Université de Montréal

Auditory and visual event-related potential alterations in fragile X syndrome

par Inga Sophia Knoth

Département de psychologie
Faculté des arts et des sciences

Mémoire présenté à la Faculté des études supérieures
en vue de l'obtention du grade de maîtrise (M.Sc.) en psychologie

Août, 2013

Résumé

Le syndrome du X fragile (SXF) est la première cause héréditaire de déficience intellectuelle et également la première cause monogénique d'autisme. Le SXF est causé par l'expansion de la répétition du nucléotide CGG sur le gène FMR1, ce qui empêche l'expression de la protéine FMRP. L'absence du FMRP mène à une altération du développement structurel et fonctionnel de la synapse, ce qui empêche la maturation des synapses induite par l'activité et l'élagage synaptique, qui sont essentiels pour le développement cérébral et cognitif. Nous avons investigué les potentiels reliés aux événements (PRE) évoqués par des stimulations fondamentales auditives et visuelles dans douze adolescents et jeunes adultes (10-22) atteints du SXF, ainsi que des participants contrôles appariés en âge chronologique et développemental. Les résultats indiquent un profil des PRE altéré, notamment l'augmentation de l'amplitude de N1 auditive, par rapport aux deux groupes contrôle, ainsi que l'augmentation des amplitudes de P2 et N2 auditifs et de la latence de N2 auditif. Chez les patients SXF, le traitement sensoriel semble être davantage perturbé qu'immature. En outre, la modalité auditive semble être plus perturbée que la modalité visuelle. En combinaison avec des résultats anatomique du cerveau, des mécanismes biochimiques et du comportement, nos résultats suggèrent une hyperexcitabilité du système nerveux dans le SXF.

Mots-clés : Syndrome du X Fragile, déficience intellectuelle, traitement des informations sensorielles, potentiels reliée aux évènements, N1

Abstract

We investigated early auditory and visual information processing in Fragile X Syndrome (FXS), the most common form of X-linked Intellectual Disability (ID) and the only known monogenetic cause of autism. FXS is caused by a trinucleotide repeat expansion in the FMR1 ('Fragile X mental retardation 1') gene, which prevents expression of the 'fragile X mental retardation protein' (FMRP). FMRP absence leads to altered structural and functional development of the synapse, while also preventing activity-based synapse maturation and synaptic pruning, which are essential for cerebral and cognitive development. We review the contribution of electrophysiological signal studies for the understanding of information processing in FXS and compare event-related potential (ERP) findings to those concerning other clinical populations that share symptoms with FXS. In our research project, we investigated ERPs evoked by basic auditory and visual stimulation in twelve adolescents and young adults (10-22) with FXS, as well as healthy chronological- and developmental- age matched controls. We found an altered ERP profile in FXS, including increased auditory N1 amplitude, relative to both control groups, as well as increased auditory P2 and N2 amplitudes and increased auditory N2 latencies. Rather than being immature, sensory processing appears to be specifically disrupted in FXS. Furthermore, the auditory modality seems to be more affected than the visual modality. In combination with brain anatomical, biochemical and behavioural findings, our results suggest a hyperexcitable nervous system in FXS.

Key words: Fragile X Syndrome, intellectual disability, sensory information processing, event-related potentials, N1

Table of Contents

List of tables

First article

Second article

List of figures

Second article

List of abbreviations

ADHD:	Attention Deficit Hyperactivity Disorder
DS:	Down Syndrome
FXS :	Fragile X Syndrome
ID :	Intellectual Disability
IQ:	Intelligence Quotient
DTI:	Diffusion Tensor Imaging
EEG:	Electroencephalography
ERP:	Event-Related Potential
fMRI:	functional Magnetic Resonance Imaging
MEG:	Magnetoencephalography
MMN:	Mismatch Negativity
MRI:	Magnetic Resonance Imaging
PET:	Positron Emission Tomography
FMR1:	Fragile X Mental Retardation 1
FMRP:	Fragile X Mental Retardation Protein
GABA:	Gamma-Aminobutyric Acid
mGluR:	metabotropic Glutamate Receptors
mRNA:	messenger Ribonucleic Acid
Ag/AgCl:	Silver chloride electrode
ANOVA:	Analysis of Variance
cd/m^2:	candela per square meter
cm:	centimeter
Hz:	Hertz
dB:	decibel
kΩ:	kiloohm
μV:	microvolt

M:	mean
ms:	millisecond
N.A.:	not available
n.s.:	not significant
s:	second
SD:	Standard Deviation

'Everything makes sense a bit at a time.
But when you try to think of it all at once,
it comes out wrong.'
- Terry Pratchett

Acknowledgements

First of all I thank my supervisor Sarah Lippé, without whom I would have not been able to realize my dream of studying and doing research in Québec. I thank her for having seen my potential from afar and fully supporting me for the whole duration of my stay. I thank her for sharing her academic wisdom with me, for her patience and generosity, for her confidence and extraordinary flexibility. Without a supervisor as passionate and dedicated as her, this project would not have been possible. A very special thanks is due to Phetsamone Vannasing, without whose magic social skills and kindness I would have hardly succeeded in testing as many patients. I also thank her for helping me with every imaginable problem I encountered and for cheering me up when needed.

I thank my colleagues for being amazingly inspiring people who gave me valuable advice and encouraged me no matter how insurmountable the obstacle seemed to be. I especially thank all of you who looked over my French and helped me progress.

I thank J. Arthur White, not only for correcting my English in every important piece of writing over the past two years, but also for all the fascinating conversations, hilarious moments and adventurous trips we had together.

I thank the Montreal Improv theatre and its community for becoming my family away from home. I cannot say enough good things about these witty, brilliant and caring people who are now among my best friends. Learning and performing at the Montreal Improv theatre is a delightful pleasure for me that I am very grateful for. Improv is my therapy.

My studies in Canada were financed by excellence scholarships from the German National Academic Foundation, the German Academic Exchange Service and an International Fee Exemptions scholarship awarded by the government of Québec.

General introduction

Learning mechanisms

Learning mechanisms and their underlying brain functions are a major field of interest and research in cognitive neuroscience. One of the most influential attempts to account for the neuronal processes underlying associative learning was introduced in the late 1940s by Donald Hebb in his book *The Organization of Behaviour* (Hebb, 2002). His theory explains the formation of memory traces through synaptic plasticity. Frequent reverberatory activity between neurons is believed to stabilize the connection between these neurons through growth or metabolic changes. Thus, memory traces are represented through neural networks (Hebb, 2002). Modern neuroimaging techniques enable us to non-invasively investigate information processing and learning mechanisms in the human brain. However, not all of these techniques have a sufficient temporal resolution to allow the investigation of rapid neural processes like postsynaptic potentials, which usually last between tens and hundreds of milliseconds (ms) (Luck, 2005). Hemodynamic measures like functional magnetic resonance imaging (fMRI) and positron emission tomography (PET), for example, are limited to a resolution of several seconds and are thus not suited for the investigation of synaptic processes. The method of choice to address neural processes is electroencephalography, the measurement of electrical activity in the brain through electrodes placed on the scalp, which is plotted in voltage over time. Neural responses associated with specific sensory, cognitive and motor events can be extracted from the electroencephalogram (EEG) using averaging techniques in order to discard random brain activity from the specific event-related potential (ERP) (Luck, 2005). ERPs are currently believed to reflect cerebral local field potentials, which are summarized postsynaptic potentials from large groups of neurons (Luck, 2005). ERP waveforms consist of a series of positive and negative voltage deflections, which are called 'ERP components' and named according to the order or latency-window in which they occur. However, it is important to note that some authors refer to underlying cerebral generator processes, which contribute to the polarity of the recorded voltage deflection, when they talk about ERP components

