, peak amplitude was found at Pz and Oz (auditory and deviant modality, respectively). Deviant minus standard P3b peak amplitude (C) and latency (D) is depicted for controls and FXS to show auditory vs. visual modality differences in P3b generation. Asterisks (*) represent significant differences at $p < .05$. 
5. Cortical activity during selective attention

5.3.5 ERP components and task performance.
To assess whether auditory and visual oddball task performance could be predicted by their corresponding ERP components (N1, P2, N2b, and P3b), hierarchical regression analyses were performed, separately, for each modality, and Bonferroni correction (alpha .01) was applied for multiple comparisons. As we expected that behavioral performance in FXS males would be impaired due to a cascade of information processing deficiencies underlying stimulus change detection, ERP difference scores (i.e., deviant minus standard peak amplitudes) were calculated for each component. The subsequent P3b, N2b, P2, and N1 difference scores were used as predictor variables, and the oddball task performance indices (RT to deviant stimuli, % hit rate, and % false alarms) were taken as dependent variables.

First, we computed the correlations between the sensory change detection components (N1 and P2) with the active attentional ERP components (N2b and P3b). A significant positive correlation was observed for controls in the auditory condition between the N1 and P3b difference scores, \( r(20) = .50, p < .01 \), and between P2 and N2b difference scores, \( r(20) = .57, p < .01 \).

Regression analyses revealed that auditory P3b amplitude significantly predicted RT to deviant stimuli in controls \( (R^2 = .22, F(1, 20) = 5.60, p < .05, \text{Beta} = -.46) \). In FXS, auditory P3b amplitude significantly predicted TRT \( (R^2 = .66, F(1, 9) = 17.15, p < .01, \text{Beta} = -.81) \), hit rate \( (R^2 = .63, F(1, 9) = 17.94, p < .0001, \text{Beta} = .88) \), and % false alarms \( (R^2 = .50, F(1, 9) = 11.0, p < .01, \text{Beta} = -.76) \). In both groups, the N1, P2, and N2b components failed to significantly explain the variance in auditory oddball performance indices \( (p'\text{s} > .05) \). Correlations of the ERP difference scores with the auditory performance indices are presented in Table 3.

In the visual modality, the ERP components failed to significantly predict any of the visual oddball performance indices in controls \( (\text{all } p'\text{s} > .01) \). In FXS males, however, the model including both P3b and N2b amplitude best predicted visual TRT \( (R^2 = .68, F(1, 9) = 8.61, p < .01) \), explaining an extra 33% of the variance in TRT as in the model with P3b alone. Correlations of the ERP difference scores with the visual performance indices are presented in Table 4.
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Table 3. Correlations of the ERP difference scores with the auditory oddball performance measures.

<table>
<thead>
<tr>
<th>Amplitude</th>
<th>Controls</th>
<th>FXS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TRT</td>
<td>Hit rate</td>
</tr>
<tr>
<td>N1</td>
<td>-.10</td>
<td>.16</td>
</tr>
<tr>
<td>P2</td>
<td>.27</td>
<td>-.12</td>
</tr>
<tr>
<td>N2b</td>
<td>.24</td>
<td>.04</td>
</tr>
<tr>
<td>P3b</td>
<td>-.47</td>
<td>.31</td>
</tr>
</tbody>
</table>

Note: Amplitude reflects the difference scores (deviant minus standard) of the ERP components. Bold text represents significant difference at $p < .01$.

Table 4. Correlations of the ERP difference scores with the visual oddball performance measures.

<table>
<thead>
<tr>
<th>Amplitude</th>
<th>Controls</th>
<th>FXS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TRT</td>
<td>Hit rate</td>
</tr>
<tr>
<td>N1</td>
<td>-.25</td>
<td>.13</td>
</tr>
<tr>
<td>P2</td>
<td>.13</td>
<td>-.06</td>
</tr>
<tr>
<td>N2b</td>
<td>.28</td>
<td>-.19</td>
</tr>
<tr>
<td>P3b</td>
<td>-.36</td>
<td>.00</td>
</tr>
</tbody>
</table>

Note: Amplitude reflects the difference scores (deviant minus standard) of the ERP components. Bold text represents significant difference at $p < .01$.

5.4 Discussion

The aim of the present study was threefold. First, we examined the electrocortical correlates of early information processing to assess the alleged information processing deficits in FXS males. Second, we examined whether these deficits are typical for the auditory modality or extend also to the visual modality. Third, we examined whether early sensory processing deficits in FXS males are associated with higher-level information processing. These issues were addressed by recording ERPs to simple auditory and visual stimuli using a standard oddball paradigm.

The performance data that emerged from the oddball tasks were clear-cut. The control participants outperformed the FXS males on all measures derived from the oddball tasks. That is, FXS males responded slower to deviant stimuli, their hit rate to deviant stimuli was lower and the proportion of false alarms to standard stimuli was higher. Moreover, FXS males committed significantly more false alarms on the auditory than on the visual oddball task.
In fact, a substantial proportion of our sample of FXS males (31%) performed below chance level on the auditory oddball task and was therefore excluded from further analysis of auditory stimulus processing. Interestingly, their performance on the visual oddball task was much better; they were more accurate and exercised better inhibitory control as indicated by lower false alarm rates.

It could be argued, however, that the substantial performance difference between the auditory vs. visual oddball task in FXS males was due to the stimuli used in the oddball tasks. Pure tones were used in the auditory oddball task whereas meaningful symbolic stimuli (i.e., smileys) were used in the visual oddball task. For example, smileys could be more attractive (‘attention-grabbing’) than pure tones, which might account for the huge performance difference of FXS males (total sample) in processing auditory vs. visual stimuli. This interpretation is challenged, however, by our observation that FXS males who performed above chance level on the auditory task did not show a significant difference in their response latencies to auditory vs. visual deviant stimuli. That is, paying less attention to a particular class of stimuli would be expected to result in slower RTs. Thus, poor discriminative ability between standard and deviant auditory stimuli is more likely to explain the auditory vs. visual performance discrepancies, rather than poor auditory task engagement. This interpretation is supported by previous reports showing modality effects on (cognitive) performance of FXS males. For example, FXS males have been reported to perform worse on auditory than on visual tasks requiring attentional control (Sullivan et al., 2007) and short-term memory capacity (Van der Molen et al., 2010). Furthermore, our current findings indicate that the modality differences related to task performance are matched by electrocortical discrepancies in stimulus processing within the auditory vs. visual modality. Further investigation of modality differences in FXS information processing by calibrating carefully auditory and visual stimuli is an important goal for further research.

The ERP findings showed clear differences between FXS males and control participants. FXS males showed exaggerated N1 and N2b components
whereas the P3b component was substantially attenuated compared to controls. The observation of exaggerated N1 amplitudes to auditory stimuli is consistent with previous findings suggesting increased neural sensitivity to auditory stimulation in FXS humans (Castrén et al., 2003; Rojas et al., 2001; Van der Molen et al., 2011), as well as in *fmr1* knockout mice (Chen & Toth, 2001; Moon et al., 2006). The current findings extend previous reports in showing a similar enhancement of N1 to visual stimuli. The finding of an augmented N1 in FXS males is suggestive of a 'hypersensitive' frontally oriented neural circuitry associated with the pre-attentive detection of both auditory and visual stimuli, as N1 augmentation in FXS males was observed over frontocentral electrode leads in both modalities. Particularly in the auditory modality, neural hypersensitivity is a well-documented phenomenon in FXS (Frankland et al., 2004; Hessl et al., 2009), and could result from impaired post-stimulus inhibition that typically decreases the excitability of the neurons responding to a particular stimulus (May et al., 1999; Sable, Low, Maclin, Fabiani, & Gratton, 2004). Post-stimulus inhibition is mediated by excitatory and inhibitory neural activity in the brain, largely regulated by glutamatergic and GABAergic neurotransmission (Ben-Ari, Gaiarsa, Tyzio, & Khazipov, 2007). In FXS, there is evidence of a structural imbalance between the excitatory and inhibitory drive on neural activity (Bear, 2005; Bear, Huber, & Warren, 2004; D’Hulst et al., 2006; D’Hulst & Kooy, 2007). Putatively, this imbalance results in hypersensitivity to sensory stimulation, which in turn may compromise efficient stimulus discrimination. Indeed, greater N1 and P2 difference scores resulted in an increase of P3b and N2b in controls, respectively. Although this finding was not observed in the visual modality, this may indicate a relation of the sensory change detection mechanisms with later, active attentional stimulus processing. Additionally, our results showed that auditory N2b amplitude in FXS males was smaller to deviant compared to standard stimuli, whereas the opposite was seen in control participants. Although these differences were not significant, the current N2b findings might reflect a deficiency in mismatch detection (Escera et al., 1998; Näätänen et al., 2007),
hinder the allocation of attentional resources necessary for stimulus classification (Polich, 2007).