(Näätänen & Picton, 1987). Usually, the early components are associated with basic sensory processing and differ according to modality, whereas the later components (starting with N2) are expected to reflect more cognitive phenomena, like face (N170) and language (N400, P600) processing or error detection (error related negativity). It is possible to investigate sensory and cognitive processing using ERPs since an extensive body of research identifies a number of factors that influence the amplitude and latency of specific ERP components and can be manipulated in experiments. Further, ERP components have been found to specifically change with brain development, which makes them valuable instruments in the investigation of brain maturation (Lippé, Roy, Perchet, & Lassonde, 2007).

Intellectual disability

A complementary approach to the study of brain mechanisms in healthy individuals is to investigate pathologies presenting deficits in these mechanisms. Results obtained from pathology research can provide insight toward understanding the proper functioning found in healthy individuals, while also serving as a basis for developing treatments for the condition in question. In the study of information processing and learning mechanisms, Intellectual Disability (ID) (formerly mental retardation) is thus a disorder of particular interest, since it is 'characterized by significant limitations both in intellectual functioning and in adaptive behavior as expressed in conceptual, social and adaptive skills.' (Schalock et al., 2007). Generally, ID can be assessed using Standard Intelligence Quotient (IQ) tests with a mean of 100 and a standard deviation of 15 in combination with the Vineland adaptive Behavior Scales, and must be diagnosed before the age of 18 (Schalock et al., 2007). In this context, ID is diagnosed when IQ is assessed as <70 (i.e., at least 2 standard deviations below the mean) (Ropers, 2010) and a significant deficit in adaptive functioning is identified (Perry & Factor, 1989). However, ID cannot be considered a homogeneous condition, since it can be caused by numerous genetic and environmental factors. In 30 to 50% of cases, the cause of ID remains unknown (Daily, Ardinger, & Holmes, 2000). In order to study the underlying mechanisms of a disorder, a certain extent of phenotypic, genetic and mechanistic homogeneity is required. Thus, it is reasonable to investigate a condition with an identified cause. Among genetic

causes, X-linked recessive gene defects are believed to be accountable for approximately 10-12% of ID found in males (Ropers & Hamel, 2005). The most common form of X-linked intellectual disability is Fragile X Syndrome (FXS), which affects about 2% of male ID patients (Ropers & Hamel, 2005). Since FXS is caused by a single gene mutation, it is regarded as an important pathology in the investigation of gene-brain-behavior relationships.

Fragile X Syndrome

The physical phenotype of FXS is rather subtle, including a long face, prominent ears and hyperextensible joints (Hull & Hagerman, 1993). Over 90% of male and over 50% of female FXS individuals meet the criteria for an ID, ranging from mild to severe for male patients and from mild to moderate in females (Hessl et al., 2009). FXS patients who do not meet the criteria for ID often present learning disabilities (Loesch et al., 2003). Since cognitive growth in children with FXS is significantly slower than in typical developing children (Hall, Burns, Lightbody, & Reiss, 2008), the intellectual discrepancy increases with age, resulting in an age-dependent gradual decline in IQ (Schneider, Hagerman, & Hessl, 2009). The most severely impaired cognitive domain in FXS is executive functions, including deficits in working memory, planning and set shifting, attentional control and inhibition (K. M. Cornish et al., 2008; Schneider et al., 2009). Further, visual-spatial cognition is often impaired, including visual-spatial reasoning, object occlusion and arithmetical problem solving (K. M. Cornish et al., 2008; Farzin & Rivera, 2010; Loesch et al., 2003). Over 50% of male FXS patients meet the behavioral criteria for Attention-Deficit/Hyperactivity Disorder (ADHD), as reported by parents and teachers (Sullivan et al., 2006). Hyperarousal to sensory stimuli is especially common in FXS, while hyperactivity, impulsivity, impairment of inhibitory control and short attention span are also frequently found (Schneider et al., 2009; Sullivan et al., 2006). Many of the symptoms found in FXS are typical of the autistic spectrum; about 30% of male individuals with FXS meet the full diagnostic criteria for autism, with FXS considered the only known monogenetic cause of autism (Rogers, Wehner, & Hagerman, 2001). The main symptoms shared between FXS and autism concern abnormal behavior, lack of social cognition and language deficits (Schneider et al., 2009). Abnormal behavior in FXS includes

stereotyped behavior, self-injury, perseverative preoccupations and interests (Bregman, Leckman, & Ort, 1988), as well as delayed socialization and avoidance (Budimirovic et al., 2006). Delayed echolalia, idiosyncratic responses, abnormalities in intonation and rhythm, verbal perseveration, tangential language and cluttering of speech are examples of language deficits found in FXS (Bregman et al., 1988; K. M. Cornish et al., 2008; Sudhalter & Belser, 2001). Even though many FXS males show a broad spectrum of anxiety symptoms, they often do not meet the criteria for an established anxiety disorder enumerated in the Diagnostic and Statistical Manual of Mental Disorders. However, anxiety symptoms most frequently observed in FXS, such as poor eye contact, gaze aversion and excessive shyness are reminiscent of social phobia (Tranfaglia, 2012). Thus, a broad spectrum of functioning is found to be impaired in FXS, even though the specific symptoms, as well as their intensity, vary considerably from case to case (Schneider et al., 2009). On the other hand, vocabulary, verbal working memory and long-term memory for meaningful information appear to be well preserved in most cases (K. Cornish et al., 2005b).

Genetic mechanisms underlying FXS

FXS is caused by a CGG trinucleotide repeat expansion in the fragile X mental retardation 1 (FMR1) gene, which is located on the X-chromosome. Generally, it follows the hereditary transmission of X-chromosomal inheritance, but with some particular features. Firstly, despite their existing non-mutated X-chromosome, women can also be affected (approximately half of the prevalence found in men) but with greater variation in the phenotype expression (Bennetto, Pennington, Porter, Taylor, & Hagerman, 2001). Besides the full mutation of more than 200 trinucleotide repeats (compared to the normal length of approximately 30 triplets), a premutation with an intermediate length of between 55 and 200 repeats also exists. This premutation leads to non-penetrant carriers, who may pass on a full mutation to their child, due to the instability of the premutation in meiosis (Bassell & Warren, 2008). Normally, the FMR1 gene codes for the 'fragile x mental retardation protein' (FMRP) (Verheij et al., 1993). FMRP is a messenger ribonucleic acid (mRNA)-binding protein, which has been shown to strongly inhibit translation of various mRNAs (Laggerbauer, Ostareck,

Keidel, Ostareck-Lederer, & Fischer, 2001). The FMR1 mutation found in FXS silences the transcription of FMRP, resulting in an FMRP absence (Laggerbauer et al., 2001). According to the mGluR theory of FXS, FMRP deficit results in an exaggerated mRNA translation and thus causes continuous enhanced mGluR-dependent Long Term Depression. Consequently, the protein-synthesis in the synapses is not modified specifically to stimuli induction, resulting in a loss of protein synthesis-dependent plasticity (Bassell & Warren, 2008). Further, FMRP absence leads to altered axonal development, including increased density of dendritic spines, weak, elongated dendritic spines and immature synaptic connections (Comery et al., 1997). Based on the assumed molecular mechanisms, mGluR5 inhibitors were investigated as possible medical treatments for the FXS phenotype in several animal models (Krueger & Bear, 2011). Subsequent to findings that a number of phenotypes were reversed in animal models, a clinical pilot with human patients was carried out. No clinically significant adverse effects were detected in FXS patients after the administration of a single dose of the mGluR5 inhibitor fenobam and potentially beneficial clinical effects were discovered in half of the patients (Berry-Kravis et al., 2009). However, to this day no double-blind randomized trial with fenobam in FXS patients has been completed.

Brain anatomy in FXS

A number of structural studies have investigated brain anatomy in FXS. One of the most frequently and consistently found differences between FXS and age-matched controls is a significantly enlarged caudate nucleus in FXS patients (Lightbody & Reiss, 2009). The caudate nucleus is a structure located in the basal ganglia believed to be involved in movement, learning and memory, notably in associative learning (Packard & Knowlton, 2002), as well as in transferring information to the frontal lobe (Ring & Serra-Mestres, 2002). The alterations observed in the caudate nucleus might therefore be connected with deficits in learning, motor coordination and attention found in FXS (Lightbody & Reiss, 2009). The enlarged caudate nucleus is found early on in FXS and not only in comparison to healthy controls, but also when compared to children with idiopathic developmental delay and autism (Hazlett et al., 2009). Some authors found a difference in relative volume increase between

male and female FXS patients (Eliez, Blasey, Freund, Hastie, & Reiss, 2001; Gothelf et al., 2008), whereas others did not find these gender differences (Lee et al., 2007). Further, a positive correlation between caudate nucleus volume and aberrant behavior, as assessed by the Aberrant Behavior Checklist and the Stereotypy subscale of the Autism Behavior Checklist, has been found (Gothelf et al., 2008). The second structure in which volume alterations have been detected is the cerebellar vermis, which has been found to be consistently smaller in FXS (Lightbody & Reiss, 2009). While the cerebellum has traditionally been mainly associated with motor functioning, evidence has accumulated that it may also play a role in cognitive processes, especially spatial cognition, language production and executive functions (Rapoport, van Reekum, & Mayberg, 2000), all of which have been found to be disturbed in FXS. Since the reduced volume of the cerebellar vermis has been found early on and consistently in FXS, it has been suggested as a distinguishing feature of brain anatomy for the disorder (Hoeft et al., 2008). Further, a positive relationship between cerebellar vermis size and IQ has been found in FXS, but not in healthy controls (Gothelf et al., 2008). However, results concerning the cerebellar vermis and autistic behavior in FXS have been inconsistent, possibly due to differences in group size and diagnostic criteria (Lightbody & Reiss, 2009). Studies of FXS brain anatomy have also found a smaller superior temporal gyrus (Gothelf et al., 2008), which is involved in auditory processing, including language, and also in social cognition (Bigler et al., 2007). The amygdala has been found to be significantly smaller in children affected with FXS, even at very young ages (Hazlett et al., 2009; Kates, Abrams, Kaufmann, Breiter, & Reiss, 1997). The amygdala is known to play a central role in the mediation of emotions, particularly fear (LeDoux, 1995), and in the organization of social behavior (Adolphs, Tranel, & Damasio, 1998). Considering the autistic symptoms often found in FXS, such as social avoidance and gaze aversion, it is not surprising that the amygdala has been found to be reduced in FXS patients with autism diagnosis (Lightbody & Reiss, 2009). However, the amygdala also appears to be reduced in patients with FXS who do not show signs of autism (Hazlett et al., 2009). Alterations found in the size of the hippocampus, a structure important for memory and learning, were too inconsistent in the case of FXS to establish any general conclusions, since the hippocampus of FXS patients has been found to be larger, smaller and not different from that of controls (Lightbody & Reiss, 2009). Hoeft and colleagues found an enlarged fusiform gyrus and a decreased insula in young children with

FXS in comparison to normally developing and developmentally delayed control children (Hoeft et al., 2008). The fusiform gyrus is a cortical region specialized in face processing (Kanwisher & Yovel, 2006), while the insula is believed to be involved in interoceptive awareness, emotional responses, empathetic processes, as well as salience and cognitive control (Menon & Uddin, 2010). Aberrant maturation of the prefrontal gyri has been linked to abnormal intellectual development in FXS (Bray et al., 2011). Diffusion tensor imaging (DTI), an MRI method that maps molecular diffusion in the brain, showed a decreased white matter tract connectivity in frontostriatal pathways and parietal sensory-motor tracts in FXS females, relative to healthy controls (Barnea-Goraly et al., 2003). These pathways are believed to be involved in the mediation of sensory processes, while also affecting regulation, executive functions and motor programming (Hessl, Rivera, & Reiss, 2004) - domains that have been found to be impaired in FXS.

Functional neuroimaging in FXS

Given that, in contrast to structural studies, functional studies do not allow for sedation of participants, it is more difficult to obtain functional data from FXS patients. The behavioral phenotype of FXS patients, often including hyperactivity, anxiety, impulsivity and stereotyped behavior, makes it nearly impossible for some of them to stay motionless and attentive in an unknown and somewhat intimidating setting, as required in fMRI. Thus, most of the earlier studies focus on the more functional female FXS patients (Lightbody & Reiss, 2009). However, advantages in behavioral training and pre-test preparation in recent years have made it possible to test some male patients as well (Lightbody & Reiss, 2009). Given the gaze aversion frequently found in FXS, face and gaze processing are of particular interest in functional imaging studies. While female FXS patients appeared to process face stimuli in a relatively appropriate manner, they did not show a preference for the more socially relevant forward faces in terms of brain activation, as found in healthy controls (Garrett, Menon, MacKenzie, & Reiss, 2004). In a follow-up study with male patients, it was found that face processing was accompanied by less prefrontal cortex activity, while activity in the insula and amygdala were enhanced (Watson, Hoeft, Garrett, Hall, & Reiss, 2008). Thus, FXS patients