The current findings are consistent with a recent study in which the involuntary aspects of auditory change detection in FXS males were investigated (Van der Molen et al., 2011). Results revealed that both the MMN (associated with sensory change detection) and the P3a (associated with the involuntary or passive triggering of attention) were significantly reduced in FXS males relative to chronologically age-matched controls. These findings are in line with the assumption that electrocortical deficiencies during passive attention may impact on later, active attentional decision-making processes, as demonstrated by the absence of correlations between early sensory ERP components (N1 and P2) with later, cognitive ERP components (N2b, P3b).

The current P3b findings are consistent with the notion of a cascade of processing deficiencies in FXS males. We observed a considerably attenuated auditory P3b to deviants in FXS males compared to controls. This finding is in accord with previous reports of a reduced P3b in FXS individuals (St Clair, Blackwood, Oliver, & Dickens, 1987). The difference in P3b amplitude between FXS males and controls was less pronounced for visual relative to auditory stimuli. But the current findings showed that, for both modalities, the amplitude of P3b was smaller in FXS males compared to controls. A similar attenuation of the P3b component has been reported for individuals with other types of intellectual disability, such as Prader-Willi (Stauder, Brinkman, & Curfs, 2002) and Rett syndrome (Stauder, Smeets, van Mil, & Curfs, 2006). Recent developments in the P3b literature suggest that the P3b reflects stimulus evaluation and decision-making processes (Nieuwenhuis et al., 2005; Nieuwenhuis et al., 2010). These information-processing functions of the P3b have been attributed to a widespread neural network, including the prefrontal cortex, anterior insula, cingulate gyrus, temporoparietal junction, medial temporal cortex and the hippocampal formation (Nieuwenhuis et al., 2010; Ranganath & Rainer, 2003). The efficiency of this ‘stimulus evaluation/decision network’ is argued to be dependent on neuromodulatory processes (e.g., the locus coeruleus- norepinephrine system), which facilitate the (behavioral)
response to potentially significant events (Nieuwenhuis et al., 2005; Nieuwenhuis et al., 2010; Ranganath & Rainer, 2003). Importantly, abnormal network connectivity has been widely documented in mental retardation syndromes, such as FXS (Galvez, Gopal, & Greenough, 2003; Greenough et al., 2001; Hinton, Brown, Wisniewski, & Rudelli, 1991; Irwin et al., 2001; Pfeiffer & Huber, 2007), Down syndrome (Dierssen & Ramakers, 2006; Hanson, Blank, Valenzuela, Garner, & Madison, 2007) and Rett syndrome (Dani & Nelson, 2009). In FXS, abnormal network connectivity is particularly characterized by altered dendritic spine density and/or morphology, suggesting deficient synaptic pruning (Greenough et al., 2001; Irwin et al., 2001). Thus, the P3b attenuation in FXS might result from abnormal network connectivity during stimulus-driven brain activation, consequently hindering stimulus evaluation processes. This interpretation, albeit speculative, is consistent with theoretical accounts of P3b generation (Nieuwenhuis et al., 2005; Nieuwenhuis et al., 2010; Polich, 2007), as well as of information processing in mental retardation syndromes (Dierssen & Ramakers, 2006; Ramakers, 2000, 2002).

Finally, we examined the relation between ERP components and performance measures. Our results showed that P3b amplitude to deviant auditory stimuli predicted the speed of responding to these stimuli in both FXS males and controls. In FXS males, P3b amplitude to deviant stimuli predicted also hit rate to deviant stimuli and the proportion of false alarms to standard stimuli. This pattern of findings was absent for the visual oddball task. However, both N2b and P3b difference scores best explained the variance in RT to visual deviant stimuli in FXS males. The latter finding may be interpreted to suggest that visual deviant stimuli (i.e., smiley faces) resulted in increased triggering of attention (N2b; Näätänen, Kujala, & Winkler, 2011) necessary for efficient network-level decision-making (P3b; Nieuwenhuis et al., 2005) in FXS males. Although our experimental design precludes conclusions in terms of causative relations, the correlations between the P3b and performance in auditory and visual modalities, suggest that information processing deficiencies manifested by the P3b may underlie FXS task performance at the behavioral level. Importantly, to accurately determine whether the observed behavioral
5. Cortical activity during selective attention

and electrocortical findings are specific to the FXS etiology, as opposed to more general developmental changes affecting intellectual ability, future investigations should preferably include an additional neurodevelopmental disorder matched on both chronological age and intellectual ability. Future investigations may also address whether similar findings can be observed for FXS individuals with lower intellectual performance levels.

Together, the current data pattern shows that information processing at a network level can be characterized by a cascade of processing abnormalities in FXS. The exaggerated N1 and N2b amplitudes are consistent with the sensory hypersensitivity that is common in FXS (Castrén et al., 2003; Chen & Toth, 2001; Hagerman & Hagerman, 2002; Hessl et al., 2009; Moon et al., 2006; Rojas et al., 2001), which may result from hyperexcitable circuitry in the sensory cortical areas (Pfeiffer & Huber, 2007), possibly interfering with the efficiency of sensory-gating processes (Chen & Toth, 2001; Hessl et al., 2009). In contrast, P3b was attenuated in FXS males and exhibited a more diffuse topographic distribution compared to controls. As suggested by our current data, the attenuated P3b in FXS males might result from deficient low-level sensory processing and the flawed stimulus discrimination that results from it. In particular in the auditory modality, this notion is supported by the observation that an increase of sensory change detection (N1, P2) is associated with an increase of attentive deviancy detection (N2b, P3b). Interestingly, this relation was absent in FXS males. Alternatively, the absence of FMRP in FXS might have a direct effect on the neural sources implicated in the generation of P3b, most notably the temporoparietal junction (Strobel et al., 2008). Interestingly, recent neuroimaging research demonstrated frontal and temporal lobes to be smaller and parietal and occipital lobes to be larger in FXS individuals relative to controls (Gothelf et al., 2008; Hallahan et al., 2011). Future, combined neuroimaging and electrocortical studies should reveal whether the aberrant ventrodorsal gradient observed in FXS individuals contributes to the reduced magnitude of the P3b seen in the current report. Those studies might also address the issue of why the FXS system involved in
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processing an auditory deviant stimulus is more vulnerable than its visual counterpart.
Chapter 6

Summary and discussion
Summary and Discussion
The primary objective of this thesis was to provide a detailed characterization of cognitive functioning in FXS and to investigate its underlying information processing characteristics. Results of these investigations could further our understanding of the basic information processing characteristics that go astray in FXS and lead to important hypotheses on the causal neural mechanisms that can account for the cognitive and behavioral abnormalities. We first characterized the cognitive profile of relative strengths and weaknesses in cognitive abilities in adult males with FXS, using a battery of tests measuring cognitive abilities in verbal, non-verbal, memory and executive functioning domains (Chapter 2). This cognitive profile was subsequently used as starting point for in-depth analysis of (1) the underlying information processing characteristics in the attentional domain (Chapter 3), as well as (2) the basic information processing characteristics associated with stimulus perception and discrimination in the FXS brain (Chapters 4 and 5). Results of these investigations could provide a link between cognitive deficits and their possible underlying neurobiological mechanisms.

6.1 The cognitive profile in fragile X syndrome
Cognitive functioning in FXS has been widely investigated. However, some key issues regarding profiling relative strengths and weaknesses in cognitive functioning were unexplored. For example, cognitive functioning in FXS is subject to high levels of variability (Dykens, Hodapp, & Leckman, 1987), which raises the important question whether cognitive profiles are similar for individuals of various performance levels. In addition, in chapter 2, we addressed the critical issue whether the use of the frequently employed verbal mental age reference measure (i.e., receptive vocabulary) could bias the interpretation of the cognitive profile in terms of relative strengths and weaknesses. To this end, we contrasted cognitive performance to both a verbal and non-verbal mental age reference measure.