showed an increased sustained activation in brain regions related to emotion perception and arousal, in comparison to typically developing controls and controls with non-syndromic developmental delay. A study investigating anxiety and face processing in FXS showed that FXS patients with high reported anxiety recruited encoding, social cognition and memory related areas of the brain significantly less during face processing than FXS patients with lower levels of anxiety (Holsen, Dalton, Johnstone, & Davidson, 2008). The relationship between autism and FXS with regard to face processing has been examined by Dalton and colleagues (Dalton, Holsen, Abbeduto, & Davidson, 2008). While activation in the fusiform gyrus was comparable across FXS and autism groups, FXS patients showed more activation in the left hippocampus, the right insula, left postcentral gyrus and superior temporal gyrus than healthy and idiopathic autism control groups. These findings suggest that, despite similar behavioral outcomes, the underlying cerebral mechanisms in FXS and autism might differ. Working memory is one of the executive functions that has been investigated in FXS. Two studies with female FXS patients showed that brain areas associated with working memory (inferior and middle frontal gyri, superior parietal lobule and supramarginal gyrus) are activated during specific working memory tasks (N-back task and math calculations) (Kwon et al., 2001; Rivera, Menon, White, Glaser, & Reiss, 2002). However, unlike the healthy control group, the FXS females in both studies did not show increased brain activation in response to greater task difficulty, suggesting a failure to recruit additional resources when demanded. With regards to attention and impulse control, Hoeft and colleagues carried out a study investigating inhibition in male adolescent FXS patients using a Go/No Go fMRI task (Hoeft et al., 2007). While developmentally delayed and normally developed controls recruit a right fronto-striatal network during the response inhibition task, the FXS group demonstrated what may have been a compensatory strategy through increased ventrolateral pre-frontal cortex activity.

Electrophysiology in FXS

The recording of spontaneous electrical activity over a short period of time is generally used in a clinical context in order to diagnose epileptic activity. Epilepsy describes a set of neurologic syndromes whose predominant feature is a predisposition to recurrent unprovoked seizures (Chang & Lowenstein, 2003). In FXS, epilepsy is reported in 10 to 20% of cases, an incidence significantly larger than in the general population (<1%) (Berry-Kravis, 2002). The seizure pattern most frequently resembles benign focal epilepsy of childhood, an idiopathic age-specific epileptic syndrome that usually goes into remission in adolescence. The EEG pattern in benign childhood epilepsy features centrotemporal epileptiform foci with wide spikes that appears bi- or triphasic with a relatively high amplitude (Kramer, 2008). Thus, FMRP absence seems to cause increased neuronal excitability and susceptibility to epilepsy (Berry-Kravis, 2002).

Van der Molen and Van der Molen investigated the oscillatory dynamics during resting-state EEG in male FXS patients (M. J. Van der Molen & Van der Molen, 2013). They found an increased relative theta power and a decreased relative upper alpha power in FXS when compared to healthy controls. This is a pattern also typically found in children and adults with ADHD (Barry, Clarke, & Johnstone, 2003). Alpha rhythm in EEG is believed to play a pulsed inhibition role in cognitive processing, which gates information by reducing the processing capabilities of a given area (Jensen & Mazaheri, 2010). The reduction of alpha activity in FXS may thus be a neural marker of a hyperexcitable nervous system, since the neural inhibitory mechanism that regulates incoming sensory information is aberrant (M. J. Van der Molen & Van der Molen, 2013). The pulsed inhibition reflected by the alpha rhythm is believed to be influenced by gamma-Aminobutyric acid (GABA)ergic input from the interneural network. This supports the observation of reduced alpha oscillations in FXS, since the GABAergic system appears to be dysfunctional in FXS patients and in animal models (Paluszkiewicz, Martin, & Huntsman, 2011). The alpha/theta power abnormalities may underlie further information processing deficits, since alpha/theta synchronization has also been associated with cognitive and memory performance (Klimesch, 1999). The results obtained during resting state in FXS already indicate that electrophysiology is a promising method of investigating information processes in FXS.

After having described the behavioral and cognitive phenotype of FXS, the underlying genetic mechanisms, as well as alterations in brain anatomy and activation, the question arises: how does FXS affect the synaptic mechanism underlying information processing? How does the reduction in synaptic plasticity caused by FMRP absence affect basic sensory processing and more sophisticated cognitive processing? As described in the beginning of this section, ERPs enable us to non-invasively investigate sensory and cognitive processing in humans. A review presenting and discussing relevant ERP studies conducted with full mutation FXS patients is the first of two articles presented within the framework of this master's thesis and logically precedes the research article, which is presented second.

ERP alterations in FXS

The aim of writing the review article was to collect and discuss all results obtained thus far in relevant ERP studies investigating information processing in FXS. We then designed a study based on what has previously been found, while also adding new elements, in order to broaden knowledge of specific brain responses in FXS. Surprisingly, only five relevant ERP studies with FXS full mutation patients have been published since the 1980s. The explanation for this is most likely found in the difficulty of testing FXS patients due to the behavioral phenotype they present, a problem we also encountered in our own study. In contrast to clinical EEG, the participant cannot be sedated during EEG if brain responses to specific sensory events are to be evoked and recorded. The installation of electrodes often poses a problem, since most of the patients present social anxiety and do not like to be touched by a stranger or restricted by an EEG net. Further, participants need to remain still during the testing, since movement artifacts distort the data and recorded segments during which the participant has moved must be rejected in the analysis.

The review first gives a brief description of FXS and its underlying genetic mechanisms, as well as its cognitive profile. After a short introduction of the ERP method, the five selected studies are presented and a detailed comparison of their methods is given. In the main part of the review, each of the following ERP components is presented in detail: N1, P2, MMN, N2 and P3. A general description is given for each component, followed by the

findings of each study concerning them and possible alterations in FXS. These are then compared to findings in syndromes sharing symptoms with FXS, namely other IDs and autism. Further, the maturation of every component is described, in case the alterations found in FXS reflect an immature brain response resembling that of a younger child with the same level of cognitive functioning. Possible factors that might have influenced or caused the deviances are discussed and hypotheses concerning underlying neuronal mechanisms are proposed. Finally, controversies between studies are addressed. In the discussion and conclusion the ERP profile specific to FXS is presented, including all reported alterations. While parameters of the more cognitive components MMN, N2 and P3 appear to be generally altered in ID, the basic sensory components N1 and P2 seem to be altered more specifically in FXS. In conclusion, the review article suggests that basic stimulus processing, attentional processing, and memory formation are impaired, which is consistent with symptoms found in FXS.

Implications for our study design

Since basic sensory processing seemed to be especially impaired in FXS, we decided to choose a simple auditory and visual stimulation paradigm in order to evoke basic stimulus processing brain responses. The tasks involved were used in two earlier studies investigating the maturation of infant auditory and visual processing realized by my supervisor, Sarah Lippé (Lippé, Martinez-Montes, Arcand, & Lassonde, 2009; Lippé et al., 2007). So far, only oddball paradigms have been studied in FXS patients with full mutation. Furthermore, only one study investigated visual ERPs and the results seemed to suggest that stimulus processing in the visual modality is less affected than in the auditory modality in FXS, implying an important modality difference. Thus, we wanted to further examine visual processing in FXS by investigating basic visual ERPs.

While a number of imaging studies contains a control group with non-syndromic ID or with younger controls matching the developmental age of the patients in order to control for general effects of ID or brain immaturity, this has only been done in one of the ERP studies of FXS. It is therefore difficult to determine whether brain development in FXS remains

immature, causing ERP profiles to appear similar to younger children with the same level of cognitive functioning as the patients, or if the absence of FMRP further disrupts sensory processing, leading to an ERP profile specific to FXS. In order to differentiate these two possibilities, we not only tested a healthy age and gender matched control group, but also healthy controls in the age of cognitive functioning (developmental age) of the patients tested.

The ethics, scientific and administrative committee at the CHU Sainte-Justine Mother and Child University Hospital Center reviewed the research protocol and asked for minor specifications concerning recruitment methods, data access, neuropsychological testing, statistical analysis and number of participants as well as group sizes. In consequence, we modified the protocol and the consent form in order to obtain final permission from all committees.

Considering the expected difficulties in testing FXS patients, we decided to administer the IQ test during a home visit preceding the EEG recording at the CHU Sainte-Justine Mother and Child University Hospital Center. This allowed the patient to feel safe while meeting us first in a well-known environment and thus to develop a positive relationship with us, which was especially important for the second visit when the EEG was recorded. During the first visit, we prepared the patients for the EEG by showing them pictures and explaining the procedure in simple words. During the EEG testing we created a pleasant environment, by playing a movie in the beginning and offering the patient snacks and toys while they got used to the environment. While installing the electrodes, we proceeded effectively but carefully in order to avoid disturbing the patient more than necessary. Whenever the patient was continuously dissatisfied with the situation, we stopped the testing.

Given the high prevalence of epilepsy in FXS, we presented all patient EEG data to a neurologist. In the case of epileptic activity, data was excluded from analysis and the family doctor of the patient was contacted in order to inform the patient and schedule a follow-up meeting for an accurate diagnosis at the hospital.

Hypotheses

Based on the structural and functional alterations in neurons and synaptic plasticity caused by the FMRP absence, the cognitive phenotype, as well as the alterations in brain anatomy and brain activation described above, and in particular the ERP alterations summarized in our literature review, we assume that basic neuronal information processing is impaired in FXS.

Therefore, we expect to find an auditory and visual ERP profile in our FXS patient population that differs in several components, notably in auditory N1, P2 and N2 and visual N70, P1 and N2 amplitude, as well as in N2 latency, from the healthy control group matched to the patient group on the basis of chronological age and gender.

Since the absence of FMRP is believed to lead to altered neurodevelopment, and since aberrations in brain anatomy are found as early as one year of age, we expect that at least some of the components will not only appear immature, but specifically altered, in FXS. Thus, we expect that the auditory components N1 and P2 will not only be altered relative to the chronological control group, but also relative to a healthy control group that is matched to the developmental age of cognitive functioning of patients with ID. Component N2, however, might not differ from the developmental control group, since it is typically altered in ID and could therefore reflect an immature brain response associated with the level of cognitive functioning.

A modality difference in basic processing impairments between the auditory and visual modality in FXS has been suggested in a previous ERP study. This modality difference seems to gain further support through language deficits often found in FXS, which could be partially explained by impairments in auditory processing. Thus, we expect visual ERPs to be less aberrant in FXS than auditory ERPs, meaning that fewer components differ significantly from the control groups in the visual compared to the auditory modality.

Contributions to the articles

First article

The first article is a literature review presenting all relevant ERP studies that have thus far been conducted with patients with FXS full mutation. The objective of this article was to unveil the contribution of electrophysiological signal studies for the understanding of the information processing impairments in FXS. The literature review, as well as the initial draft of the article, were entirely carried out by Inga Sophia Knoth. Sarah Lippé's corrections and commentaries were taken into account before the manuscript was submitted to *Frontiers in Human Neuroscience* in April 2012. The article was accepted and published in November 2012.

Second article

The second article describes the research project that was realised by Inga Sophia Knoth in the framework of her Master's degree. The initial idea of the project originated from Sarah Lippé and Jacques Michaud. The EEG paradigm was created by Sarah Lippé in the framework of her PhD studies. Jacques Michaud provided his database of FXS patients for the project. Recruitment and screening of patients and controls, IQ testing and evaluation, EEG recording, EEG pre-treatment, ERP and statistical analysis were mainly carried out by Inga Sophia Knoth. Phetsamone Vannasing helped with patient EEG recording and EEG pre-treatment. Some patients were tested by neuropsychologist Domitille Malfait. Bachelor students Maude Joannette and Patricia Laniel helped with recruitment and EEG recording of control participants. The first draft of the article was entirely written by Inga Sophia Knoth. Corrections and commentaries of the co-authors have been taken into account and the manuscript is ready for submission to the *Journal of Neurodevelopmental Disorders*.

First article

Event-related potential alterations in fragile X syndrome

Published in *Frontiers in Human Neuroscience*, volume 6, september 2012

Inga Sophia Knoth[1,2] & Sarah Lippé[1,2]

[1]*Centre de Recherche CHU Ste-Justine, University of Montreal, Montreal, QC, Canada*

[2]*Centre de Recherche en Neuropsychologie et Cognition, University of Montreal, Montreal, QC, Canada*

Abstract

Fragile X Syndrome (FXS) is the most common form of X-linked intellectual disability (ID), associated with a wide range of cognitive and behavioral impairments. FXS is caused by a trinucleotide repeat expansion in the FMR1 gene located on the X-chromosome. FMR1 is expected to prevent the expression of the "fragile X mental retardation protein (FMRP)", which results in altered structural and functional development of the synapse, including a loss of synaptic plasticity. This review aims to unveil the contribution of electrophysiological signal studies for the understanding of the information processing impairments in FXS patients. We discuss relevant event-related potential (ERP) studies conducted with full mutation FXS patients and clinical populations sharing symptoms with FXS in a developmental perspective. Specific deviances found in FXS ERP profiles are described. Alterations are reported in N1, P2, Mismatch Negativity (MMN), N2, and P3 components in FXS compared to healthy controls. Particularly, deviances in N1 and P2 amplitude seem to be specific to FXS. The presented results suggest a cascade of impaired information processes that are in line with symptoms and anatomical findings in FXS.