Results of the Chapter 2 study corroborated previous findings (Dykens et al., 1987; Maes, Fryns, Van Wallegem, & Van den Berghe, 1994) by showing
that cognitive performance is particularly weak on measures of reasoning and performat abilities confined to abstract item content. In contrast, visuo-perceptual recognition and receptive vocabulary were relative strengths in FXS cognitive functioning. Model-based cluster analyses furthermore revealed that this pattern of findings was similar for FXS males of different overall performance levels/IQs. Although future investigations should aim to replicate similar findings for domains of memory and executive functioning, our current results strengthen the notion that cognitive functioning in FXS males can be characterized by a profile of relative strengths and weaknesses in cognitive functioning that is similar for FXS individuals of various levels of performance.

In addition, FXS males exhibited a pronounced deficit in verbal short-term memory. By using both non-verbal and verbal mental age reference measures, we were able to demonstrate that the significance of this discrepancy between verbal and visual short-term memory could only be observed when contrasting short-term memory abilities with the non-verbal reference measure. Also, for the remaining sub-domain analyses, the choice of an appropriate reference measure revealed to be critically important in examining cognitive profiles. That is, when compared to the verbal mental age reference, most cognitive abilities showed as relative weakness, whereas comparison with the non-verbal mental age reference exposed more subtleties in relative strengths and weaknesses in cognitive performance. In this regard, the use of receptive vocabulary as a single reference measure will result in an overabundance of cognitive weaknesses in FXS individuals. This in turn may compromise identifying relative strengths and weaknesses in cognitive performance.

Together, the findings that emerged from this study revealed that adult FXS males can be characterized by an overall impairment in cognitive functioning, based on mental age level performance. In addition, those cognitive abilities with a high demand on executive control seemed to be more significantly impaired than perceptual functions. However, based on the findings reported in the ERP studies (Chapters 4 and 5), we provide evidence that information processing in the FXS brain is already compromised during the
bottom-up stages of information processing, which could impact on the later, cognitive stages of information processing.

6.2 Attentional set-shifting in simple and complex stimulus environments

To complement the cognitive profiling study (chapter 2), we next characterized attentional set-shifting ability in FXS males using the IED from the CANTAB test battery. As each subsequent stage on the IED imposes a higher demand on attentional control, we were able to investigate in more detail those cognitive processes that contribute to the alleged attentional control deficiencies in FXS. In order to interpret results in terms of syndrome-specific signatures, we compared IED performance of FXS males to that of three control groups, comprising typically developing adults and children matched on chronological and mental age, respectively, as well as individuals with Down syndrome matched on both chronological and mental age.

Based on overall IED stage performance, a significant proportion of the FXS participants already failed before the crucial intra- and extra-dimensional set-shifts. This finding may suggest that the key weakness characterizing cognitive flexibility in FXS is not shifting attentional set between stimulus dimensions, but can be traced back to more basic reversal learning. Indeed, with a novel approach to investigate IED stage performance in terms of repetition, maintenance, and discrimination errors, we were able to show that attentional set-shifting ability in FXS males differed from the control groups based on a significant impairment in reversal learning, as a result of enhanced repetitive decision-making. Although feedback indicated that a formerly correct stimulus now had become incorrect, FXS males continued to respond to this previously reinforced stimulus. Interestingly, this increased repetitive behavior during reversal learning was particularly observed when faced with simple stimulus configurations.

A potential explanatory mechanism of this enhanced repetitive decision-making could be the perseverative responding due to a failure to disengage attention from a previously reinforced, but now incorrect stimulus.
Another potential, but contrasting, underlying mechanism is learned irrelevance, which refers to reduced attention directed towards a particular stimulus that has proven to be irrelevant (Mackintosh, 1975). For example, FXS males may have a specific weakness in reorienting attention towards a stimulus for which a former association with irrelevance exists. A limitation in the IED experimental design is that perseverations and learned irrelevance cannot be accurately disentangled. Therefore, future studies should preferably design their experimental paradigms in a way that allow to investigate these cognitive phenomena separately.

Besides repetitive decision-making, FXS males also showed enhanced distractibility to irrelevant stimuli. In contrast to all control groups, FXS males exhibited greater difficulty with completing the compound discrimination stages, in which distractor stimuli were introduced from a non-reinforced, novel stimulus dimension. When presented with this more complex stimulus configuration, FXS males engaged in increased random search behavior, which was particularly observed during the reversal of the compound discrimination stage.

This increased distractibility to irrelevance could possibly be explained by an underlying deficiency in stimulus perception, compromising stimulus discrimination, and perhaps, novelty processing. In more detail, increased neural and behavioral sensitivity to the perception of sensory stimulation has been widely described in humans with FXS (Castrén, Paakkonen, Tarkka, Ryynanen, & Partanen, 2003; Ferri et al., 1994; Frankland et al., 2004; Hessl et al., 2009; Miller et al., 1999; Rojas et al., 2001), as well as in the fmr1 knockout mouse (Chen & Toth, 2001; Frankland et al., 2004; Moon et al., 2006). In particular, neural hyperexcitability has been demonstrated in the FXS brain by augmented event-related brain potentials to auditory stimuli (i.e., tones) (Castrén et al., 2003; Rojas et al., 2001). Also on a behavioral level, FXS males are hypersensitive in loud or crowded environments (Hagerman & Hagerman, 2002), possibly a reflection of aberrant stimulus perception in the brain. This increased neural activity to incoming stimuli may hinder efficient downstream information processing necessary to distinguish between relevant and
irrelevant stimuli. Indeed, by investigating the pre-attentive and attentive stages of stimulus perception and discrimination in chapters 4 and 5, we found support for this notion.

6.3 Stimulus processing in the FXS brain: evidence from passive and active information processing paradigms

The investigations in both Chapters 2 and 3 demonstrated that cognitive and attentional functioning is particularly impaired for those functions with a high demand on attentional or executive control. However, based on increased distractibility to irrelevant stimuli (Chapter 3), as well as prior evidence of enhanced sensitivity to stimulus perception, it could be speculated that information processing in FXS is already compromised during the early (pre-attentive) stages of information processing. These alleged early stimulus perception abnormalities could result in a cascade of information processing deficiencies, compromising efficient decision-making and goal-directed behavior (Nieuwenhuis, Aston-Jones, & Cohen, 2005; Polich, 2007).

In Chapter 4 we investigated passive stimulus detection and discrimination in the FXS brain using the passive variety of the oddball paradigm in which infrequent (deviant) tones were embedded in a sequence of frequent (standard) tones. Results corroborated the previously reported N1 augmentation to stimulus detection (Castrén et al., 2003; Rojas et al., 2001) and revealed the novel finding that N1 amplitude did not differentiate between standard and deviant tones in FXS males. More specifically, in control participants we observed significantly larger N1 amplitudes to deviant than standard tones, whereas in FXS males this difference was absent. Normally, stimulus repetition leads to a decrement in N1 generation, a process referred to as habituation (May et al., 1999; Sable, Low, Maclin, Fabiani, & Gratton, 2004). The current data suggest that this habituation to stimulus repetition is impaired in FXS males.

Dishabituation to stimulus repetition has been previously reported in FXS children (Castrén et al., 2003) and could be related to elevated levels of neural excitability resulting from imbalanced excitatory (glutamatergic) and
inhibitory (GABAergic) neurotransmission (Bear, Huber, & Warren, 2004; D’Hulst et al., 2006) which in turn compromises the efficiency of pre-attentive change detection mechanisms in the FXS brain. Indeed, results showed that the pre-attentive change detection mechanism (MMN) was significantly attenuated in FXS males. Interestingly, this MMN attenuation was followed by augmented N2b and reduced P3a activity, which are ERP components associated with the allocation of attentional resources to salient or novel stimuli (Escera, Alho, Winkler, & Näätänen, 1998; Escera, 2007). The observed reduction of MMN amplitude is likely a resultant of impaired sensory stimulus representation, subsequently compromising the efficient triggering of attentional resources to a potential important event, as well as the active inhibition of information processing related to irrelevant stimuli (Polich, 2007).

This notion of a cascade of information processing deficiencies as a potential explanation for impaired decision-making at the behavioral level was supported by our findings from the selective attention study described in Chapter 5. On both auditory and visual tasks, we found an augmentation of the N1 response to standard stimuli, which was followed by a significant reduction in P3b activity to deviant stimuli. At the behavioral level, FXS participants showed impaired performance on both auditory and visual tasks, with a considerable proportion of participants performing below chance level on the auditory task. Results furthermore showed that, in the FXS group, variation in P3b activity to deviant stimuli best explained performance on the oddball tasks. That is, larger P3b difference scores were related to better task performance. As P3b activity is posited to reflect the outcome of a decision-making process in the brain (Nieuwenhuis et al., 2005), it could be argued that deficient information processing at the network level underlies impairments observed at the behavioral level.