Key words: fragile X syndrome, event-related potential, cognition, intellectual disability, autism spectrum disorders

Introduction

Intellectuel Disability and Fragile X Syndrome

Intellectual disability (ID) is among the most common and severe handicaps of childhood. It is defined as "a condition of arrested or incomplete development of the mind, which is especially characterized by impairment of skills manifested during the developmental period, skills which contribute to the overall level of intelligence, i.e., cognitive, language, motor, and social abilities" (World Health Organization, 2004). Generally, Standard Intelligence Quotient (IQ) tests with a mean of 100 and a standard deviation of 15 are used for diagnosis. In this context, ID is determined by assessing an IQ <70 (i.e., less than 2 standard deviations below the mean) (Ropers, 2010). Numerous genetic and environmental factors can cause ID. They remain unknown in 30–50% of cases (Daily et al., 2000). Among genetic causes, X-linked recessive gene defects are believed to be responsible for approximately 10–12% of ID found in males (Ropers and Hamel, 2005). The most common form of X-linked mental retardation is the Fragile X Syndrome (FXS), which affects about 2% of male ID patients (Ropers and Hamel, 2005). FXS is caused by a trinucleotide repeat expansion in the FMR1 gene, which is located on the X-chromosome. Generally, it follows the hereditary transmission of X-chromosomal inheritance, but with some particular features. Firstly, despite their existing non-mutated X- chromosome, women can also be affected (approximately half of the prevalence found in men) but with greater variation in the phenotype expression (Bennetto et al., 2001). Besides the full mutation of more than 200 repeats which underlies FXS in comparison to the normal length of 30 triplets, there also exists a premutation with an intermediate length between 55 and 200 repeats. This premutation leads to non-penetrant carriers, who may pass on a full mutation to their child, due to the instability of the premutation in meiosis (Bassell and Warren, 2008). According to the mGluR theory of FXS, the FMR1 gene prevents expression of the encoded "fragile X mental retardation protein (FMRP)" (Bear et al., 2004). Normally, FMRP is known to repress the translation of specific mRNAs in response to the activation of metabotropic Glutamate Receptors (mGluRs). In turn, mGluRs are regulated by the inhibitory GABAergic system presynaptically, a putative altered mechanism in FXS. In Fragile X patients, the absence of FMRP leads to altered structural and

functional development of the synapse. On the structural level, altered dendritic development, including increased density of dendritic spines, weak, elongated dendritic spines, and immature synaptic connections, are found in FXS patients and FXS animal models (Comery et al., 1997). Functionally, the FMRP deficit results in an exaggerated mRNA translation and thus causes continuous enhanced mGluR-dependent long-term depression. Consequently, the protein-synthesis in the synapses is not modified specifically to stimuli induction and therefore a loss of protein synthesis-dependent plasticity occurs (Bassell and Warren, 2008). The FMRP absence might therefore prevent activity-based synapse maturation and synaptic pruning, which is essential for normal brain development (Weiler and Greenough, 1999) and cognitive development (Schneider et al., 2009). In this context, the mGluR5 inhibitors were investigated as possible medical treatments for the FXS phenotype in several animal models (Krueger and Bear, 2011). Subsequent to the finding of a number of reversed phenotypes in animal models, clinical trials with human patients have been initiated and show promising preliminary results (Berry-Kravis et al., 2009).

In this review, we aim at unveiling the contribution of electro- physiological signal studies for the understanding of information processing impairments of a common intellectual deficiency syndrome, FXS.

Cognitive impairments found in FXS

The ID in FXS does not globally extend to all cognitive domains, but concerns abilities within and across specific domains, which show stability into adulthood (Cornish et al., 2008). In most cases, vocabulary, verbal working memory and long-term memory for meaningful information are well preserved (Cornish et al., 2005), whereas the cognitive and behavioral domains listed in *table I* tend to be affected frequently. Since the FXS phenotype shows great variability from case to case, the mentioned symptoms occur in some, but not all, FXS patients. In addition, the intensity of the symptoms ranges from mild to severe (Schneider et al., 2009). The deficits shown in behavior and social cognition, marked in gray within the table, are shared with disorders belonging to the autistic spectrum; about 30% of male individuals with FXS meet the diagnostic criteria for autism (Rogers et al., 2001).

Although non-exhaustive, *table I* shows a wide range of cognitive impairments in FXS patients. Most studies have investigated patients with FXS full mutation; however, it is worth mentioning that a recent study found attentionally based enumeration impairments in premutation carriers (Goodrich-Hunsaker et al., 2011). Premutation carriers may thus also present subtle cognitive impairments.

Table I. Symptoms frequently found in FXS patients sorted by domains.

Domain	Symptoms frequently found in FXS patients
Behavior	**Pervasive hyperactivity & Impulsivity** (Baumgardner, Reiss, Freund, & Abrams, 1995; Bregman et al., 1988; Schneider et al., 2009) **Stereotyped behavior, self injury, perseverative preoccupations and interest** (Bregman et al., 1988) **Poor fine and gross motor coordination** (Loesch et al., 2003) **Delayed socialization and avoidance** (Budimirovic et al., 2006)
Social cognition	**Gaze aversion** (Bregman et al., 1988; Schneider et al., 2009) **Impaired face recognition & emotion perception** (Turk & Cornish, 1998) **Theory of mind** (Garner, Callias, & Turk, 1999)
Language	**Delayed echolalia** (Bregman et al., 1988; K. Cornish et al., 2005a; Schneider et al., 2009) **Idiosyncratic responses** (Bregman et al., 1988) **Abnormalities in intonation & rhythm** (Bregman et al., 1988) **Verbal perseveration** (Bregman et al., 1988; Schneider et al., 2009) **Cluttering of speech** (K. Cornish et al., 2005a) **Tangential language** (Sudhalter & Belser, 2001)
Executive functions	**Working memory** (K. Cornish et al., 2005a; K. M. Cornish et al., 2008; Schneider et al., 2009) **Planning & set shifting** (Schneider et al., 2009) **Deficits in attentional control** (Bregman et al., 1988; K. Cornish et al., 2005a) **Inhibition** (K. Cornish et al., 2005a) **Sequential processing** (Loesch et al., 2003)
Emotional stability	**Anxiety disorders** (Bregman et al., 1988; K. Cornish et al., 2005a; Schneider et al., 2009) **Social avoidance** (K. Cornish et al., 2005a; Schneider et al., 2009) **Aggression** (Schneider et al., 2009)
Visual-spatial cognition	**Impairments in visual-spatial reasoning** (K. M. Cornish et al., 2008; Schneider et al., 2009) **Object occlusion** (Farzin & Rivera, 2010) **Arithmetic problems** (Loesch et al., 2003)
Hyperarousal	**Hyperarousal to sensory stimuli** (Schneider et al., 2009)

ERP findings in FXS

In order to address maturational abnormalities in FXS, cortical and subcortical morphology have been studied and were found to be associated with alterations in cognition (Meguid et al., 2012). Given the availability of the Event Related Potential technique and its

capacity to record local field potentials, which are summarized postsynaptic potentials from large groups of neurons (Luck, 2005), it is surprising that only a few ERP studies have addressed FXS, in which synaptic plasticity is assumed to be impaired. Indeed, five relevant ERP studies conducted with full mutation FXS patients have been published since the 1980s (St. Clair et al., 1987; Rojas et al., 2001; Castrèn et al., 2003; Van der Molen et al., 2012a,b). After a short description of the applied study designs, their findings will be presented in an order corresponding to the investigated ERP components.

Study design

Table II shows the study population characteristics in the reviewed studies. Samples varied between 5 and 28 individuals, from children to adults, and male and female frequency varied between studies' samples[1].

Table II. Study population characteristics in the reviewed studies.

	FXS patients				Healthy controls			Down Syndrome		
Study	**N**	**Age in years**	**Mental age /IQ**	**Co-morbidity**	**N**	**Age in years**	**IQ**	**N**	**Age in years**	**Mental age**
St. Clair et al., 1987	28, 2♀, 26♂	16-66 M: 43 (±13)	1.8-4.6 M: 3.08 (±0.08)	No epilepsy, no autism	83	18-75	N.A.	90 36 54	16-66 16-37 38-66	 3(±1.5) 2.17(±1.5)
Rojas et al., 2001	11, 6♀, 5♂	M: 28.95 (±2.51)	IQ: 67.55 (±5.47)	N.A.	11, 6♀, 5♂	M: 28.83 (±2.51)	127.5 (±2.9)			
Castrèn et al., 2003	5♂	7-13 M: 11.6 (±2.8) 1x 28	N.A.	No epileptic seizures, no medication	4♂	M: 10.6 (±0.6)	N.A.			
Van der Molen et al., 2011/12	16 /11 ♂	18-42 M: 29.6	7.7 (±1.6)	No medication	20/ 22♂	19-47 M: 29.2	121.5 (±25.8)			

[1] Some of the listed specifications for St. Clair's study population were detailed elsewhere (St. Clair and Blackwood, 1985; Primrose et al., 1986).

All researchers investigated full mutation FXS patients and age-matched healthy controls. However, St. Clair and colleagues included an additional control group with ID, i.e., Down syndrome (DS). This control group enabled differentiation between obtained effects that rely on the level of brain development and effects that are specific for brain mechanisms underlying FXS. Therefore, the developmental level of the FXS patients has to be considered as a confounding variable to the results of the other four studies. Both chronological and mental age show considerable variation among the reported studies, ranging from children in Castrèn's study to patients in retirement age in St. Clair's study. This variation has to be kept in mind when results between the studies are compared, since both chronological and developmental age is expected to influence ERP waves (Courchesne, 1990). The IQs reported for the control subjects in Rojas and Van der Molen's studies are strikingly high, which probably reflects the tendency to recruit controls in the university setting, since years of education are positively correlated with IQ (Rowe et al., 1998).

The higher prevalence of FXS full mutations in men is reflected in the gender distribution in the majority of the studies. By contrast, Rojas and colleagues investigated more female FXS patients (Rojas et al., 2001), which might account for the rather moderate ID found in their population compared to the other three studies which provide maturational age for their FXS patients (St. Clair et al., 1987; Van der Molen et al., 2012a,b), since the female FXS phenotype shows more variability (Bennetto et al., 2001).

The authors reported little on possible comorbidities in the investigated patients. Only St. Clair and colleagues specifically mentioned the absence of autism in their population (Primrose et al., 1986), whereas most of the other studies mainly controlled for epilepsy and medication. All participants were tested for sufficient hearing.

Experimental procedures used in the reviewed studies are listed in *table III.* All studies investigated the auditory modality. However, Van der Molen and colleagues (2012b) investigated the visual modality in their second study. Except for Rojas and colleagues (2001), all other studies made use of oddball paradigms—active in half of the cases and passive in the other half. St. Clair's group did not report the behavioral outcomes of their task, nor did they connect them with the recorded brainwaves, since they used it predominantly to check if the participants were able to perceive the difference between the standard and the deviant tone.

Table III. Comparison of the experimental procedures used in the reviewed studies.

Study	Experimental paradigm	Stimuli parameters	Task
St. Clair et al., 1987	Active auditory oddball paradigm	– Standard/Deviant tone (1000/1500 Hz) – Ratio 9:1 – Stimulus rate 1.1/sec – Intensity: 75dB binaurally through headphones – Stimulus duration: 20ms, rise/fall time 9.9ms	Count aloud infrequent tones (check of task comprehension)
Rojas et al., 2001	Presentation of pure tones	– 1000 Hz sine-wave tone – Intensity: 80dB monaurally through headphones – Stimulus duration: 30ms, rise/fall time 5ms – 4s inter-stimulus interval	No task, participants watched silent movie
Castrèn et al., 2003	1. Passive auditory oddball paradigm (only ERP to standard tones were analysed)	– Standard/deviant tone (800/560 Hz) – Ratio 8.5:1.5 – Stimulus duration: 84ms, rise/fall time 7ms – Intensity: 60dB above subject's hearing threshold, right ear through headphones – 1s inter-stimulus interval	No task, participants watched silent movie
	2. Auditory habituation	– Trains with 4 identical standard tones – 1s inter-stimulus interval – 12s inter-train interval	
Van der Molen et al., 2011	Passive auditory oddball paradigm	– 1000/1500 Hz sinusoidal tone – Deviant/standard order counterbalanced across subjects – Ratio 9:1 – Stimulus duration: 75ms, rise/fall time 5ms – Intensity: 80dB binaurally through headphones – 1sec inter-stimulus Interval	No task, participants watched silent movie
Van der Molen et al., 2012 (Task order counterbalanced across subjects)	1. Active auditory oddball paradigm	– 1000/1500 Hz sinusoidal tone – Deviant/standard order counterbalanced across subjects – Ratio 8:2 – Stimulus duration: 100ms, rise/fall time 5ms – Intensity: 80dB binaurally through headphones – 500ms inter-stimulus Interval	– Response as quickly/accurate as possible to onset of deviant stimuli by pressing space bar – Responses (hits/false alarms, reaction times) registered within a 100-1200ms time window after stimulus onset
	2. Active visual oddball paradigm	– Blue/yellow coloured smiley faces – 9.34 cd/m2, width 3.66°, height 3.68° – Centrally presented against black background (2.19 cd/m2) on a 17-inch laptop screen, 70cm distance to screen	

Rojas and colleagues (2001) used Magnetoencephalography (MEG) as opposed to EEG. Their study is nevertheless considered in this review, since MEG signals are expected to originate from the same neurophysiological processes as EEG and offer evoked field potentials equivalent to ERPs. The details of the conducted EEG/MEG recording and analysis in the reviewed studies are summarized in *table IV*. Obviously, the time span between the first study reviewed in this article, published by St. Clair and colleagues in 1987, and the most recent studies by Van der Molen and colleagues (2012a,b) has an influence on the technical

sophistication of EEG recording and analysis equipment. The number of recording electrodes has increased as well as the computational possibilities to remove artifacts. Moreover, St. Clair and colleagues did not report separate results according to standard and deviant tones, even though they claim to have analyzed them separately.

Table IV. EEG/MEG registration and analysis in the reviewed studies.