Interestingly, the observation of auditory vs. visual performance discrepancies on the oddball task corresponded to modality differences in information processing. That is, those participants with above-chance level performance showed larger P3b activity in response to visual deviants relative to auditory deviants. In controls, however, both behavioral and ERP indices
were similar between modalities. Although the visual stimuli may have been more appealing (i.e., attention grabbing) or salient than the auditory stimuli used in the Chapter 5 study, it could be argued that due to FMR1 silencing, information processing in the auditory modality is more severely affected than in the visual modality. This is in line with the auditory vs. visual short-term memory discrepancies found in the Chapter 2 study, as well as with a host of studies reporting elevated arousal levels in response to auditory stimulation (Castrén et al., 2003; Chen & Toth, 2001; Frankland et al., 2004; Hessl et al., 2009; Moon et al., 2006; Rojas et al., 2001).

6.4 Concluding remarks

Taken together, the combined psychophysiological and neuropsychological approach in the current thesis has contributed to our understanding of how certain aspects of cognitive dysfunction in FXS may result from abnormalities in underlying information processing characteristics. Particularly, results from the ERP studies add an important dimension to explaining FXS cognitive dysfunction in terms of a primary deficiency in top-down, executive control. Although perceptual functions may seem to comprise relative strengths at the behavioral level (Chapter 2), the pre-attentive and attentive change detection studies (Chapters 4 and 5) have provided clear evidence of deficient lower-level information processing. Based on these findings we can conclude that although cognitive functioning in FXS may be characterized by executive control deficiencies at the behavioral level, lower-level perceptual impairments likely contribute to these cognitive deficits. More specifically, our results suggest that during active selective attention in the auditory modality, appropriate coupling between sensory change detection and active decision-making processes seems absent in FXS information processing. That is, in the control group we found that an increase in sensory change detection (N1 and P2 activity) was related to an increase in the active triggering of attention (N2b and P3b activity), whereas this correlation between bottom-up and top-down information processing mechanisms was absent in FXS males. However, in our passive change detection study (Chapter 4) we did not find a direct association between a
sensory change detection mechanism (MMN) and the involuntary triggering of attention (P3a). This may suggest that the alleged fine-tuning between sensory and attentive information processing (Näätänen, Kujala, & Winkler, 2011) is more influenced by top-down factors, as is the case during active decision-making.

In terms of unraveling syndrome-specific profiles of cognitive function, the question remains whether these lower-level information processing impairments are characteristics for the FXS etiology. For example, P3b attenuation has been reported in a variety of other neurodevelopmental disorders, such as Rett and Prader-Willi syndrome (Sable et al., 2004; Stauder, Smeets, van Mil, & Curfs, 2006; Strauss, Sherman, & Spreen, 2006). Although the MMN has been less frequently investigated in neurodevelopmental disorders, evidence also suggests impaired functioning of this pre-attentive change detection mechanism in autism, ADHD, and non-syndromic intellectual disability (Dunn, Gomes, & Gravel, 2008; Ikeda, Hashimoto, Hayashi, & Kanno, 2009; Ikeda, Okuzumi, Hayashi, Hashimoto, & Kanno, 2000; Kemner et al., 1996). Therefore, impairments during early stages of information processing may not be specific to FXS, but comprise a more general functional outcome of abnormal brain development. In contrast, N1 augmentation in both auditory and visual modalities may be most characteristic of the information processing deficiencies in FXS, since in a variety of other neurodevelopmental disorders (e.g., autism, ADHD, Down syndrome) an attenuation of this ERP component is observed (Barry, Johnstone, & Clarke, 2003; Bruneau, Roux, Adrien, & Barthelemy, 1999; Pekkonen, Osipova, Sauna-Aho, & Arvio, 2007). As the N1 is argued to reflect the pre-attentive extraction of the characteristics (e.g., intensity) of a detected stimulus (Näätänen & Picton, 1987), it could be argued that stimulus perception per se is not the problem, but the problem lies in the extraction of its specific properties.

From our results it appears that FXS males experience a specific difficulty with the passive or involuntary extraction of deviant or novel information from a continuous stream of stimuli within the environment (Näätänen, 2008; Näätänen & Escera, 2000; Näätänen et al., 2011; Näätänen,
Paavilainen, Rinne, & Alho, 2007). Based on the hyperexcitable state of sensory information processing, as well as the syndrome-specific difficulties in ignoring irrelevant stimuli (Chapter 3), it could be argued that the pre-attentive switching between bottom-up and top-down attentional networks (Crottaz-Herbette & Menon, 2006; Fox, Corbetta, Snyder, Vincent, & Raichle, 2006; Menon & Uddin, 2010) is impaired in FXS. In more detail, it has been suggested that efficient goal-directed behavior is enabled by the dynamic switching between a default mode network and a central executive network, in which a saliency network operates as a so-called ‘neural interface’ between these two networks to filter out relevant stimuli from the stream of irrelevant stimuli (Menon & Uddin, 2010). Based upon the observed neural hyperexcitability observed during early information processing, it could be argued that due to impaired functional connectivity in the FXS brain (Belmonte & Bourgeron, 2006), this saliency network fails in the dynamic switching between the default mode and central executive networks. That is, due to the alleged dishabituation to stimulus repetition, important stimuli can be less efficiently extracted from a continuous stream of information, probably resulting in noisy network-level decision-making. This speculative account of attentional function at the network-level requires further investigation in future studies.

**6.5 Future prospects**

Although the investigations in this thesis have contributed to our understanding of the underlying mechanisms of cognitive deficits in FXS, the top-down profiling approach (see Figure 1, Chapter 1) may be extended by promising lines of research. A first recommendation concerns investigating *developmental trajectories* of information processing in FXS. Although the current findings may be representative for adult FXS males, different results on cognitive information processing may be observed in younger and older FXS individuals. Additionally, in order to reveal ‘neurocognitive signatures’ specific for FXS, these investigations should also include other neurodevelopmental disorders, matched on general intelligence. This way, differences can be attributed to etiology, rather than general intellectual impairment, and the
results derived from these studies will provide a more accurate understanding of the gene-brain-cognition relationship in FXS.

Another interesting avenue for further research concerns delineation of the observed augmentation of sensory brain activity in FXS males. In the current thesis, the enhanced N1 and P2 activity during stimulus detection in the FXS brain was hypothesized to reflect a failure of neuronal habituation, presumably due to an imbalance in excitatory and inhibitory activity in the central nervous system. Abnormalities in the excitatory mGluR and inhibitory GABAergic systems have been well established (Bear et al., 2004; Huber, 2007) and deficiencies in these neurotransmitter systems may contribute to the behavioral symptoms observed in FXS individuals (Heulens, D'Hulst, Braat, Rooms, & Kooy, 2010; Heulens & Kooy, 2011b). For example, the experimentally validated “mGluR” theory states that increased signaling through group 1 glutamate receptors is responsible for the clinical symptoms (i.e. anxious behavior and epileptic activity) observed in FXS (Bear et al., 2004). Interestingly, by targeting the GABA<sub>B</sub> receptor using arbaclofen, a GABA<sub>B</sub> receptor agonist, diminishment of excessive glutamate signaling and a subsequent reduction of clinical symptoms was observed in both the <i>fmri</i>1 knock-out mice, as well as in individuals with FXS (Heulens & Kooy, 2011b). Putatively, pharmacological reduction of the presumed neural hypersensitivity could contribute to more efficient information processing in the FXS brain.

Another promising avenue for further research is the study of event-related brain dynamics (e.g., oscillatory cortical activity), and the imaging of structural and functional neural networks (e.g., with DTI, MRI, fMRI, EEG, MEG, and resting-state brain activity). In contrast to the work on typical development, surprisingly few investigations have studied development of structural and functional brain networks in atypical development. A possible explanation for this lack of research refers to the complications that are experienced when intellectually low-functioning individuals enter a brain-imaging scanning device, or when an EEG electrode cap is placed on their heads. Frequent obstacles to successful measurements include exaggerated
movement or anxious behavior, thereby prohibiting lengthy neuroimaging sessions.

In particular, resting-state fMRI/EEG may offer an interesting framework for studying the structural and functional characteristics of brain development in both typical and atypical populations (Uddin, Supekar, & Menon, 2010). The advantage is that brain function can be examined independent of task performance, which allows collecting informative datasets in just a few minutes. Patterns of resting-state brain activity have shown to be highly informative for investigating the development of large-scale brain networks (Douw et al., 2011; Fox & Raichle, 2007; Greicius, Supekar, Menon, & Dougherty, 2009; Stam, 2004). Using fMRI, spontaneous fluctuations in the BOLD response can be correlated between different brain regions to provide an index of functional connections (Fox & Raichle, 2007), whereas in EEG, coherence between electrodes can be calculated and/or network analysis (e.g., based on graph theory) can be performed to characterize the development of structural and functional brain networks (Bullmore & Sporns, 2009; Stam, 2004). These methods of analyzing resting-state brain activity can shed light on potentially disrupted cortical networks in neurodevelopmental disorders at any point in development.

In normal development, the analysis of resting-state brain activity has complemented theoretical views of brain development (Johnson, 2001; Johnson, 2011) by showing a transition of short-range brain connectivity networks observed in children into the advancement of long-range connectivity patterns as observed in adults (Boersma et al., 2011; Fair et al., 2007; Kelly, Uddin, Biswal, Castellanos, & Milham, 2008). Furthermore, it has been shown that specific functional neural networks, such as sensorimotor systems, precede the development of other neural systems, for example those underlying higher-level information processing (Chugani, Phelps, & Mazzotta, 1987; Durston et al., 2006; Kelly et al., 2009; Lin et al., 2008). Importantly, these findings on structural and functional brain development derived from typical development can be used as a model for interpreting trajectories of atypical brain development, such as in FXS. Currently, resting-state EEG data is being
analyzed in adult FXS males to reveal connectivity patterns of functional brain networks (Van der Molen, Van der Molen, Ramakers, & Stam, 2011). These results on ‘Small-World’ networks could then be correlated to measures of cognitive performance to study the integrity of these functional brain networks. For example, in normal development, the observed ‘path length’ in these modeled neural networks has proven to be a reliable measure of intelligence (i.e., shorter path length corresponding to higher intellectual performance levels) (Langer et al., 2011; Van den Heuvel, Stam, Kahn, & Hulshoff Pol, 2009). Based on the well-defined neurobiological background of FXS (i.e., immature cortical network development), resting-state analyses of brain activity will undoubtedly yield promising results.

In closing, I would like to argue that these proposed avenues for further research should not be studied in isolation. In order to truly understand the impact of FMRP depletion on brain development and cognitive functioning, it may be highly informative to correlate results on, for example, ‘Small World’ neural networks derived from resting-state brain activity with the patterns of GABAergic receptor expression in the FXS brain. Currently, GABAergic receptor expression is being analyzed in the brains of male adults with FXS using positron emission tomography (Heulens & Kooy, 2011a). Within an ideal multidisciplinary framework, the observed individual differences derived from these investigations could then be linked to cognitive performance levels. Importantly, when studied at different points in developmental time, a true understanding can be derived of the consequences of single-gene silencing on cognitive development.
Summary in Dutch / Samenvatting in het Nederlands
Het voornaamste doel van dit proefschrift was het bieden van een gedetailleerde karakterisering van het cognitief functioneren van mannen met het fragiele X syndroom (FXS) en het blootleggen van deficiënties in de onderliggende informatieverwerkingsprocessen. Hierdoor kan een beter inzicht worden verkregen in afwijkingen in de fundamentele informatieverwerkingsprocessen die resulteren in beperkingen in cognitie en gedrag. In Hoofdstuk 2 is het cognitieve profiel in kaart gebracht met behulp van een testbatterij voor het meten van (non-)verbale vaardigheden, geheugen en executieve functies. Dit cognitieve profiel is vervolgens gebruikt als startpunt voor een diepeanalyse van de onderliggende informatieverwerkingsprocessen in het aandacht domein (Hoofdstuk 3), alsmede voor de informatieverwerkingsprocessen gerelateerd aan stimulusperceptie en -discriminatie in de hersenen van mannen met FXS (Hoofdstukken 4 en 5). De resultaten van deze onderzoeken kunnen een belangrijke brug slaan tussen de cognitieve beperkingen en de onderliggende neurobiologische mechanismen.

7.1 Het cognitieve profiel in fragiele X syndroom
Het cognitief functioneren in FXS is op grote schaal onderzocht, maar een aantal belangrijke kwesties met betrekking tot het karakteriseren van relatieve sterktes en zwaktes in het cognitieve functioneren is in deze onderzoeken niet aan bod gekomen. Zo bestaat er een grote variabiliteit in het niveau van cognitief functioneren (Dykens, Hodapp, & Leckman, 1987), waardoor men zich kan afvragen of hoog- en laagfunctionerende FXS-mannen dezelfde cognitieve profielen laten zien. Deze gelijkenis in cognitieve profielen is onder andere van belang bij het in kaart brengen van de relatie tussen genen, hersenen en cognitie (bijvoorbeeld in de vraag of er bij FXS sprake is van een robuust profiel van relatieve sterke en zwakke cognitieve vaardigheden of dat er sprake is van meerdere subtypes, gekenmerkt door verschillende profielen). Hiernaast werd in Hoofdstuk 2 onderzocht of de veelgebruikte verbale referentiemaat voor mentale leeftijd (dat wil zeggen: receptieve woordenschat) de interpretatie van het cognitieve profiel in termen van relatieve sterktes en zwaktes zou kunnen
vertekenen. Om dit te onderzoeken werd de prestatie op de cognitieve taken vergeleken met een verbale én een non-verbale referentiemaat voor mentale leeftijd.

De resultaten laten zien dat redeneren en performale vaardigheden gerelateerd aan abstracte inhoud van het testmateriaal vooral zwak zijn in het cognitieve functioneren van mannen met FXS. Daarentegen kunnen visueel-perceptuele herkenningsvaardigheden en woordenschat als relatieve sterktes worden beschouwd. Dit profiel van relatieve sterktes en zwaktes in het cognitief functioneren blijkt gelijk te zijn voor hoog- én laagfunctionerende FXS-mannen. Voor de profielanalyse is het van belang dat ook toekomstig onderzoek dergelijke bevindingen dient aan te tonen voor het geheugen en de executieve functies. Op basis van de huidige resultaten kan echter voorzichtig worden geconcludeerd dat het cognitief functioneren in FXS kan worden gekenmerkt door een profiel van relatief sterke en zwakke cognitieve vaardigheden, ongeacht het niveau van intellectueel functioneren.

Een opvallende zwakte in het cognitief functioneren van FXS-mannen was het verbale kortetermijngeheugen. Deze beperking, die tot uiting kwam in het direct herhalen van cijferreeksen, kon alleen worden aangetoond door de inclusie van zowel een verbale als een non-verbale referentiemaat. Dat wil zeggen dat bij een vergelijking met de verbale referentiemaat zowel het visuele als het verbale kortetermijngeheugen een zwakte blijkt te zijn, terwijl bij vergelijking met de non-verbale referentiemaat alleen het verbale kortetermijngeheugen als zwakte kan worden aangemerkt. Ook voor de analyses van de overige subdomeinen blijkt de keuze van een geschikte referentiemaat van groot belang voor het bepalen van relatieve sterktes en zwaktes in het cognitief functioneren. Zo resulteerde het gebruik van de verbale mentale leeftijdsreferentie in een overdaad aan cognitieve beperkingen, terwijl bij gebruik van een non-verbale referentiemaat een grotere subtiliteit waarneembaar was in relatieve sterke en zwakke cognitieve vaardigheden. Deze resultaten tonen aan dat de keuze van een geschikte referentiemaat van essentieel belang is voor het identificeren van relatieve sterke en zwakke cognitieve vaardigheden.
Ten slotte laten de resultaten van dit onderzoek zien dat mannen met FXS kunnen worden gekenmerkt door een algemene beperking in het cognitief functioneren en dat er sprake is van een disproportioneel profiel van relatief sterkere en zwakkere cognitieve vaardigheden. De cognitieve vaardigheden die een groter beroep doen op executieve controle lijken in grotere mate te zijn beperkt dan perceptuele vaardigheden. Echter, op basis van de resultaten verkregen uit de *event-related brain potential* (ERP) -onderzoeken (Hoofdstuk 4 en 5), hebben we kunnen aantonen dat informatieverwerking in de hersenen van FXS-mannen al afwijkingen laten zien tijdens de *bottom-up* (onbewuste / stimulusgestuurde) stadia van informatieverwerking, die de latere cognitieve stadia van informatieverwerking kunnen verstoren.

### 7.2 Cognitieve flexibiliteit in een eenvoudige en complexe stimulusomgeving

Personen met FXS hebben geregeld moeite om hun gedrag aan te passen aan de wisselende omstandigheden in de omgeving. Het doel van het onderzoek in Hoofdstuk 3 was om de aard van deze problematiek in kaart te brengen met behulp van de *Intra/Extra Dimensional Set-Shifting* (IED) -taak uit de *Cambridge Neuropsychological Test Automated Battery* (CANTAB). Bij de IED moet men aan de hand van feedback herhaaldelijk kiezen voor een bepaalde stimulus (bijvoorbeeld: een paarse cirkel) terwijl een andere stimulus (bijvoorbeeld: een paars vierkant) genegeerd dient te worden. Bij het behalen van het criterium (het aantal opeenvolgende correcte keuzes) dient gekozen te worden voor de andere stimulus – men moet dan een verschuiving in aandacht toepassen, namelijk van de voorheen correcte naar de voorheen incorrecte stimulus (*reversal learning*). De IED bestaat uit verschillende stadia die opeenvolgend een groter beroep doen op aandachtscontrole en uit twee cruciale stadia waarin men de aandacht dient te verschuiven (*attentional set-shifting*) naar nieuwe stimuli van dezelfde stimulusdimensie (dat wil zeggen: nieuwe paarse vormen tijdens de intradimensionele *set-shift*) of van nieuwe stimuli van een andere stimulusdimensie (dat wil zeggen: witte lijnen tijdens de extradimensionele *set-shift*). Door de prestatie in deze stadia apart te
7. Samenvatting in het Nederlands

bestuderen is het mogelijk om de cognitieve processen onderliggend aan de vermeende aandachtscontroledeficiënties in FXS beter in kaart te brengen.

De prestatie van de FXS-groep is vergeleken met drie controlegroepen die bestonden uit (1) volwassenen en (2) kinderen gematcht op respectievelijk chronologische en mentale leeftijd en (3) een controlegroep bestaande uit personen met Downsyndroom, gematcht op chronologische én mentale leeftijd. Door de inclusie van deze drie controlegroepen was het mogelijk om beperkingen in aandachtscontrole en cognitieve flexibiliteit toe te schrijven aan de FXS-etiologie in plaats van een algemene beperking in de verstandelijke vermogens.

De onderzoeksresultaten tonen aan dat FXS-participanten al vóór de cruciale intra- en extradimensionele set-shifts de criteria niet halen. Dit zou kunnen betekenen dat attentional set-shifting tussen stimulusdimensies niet het voornaamste probleem is wat betreft de zwakte in cognitieve flexibiliteit, maar dat deze aandachtsproblemen herleid kunnen worden tot een meer basaal reversal learning-probleem. Met behulp van een nieuwe benadering voor het bestuderen van een IED-stadiumprestatie in termen van repetitie, maintenance en discriminatie fouten, zijn we inderdaad in staat geweest om aan te tonen dat attentional set-shifting in FXS-mannen verschillde van de andere controlegroepen op basis van een significante beperking in reversal learning. Dit uitte zich in verhoogd repetitief gedrag. Ondanks dat feedback aangaf dat de voorheen juiste stimulus nú onjuist was, bleven FXS-mannen herhaaldelijk kiezen voor deze eerder bekrachtigde stimulus. Een saillante bevinding was dat dergelijk repetitief gedrag voornamelijk kon worden waargenomen gedurende de eenvoudige stimulus configuraties (dat wil zeggen: wanneer er weinig stimuli op het computerscherm werden getoond).

Dit verhoogde repetitieve gedrag kan mogelijk worden verklaard door een onderliggend probleem in het verschuiven van de aandacht van een voorheen juiste stimulus naar een voorheen onjuiste stimulus. Een participant vertoont in dat geval een gedragspatroon dat niet meer past bij de huidige eisen van de omgeving, namelijk het herhaaldelijk kiezen voor de voorheen juiste stimulus in plaats van een alternatief. Dergelijk gedrag wordt ook wel
persevereren genoemd. Een andere mogelijke, maar contrasterende verklaring, refereert naar het fenomeen dat de aandacht voor een bepaalde stimulus minder wordt wanneer deze in het verleden irrelevant bleek te zijn, ook wel ‘geleerde irrelevantie’ genoemd (Mackintosh, 1975). Hierdoor zou de aandacht moeilijker te herleiden zijn naar een stimulus die voorheen incorrect of irrelevant was, met als gevolg dat men blijft reageren op de voorheen correcte (maar nu incorrecte) stimulus. Echter, door beperkingen in het design van de IED kunnen processen zoals perseveratie en geleerde irrelevantie niet goed worden ontrafeld. De taak voor toekomstig onderzoek is dan ook om deze cognitieve processen nader te onderzoeken in aangepaste experimentele paradigma’s.

Tevens lieten FXS-mannen ook zien meer afgeleid te zijn door irrelevante stimuli dan de overige controlegroepen. FXS-mannen hadden meer moeite om de criteria te behalen tijdens de compound discrimination-stadia, waarin afleidende stimuli werden geïntroduceerd uit een niet eerder bekrachtigde nieuwe stimulusdimensie. Bij deze complexe stimulus-configuraties vertoonden FXS-mannen meer distractiefouten, vooral tijdens de reversal van het compound discrimination-stadium. Deze verhoogde gevoeligheid voor distractie kan mogelijk worden verklaard door een onderliggende deficiëntie in stimulusperceptie die de efficiëntie in stimulusdiscriminatie beperkt. Zo wordt een verhoogde neurale en gedragssmatige gevoeligheid voor stimulusperceptie regelmatig gerapporteerd in FXS (Castrén, Paakkonen, Tarkka, Ryynanen, & Partanen, 2003; Ferri et al., 1994; Frankland et al., 2004; Hessl et al., 2009; Miller et al., 1999; Rojas et al., 2001), alsmede in de fmr1 knockout-muis (Chen & Toth, 2001; Frankland et al., 2004; Moon et al., 2006). Neurale hypersensitiviteit is vooral aangetoond in de hersenen van FXS-personen door een verhoogde amplitude van ERP’s als reactie op een auditieve prikkel (bijvoorbeeld: hoge en lage tonen) (Castrén et al., 2003; Rojas et al., 2001). Op gedragsniveau zijn FXS-mannen vaak hypersensitief in een luide en/of drukke omgeving (Hagerman & Hagerman, 2002). Dit is mogelijkerwijs een weerspiegeling van abnormale stimulus-verwerking in de hersenen. De verhoogde neurale activiteit tijdens
stimulusperceptie kan de efficiëntie van informatieverwerking in de hersenen beperken waardoor het moeilijker wordt om een relevante stimulus te onderscheiden van irrelevante stimuli. Evidentie voor een dergelijke assumptie kan worden aangedragen door de resultaten uit de Hoofdstukken 4 en 5 waarin passieve en actieve stadia van stimulusperceptie en -discriminatie werden onderzocht.

**7.3 Stimulusverwerking in FXS-hersen: evidentie van passieve en actieve informatieverwerkingsparadigma’s**

De onderzoeksresultaten uit de Hoofdstukken 2 en 3 laten zien dat het cognitief functioneren in FXS vooral beperkingen vertoont wanneer er een groter beroep wordt gedaan op aandachtscontrole. Dit impliceert een probleem in de cognitieve stadia van de informatieverwerking. Echter, door de grotere gevoeligheid (in herenactiviteit en gedrag) tijdens het waarnemen van stimuli en de vergrote afleiding tijdens de presentatie van irrelevante stimuli (Hoofdstuk 3) bestaat de mogelijkheid dat de informatieverwerking al gedurende de passieve stadia problemen vertoont. Dergelijke vroegtijdige informatieverwerkingsproblematiek zou kunnen resulteren in een opeenvolging van aandachtdeficiënties die een belemmering vormen voor efficiënt doelgericht gedrag (Nieuwenhuis, Aston-Jones, & Cohen, 2005; Polich, 2007).

In Hoofdstuk 4 hebben we getracht om passieve stimuliendetectie en discriminatie in de hersenen van mannen met FXS in kaart te brengen met behulp van het *oddball* paradigma. Hierin werden infrequente (deviante) tonen gepresenteerd in een lange reeks van frequentie (standaard-) tonen. De resultaten bevestigen eerdere bevindingen van een vergrote N1-amplitude tijdens stimuliendetectie (Castrén et al., 2003; Rojas et al., 2001). Een nieuwe bevinding was dat de N1-amplitude niet verschillde tussen frequentie en infrequente tonen, een verschil dat in de controlegroep wél werd gevonden. Normaal gesproken kan er bij stimuli repetitie een afname worden waargenomen in de amplitude van de N1, een proces dat ook wel habituatie wordt genoemd (May et al., 1999; Sable, Low, Maclin, Fabiani, & Gratton, 2004).
De huidige data suggereren dat deze habituatie voor stimulusrepetitie beperkt is in FXS.

Dishabituatie voor stimulusrepetitie is eerder aangetoond bij kinderen met FXS (Castrén et al., 2003) en is mogelijk gerelateerd aan een verhoogd niveau van neurale gevoeligheid, veroorzaakt door een disbalans tussen de excitatoire (glutamaterge) en inhibitoire (GABA-erge) neurotransmissie (Bear, Huber, & Warren, 2004; D’Hulst et al., 2006). Deze disbalans in neurotransmissiesystemen tast mogelijkerwijs de efficiëntie aan van het passieve veranderingsdetectiemechanisme in de hersenen. De resultaten laten inderdaad zien dat een dergelijk mechanisme (de mismatch negativity - MMN) minder goed lijkt te functioneren in hersenen van FXS-mannen. Deze reductie in MMN-amplitude wordt gevolgd door vergrote N2b-amplitudes en gereduceerde P3a-activiteit. Deze beide ERP-componenten worden geassocieerd met het toewijzen van de aandacht in de richting van een saillante of nieuwe stimulus (Escera, Alho, Winkler, & Näätänen, 1998; Escera, 2007). De reductie in MMN-amplitude is wellicht een consequentie van beperkingen in de vroegtijdige sensorische representatie van een stimulus, waardoor vervolgens de toewijzing van aandacht naar nieuwe (belangrijke) stimuli in de omgeving wordt belemmerd, alsmede de actieve inhibitie van informatieverwerking gerelateerd aan irrelevante stimuli (Polich, 2007).

Deze opeenvolging van informatieverwerkingsproblematiek in de hersenen zou een mogelijke verklaring kunnen zijn voor de aandachts- en gedragsproblematiek bij personen met FXS. Met een soortgelijk oddball-paradigma wordt in Hoofdstuk 5 onderzocht of deze problemen op het netwerk niveau (informatieverwerking in de hersenen) gerelateerd kunnen worden aan het gedrag (het reageren op een infrequente stimulus en het negeren van een frequente stimulus). De resultaten laten zien dat op zowel de auditieve als op de visuele oddball-taak de N1-activiteit voor frequente stimuli groter was in de FXS-groep dan in de controlegroep. In de FXS-groep wordt deze verhoogde N1-activiteit gevolgd door een afname in P3b-activiteit bij de presentatie van een infrequente stimulus. Dit patroon is waar te nemen op zowel de auditieve als visuele oddball-taak. Op basis van de gedragsresultaten
presteren de FXS-mannen slechter op beide taken dan de controlegroep, waarbij een aanzienlijk aantal FXS-mannen beneden kansniveau presteerde op de auditieve oddball-taak. Verder laten de resultaten zien dat een grotere P3b-activiteit in verband staat met betere taak prestatie in de FXS-groep. Aangezien P3b-activiteit in verband wordt gebracht met beslissingsprocessing in de hersenen (Nieuwenhuis et al., 2005), zouden de beperkingen in het gedrag gerelateerd kunnen worden aan deficiënties in de informatieverwerking.

Een belangrijke bevinding is dat deze discrepanties tussen taakprestatie op de auditieve en visuele oddball-taak correspondeert met de modaliteitsverschillen die zijn gevonden in de informatieverwerking in de hersenen. FXS-mannen die boven kansniveau presteerden op de beide oddball-taken vertoonden een grotere P3b-activiteit tijdens de detectie van een visuele infrequent stimulus dan tijdens een auditieve infrequent stimulus. Dit modaliteitsverschil in P3b-activiteit kon niet worden waargenomen in de controlegroep. Een mogelijke verklaring voor dit modaliteitsverschil in de FXS-groep is dat de visuele stimuli attractiever waren dan de auditieve stimuli. Een alternatieve verklaring is dat de informatieverwerking in de auditieve modaliteit meer tekortkomingen vertoont dan de visuele modaliteit als gevolg van de uitschakeling van het FMR1-gen. Deze laatste opvatting wordt ondersteund door de gevonden discrepantie tussen het auditieve en het visuele kortetermijngeheugen (Hoofdstuk 2) en de evidentie voor een verhoogd niveau van arousal tijdens auditieve stimulatie (Castrén et al., 2003; Chen & Toth, 2001; Frankland et al., 2004; Hessl et al., 2009; Moon et al., 2006; Rojas et al., 2001).

### 7.4 Concluderende opmerkingen

De psychofysiologische en neuropsychologische benadering die in dit proefschrift wordt gehanteerd draagt bij aan ons begrip van hoe cognitieve problematiek in FXS zich verhoudt tot kenmerken in de onderliggende informatieverwerking. Vooral de resultaten uit de ERP-studies voegen een belangrijke dimensie toe aan het verklaren van het cognitieve disfunctioneren in FXS in termen van een primaire deficiëntie in *top-down*, executieve controle.
Ook al lijken perceptuele functies relatieve sterktes te zijn in het cognitieve profiel (Hoofdstuk 2), de passieve en actieve veranderingsdetectiestudies hebben aangetoond dat problemen in stimulusverwerking zich al voordoen tijdens de lagere-orde stadia van de informatieverwerking. Op basis van deze gegevens kan worden geconcludeerd dat op gedragsniveau executieve controle het voornaamste probleem lijkt te zijn in het cognitieve functioneren van FXS-mannen, maar dat afwijkingen in de perceptuele stadia van de informatieverwerking hieraan kunnen bijdragen. Vooral tijdens selectieve aandachtsprocessen in de auditieve modaliteit lijkt er een probleem te bestaan in de afstemming tussen een sensorisch veranderingsdetectiemechanisme en actieve decision-making-mechanismen tijdens de informatieverwerking. In de controlegroep vonden we bijvoorbeeld in Hoofdstuk 5 dat een toename in sensorische veranderingsdetectie (N1- en P2-activiteit) gepaard ging met een toename in de activatie van aandachtsbronnen (N2b- en P3b-activiteit). Deze correlatie tussen vroege sensorische veranderingsdetectie- en aandachtmechanismen was afwezig bij FXS-mannen.

Wat betreft het ontstaan van syndroom-specifieke profielen in het cognitief functioneren blijft de vraag of deze problemen in de informatieverwerking karakteristiek zijn voor de FXS-etiologie. Een afname in P3b-activiteit is bijvoorbeeld ook waargenomen bij een aantal andere syndromen zoals Rett en het Prader-Willi syndroom (Stauder, Brinkman, & Curfs, 2002; Stauder, Smeets, van Mil, & Curfs, 2006). Hoewel de MMN minder vaak is onderzocht in ontwikkelingsstoornissen lijkt er ook evidentie te zijn voor een beperking in een dergelijk passief veranderingsdetectiemechanisme in autisme, ADHD en non-syndromale verstandelijke beperking (Dunn, Gomes, & Gravel, 2008; Ikeda, Hashimoto, Hayashi, & Kanno, 2009; Ikeda, Okuzumi, Hayashi, Hashimoto, & Kanno, 2000; Kemner et al., 1996). Problemen tijdens de vroege stadia van informatieverwerking lijken daarom niet specifiek voor FXS, maar kunnen een meer algemeen gevolg zijn van atypsische hersenontwikkeling.

Daarentegen lijkt de toename in N1-activiteit meer kenmerkend te zijn voor de informatieverwerking in FXS omdat in veel andere ontwikkelingsstoornissen (e.g., autisme, ADHD, Downsyndroom) juist minder N1-activiteit

7.5    **Perspectief voor toekomstig onderzoek**

Dit proefschrift draagt bij aan het begrip hoe cognitieve dysfuncties in FXS kunnen worden herleid naar problemen in de informatieverwerking in de hersenen. Uiteraard kan dit begrip worden vergroot door toekomstig onderzoek. Allereerst is het de taak van toekomstige studies om het ontwikkelingstraject van informatieverwerking in FXS in kaart te brengen. De huidige bevindingen kunnen namelijk representatief zijn voor volwassen mannen met FXS, maar er bestaat de mogelijkheid dat cognitieve informatieverwerking anders verloopt voor jongere of oudere personen met FXS. Daarnaast is het belangrijk om in vervolgonderzoek meerdere controlegroepen te includeren, bestaande uit personen met een andere ontwikkelsstoornis. Op deze manier kunnen mogelijke verschillen in cognitieve informatieverwerking worden toegeschreven aan FXS-etiologie, in plaats van een meer algemene beperking in de verstandelijke vermogens. Een dergelijke benadering is toegepast in het onderzoek dat is gepresenteerd in Hoofdstuk 3 en kan bijdragen aan een beter begrip van de relatie tussen genen, hersenen en cognitie in FXS.

Een andere taak in vervolgonderzoek is om de (neurale) mechanismen in kaart te brengen die verantwoordelijk zijn voor verhoogde sensorische hersenactiviteit in FXS. In dit proefschrift wordt aangenomen dat de verhoogde N1-activiteit tijdens stimulusdetectie een weergave is van neurale dishabituation, waarschijnlijk door een disbalans tussen excitatoire en inhibitoire activiteit in het centrale zenuwstelsel. Atypische mGluR-excitatie en GABA-erge inhibitie binnen FXS is duidelijk omschreven (Bear et al., 2004; Huber, 2007) en een ontregeling in deze neurotransmissiesystemen kan bijdragen aan de gedragsproblematiek bij personen met FXS (Heulens, D’Hulst, Braat, Rooms, & Kooy, 2010; Heulens & Kooy, 2011b). Op basis van de experimenteel gevalideerde “mGluR”-theorie wordt gesuggereerd dat een verhoogde signalering tussen groep-1-glutamaatreceptoren verantwoordelijk is voor de klinische symptomen (dat wil zeggen: angstig gedrag en epileptische activiteit) in FXS (Bear et al., 2004). Opmerkelijk is dat door stimulatie van GABA_B-receptoren middels arbaclofen, een GABA_B-receptoragonist, een
vermindering waarneembaar is in de glutamaat-signalering met als gevolg een afname van klinische symptomen in zowel de fmr1 knockout-muis en personen met FXS (Heulens & Kooy, 2011b). Mogelijkerwijs kan een farmacologische afname van de vermeende neurale hypersensitiviteit bijdragen aan efficiëntere informatieverwerking in hersenen van personen met FXS.

Een laatste suggestie voor vervolgonderzoek betreft het bestuderen van event-gerelateerde processen in de hersenen (bijvoorbeeld: oscillaties in hersenactiviteit) en beeldvormend onderzoek van structurele en functionele neurale netwerken. Bijvoorbeeld door *structural en functional magnetic resonance imaging* (sMRI en fMRI), *diffusion tensor imaging* (DTI), elektro- en magnetoencephalogram (EEG en MEG), en rustactiviteit in de hersenen. In tegenstelling tot onderzoek naar de normale ontwikkeling is er opmerkelijk weinig onderzoek gedaan naar de ontwikkeling van structurele en functionele hersennetwerken in de atypische ontwikkeling. Een mogelijke verklaring hiervoor is dat er bij dergelijk onderzoek complicaties kunnen ontstaan wanneer laagfunctionerende personen een MRI-apparaat betreden of wanneer een EEG-*cap* op hun hoofd wordt aangebracht. Overdagig bewegen en/of angstig gedrag zijn veel voorkomende obstakels voor succesvolle metingen. Hierdoor zijn langdurige meetsessies vaak niet mogelijk.

Het meten van rustactiviteit met fMRI/EEG biedt mogelijk een interessant raamwerk voor het bestuderen van de structurele en functionele kenmerken van hersenontwikkeling in zowel normale als atypische populaties (Uddin, Supekar, & Menon, 2010). Het grote voordeel van dergelijk onderzoek is dat hersenactiviteit onafhankelijk van taakprestatie bestudeerd kan worden en dat de data in een betrekkelijk korte periode kunnen worden verzameld. Patronen van rustactiviteit in de hersenen blijken erg informatief als het gaat om het onderzoeken van grootschalige structurele en functionele hersennetwerken (Douw et al., 2011; Fox & Raichle, 2007; Greicius, Supekar, Menon, & Dougherty, 2009; Stam, 2004). In fMRI-onderzoek kunnen spontane fluctuaties in het *blood oxygen level-dependent* (BOLD)-signaal worden gecorreleerd aan verschillende regio's in de hersenen waarmee vervolgens een index kan worden verkregen voor functionele netwerkconnecties (Fox &

In normale ontwikkeling is onderzoek naar rustactiviteit in de hersenen een belangrijke aanvulling geweest op het verbreden van het theoretische inzicht in hersenontwikkeling (Johnson, 2001; Johnson, 2011). Men heeft kunnen aantonen dat de sterke korte-afstand connectiviteit (vooral waar te nemen in de hersenen van kinderen) geleidelijk de lange-afstand connectiviteit bevordert (Boersma et al., 2011; Fair et al., 2007; Kelly, Uddin, Biswal, Castellanos, & Milham, 2008). Sterkere lange-afstand connectiviteit wordt juist meer waargenomen in de hersenen van volwassenen. Daarnaast heeft onderzoek aangetoond dat functionele neurale netwerken, zoals sensomotorische systemen, voorafgaan aan de ontwikkeling ten opzichte van andere neurale systemen, zoals hersennetten die betrokken zijn bij hogerorde informatieverwerkingsprocessen (Chugani, Phelps, & Mazziotta, 1987; Durston et al., 2006; Kelly et al., 2009; Lin et al., 2008). Dergelijke bevindingen over structurele en functionele hersenontwikkeling verkregen vanuit de normale ontwikkeling, kunnen worden gebruikt voor de theorievorming van de hersenontwikkeling in atypische populaties, zoals FXS. Op dit moment wordt rustactiviteit in de hersenen onderzocht bij volwassen mannen met FXS om connectiviteitspatronen in kaart te brengen van functionele hersennetten (Van der Molen, Van der Molen, Ramakers, & Stam, 2011). Resultaten uit dit onderzoek over ‘Small-World’-netwerken kunnen vervolgens worden gecorrereerd aan cognitieve prestatie om de integriteit van dergelijke functionele hersennetten te bestuderen. In de normale ontwikkeling is bijvoorbeeld aangetoond dat de path length (dat wil zeggen: de afstand tussen verschillende centra in de hersenen) in deze gemodelleerde neurale netwerken een representatieve maat is voor intelligentie (dat wil zeggen: een kortere path
length staat voor een hoger intelligentieniveau) (Langer et al., 2011; Van den Heuvel, Stam, Kahn, & Hulshoff Pol, 2009). Aangezien de neurobiologische achtergrond van FXS goed in kaart is gebracht (bijvoorbeeld: een immature ontwikkeling van corticale netwerken) zal dergelijk onderzoek naar rustactiviteit in de hersenen veelbelovende resultaten opleveren.

Tot slot wil ik graag benadrukken dat de hierboven genoemde suggesties voor vervolgonderzoek niet afzonderlijk bestudeerd dienen te worden. Om een duidelijk beeld te kunnen vormen over de impact van de afwezigheid van FMRP op de hersenontwikkeling en het cognitief functioneren kan het bijvoorbeeld informatief zijn om de ‘Small World’-resultaten uit het onderzoek van rustactiviteit van de hersenen te correleren aan patronen van GABA-erge expressie in de hersenen van personen met FXS. Op dit moment wordt met positron emissie tomografie (PET) GABA-erge expressie onderzocht in de hersenen van mannen met FXS (Heulens & Kooy, 2011a). Binnen ideaal multidisciplinair onderzoek kunnen de individuele verschillen die worden verkregen uit deze studie worden gekoppeld aan het niveau van het cognitief functioneren. Wanneer een dergelijke benadering in een ontwikkelingsperspectief wordt geplaatst, dan kan er een beter begrip ontstaan wat betreft de consequenties van de uitschakeling van één gen op cognitieve ontwikkeling.
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