Study	Electrodes	Processing	Component Analysis
St. Clair et al., 1987	1 Ag/AgCl-electrode at Cz, earlobe electrode as reference	– Separated average for standard/deviant tones – 500 trials total	– **N1, P2, N2, P3** determined through 2 independent rater – Latencies/ amplitudes calculated separately for each FXS patient
Rojas et al., 2001	4D Neuroimaging Magnes I neuro-magnetometer system, 37 axially-wound, first-order gradiometers, right-handed Cartesian coordinate system as reference	– Signal averaged separately for each hemisphere to obtain averaged auditory evoked magnetic field – Min. 150 trials/ear	– **P50m, N100m, P200m** observed in auditory evoked field data – Source analysis
Castrèn et al., 2003	19 Ag/AgCl electrodes, 10-20 system, right mastoid electrode as reference	– Signal averaged for standard tones	– **N1, N2** determined at the highest peak amplitude site (Fz) – **Global field power**
Van der Molen et al., 2011	EasyCap electrode cap with 28 Ag/AgCl ring electrodes, left & right mastoid electrode as linked references	– Average: 895/99 resp. 892/99 (standard/ deviant) trials in controls resp. FXS patients	– **N1, P2, MMN, N2b, P3a** at F3, Fz, F4, FC1, FCz, FC2, C3, Cz, C4, P3, Pz, P4, O1, Oz, and O2 – Peak amplitude defined by the *method of local peak amplitude measurement* (Luck, 2005), relative to the pre-stimulus baseline
Van der Molen et al., 2012		– Average: Auditory task: 236/58 resp. 234/59 (standard/deviant) trials for controls resp. FXS Visual task: 216/48 resp. 212/48 trials for controls resp. FXS	

ERP components investigated

ERPs enable us to extract neural responses associated with specific sensory, cognitive, or motor events from the overall EEG (Luck, 2005). Currently, ERPs are believed to reflect cerebral local field potentials, which are summarized postsynaptic potentials from large groups of neurons (Luck, 2005). Whereas the ERP technique enables an excellent temporal solution of 1ms or better under optimal conditions, the spatial solution has to be studied with caution since the voltage measured at an electrode always reflects the summarized contributions from several different ERP generator sources (Luck, 2005).

The reviewed studies compare ERP components between FXS patients and control groups. The term "ERP component" can either simply describe the positive and negative voltage deflections within an ERP waveform according to the order or latency-window in which they occur (Luck, 2005) or it can refer to underlying cerebral generator processes, which contribute to the polarity of the recorded voltage deflection (Näätänen and Picton, 1987). Usually, the early components are related to sensory events and thus differ among modality, whereas the later components (starting with N2) are expected to reflect more cognitive phenomena. The reviewed studies reported results regarding auditory N1 and N2 (St. Clair et al., 1987; Castrèn et al., 2003; Van der Molen et al., 2012a,b), auditory P2 and P3 (St. Clair et al., 1987; Van der Molen et al., 2012a,b) and auditory and visual MNN, visual N1, P2, N2, and P3 (Van der Molen et al., 2012b). This covers most of the commonly investigated auditory components and some of the cognitive components; however, it should be mentioned that other components exist, which might also allow interesting contributions to FXS research. Some of the predominantly cognitive ones will be addressed in the discussion toward the end of this article.

N1

Description of N1

The N1 is usually not the first major sensory response. In the auditory modality, brainstem evoked responses occur within the first 10ms after stimulus onset, which are followed by midlatency components at around 10–50ms and finally an auditory P1 at about 50ms before the auditory N1 (Luck, 2005). In the visual modality, the first ERP component, the C1 wave, typically arises 40–60ms after stimulus onset and shows a positive or negative deflection depending on which part of the visual field the stimulus is presented in (Luck, 2005). So far, no study has investigated the very early sensory components in FXS patients. Nevertheless, the main purpose of studying N1 in FXS is detecting alterations in early sensory stimulus processing. The auditory N1 peaks frontocentral at around 100ms after the onset of an auditory stimulus, whereas the visual N1 peaks 30–40ms later, at about 135ms after the onset of a visual stimulus (Näätänen and Picton, 1987). Näätänen and Picton (1987) conclude

in their review that the auditory N1 consists of three "true" components upon which three other stimulus-dependent components overlap. The first subcomponent is supposed to be a frontocentral negativity generated in the auditory cortex on the superior part of the temporal lobe. The second subcomponent, the T-complex, which peaks at temporal sites and consists of a positive wave at around 100ms and a negative wave at 150ms, probably stems from the auditory association cortices in the superior temporal gyrus. Lastly, there is a subcomponent of unknown source, generating a negative wave at the vertex at around 100ms after stimulus onset, which is believed to reflect an unspecific reaction to sensory stimulation and often overlaps with the first described subcomponent.

The visual N1 was decomposed by Di Russo et al. (2002) into four subcomponents to find pairs of generator dipoles which fit the N1 complex. They suggest an occipital source for the early N150, which peaks at occipito–parietal sites and has a centro-parietal source for the fronto-central N155. The later temporo-parietal N180 and occipito-parietal N200 are expected to be associated with the early P1 sources in the lateral extrastriate cortex and the late P1 source in the ventral occipito–temporal cortex (Di Russo et al., 2002). Research interest has been focused on the effects of spatial attention (Luck et al., 2000) and discrimination processing (Vogel and Luck, 2000).

N1 findings in FXS

St. Clair and colleagues (1987) reported that N1 latency in FXS did not differ from that in healthy controls, whereas it has been found to be significantly longer in patients with DS, during the active auditory oddball paradigm. N1 amplitude was found to be generally enhanced at vertex electrode Cz in response to both standard and deviant tones in FXS patients, compared to patients with DS and healthy controls. Rojas et al. (2001) considered the N1 equivalent in MEG, the N100 m auditory-evoked field potential, in response to pure tones and also found a significantly higher amplitude in FXS patients than in healthy aged matched controls. They further observed a difference in the lateralization of the N100 m source. While healthy adults show N100 m source location asymmetry (right anterior to left), a reduction in lateralization is found in FXS patients. The authors proposed that the reduced asymmetry either reflects a non-specific neurodevelopmental disturbance which occurs during prenatal

development of cerebral asymmetry, since the phenomenon has also been found in schizophrenia (Reite et al., 1989, 1997), or stems from postnatal influences of the FXS mutation on the temporal lobe (Reiss et al., 1994). In either case, reduced N100 m source location asymmetry would be an outcome of disrupted brain development. Castrén and colleagues (2003) also found significantly larger auditory N1 amplitudes in FXS patients compared to healthy age matched controls in response to standard tones in their auditory oddball paradigm. This difference in N1 amplitude was most prominent in the frontal site Fz and was confirmed through global field power analysis. Van der Molen and colleagues (2012a) did not find any group differences of N1 latency at FCz. As for amplitude, they reported a significantly larger N1 amplitude to standard tones in FXS in a passive auditory oddball paradigm. This difference could be observed at electrodes Fz, the fronto-central FCz and Cz, whereas no differences were found for posterior sites. Further, the N1 amplitudes in controls were significantly larger for deviant than for standard tones, a difference which could not be found in FXS. Using an active oddball paradigm, a second study of Van der Molen et al. (2012b) again did not find any differences in N1 latency, neither in the auditory, nor in the visual modality. In the active auditory oddball paradigm, they reported larger N1 amplitudes for standard and deviant tones in FXS. In the active visual oddball paradigm, they found N1 peak amplitudes to be maximal at occipito-central electrode Oz in controls, but at FCz in FXS patients. At FCz the visual N1 amplitude was significantly larger for both stimuli in FXS than in controls. In both groups, visual N1 amplitude was larger at FCz than at Oz.

In addition, two groups tested habituation of N1 in response to stimulus repetition (Castrèn et al., 2003; Van der Molen et al., 2012a). Castrèn and colleagues (2003) tested short-term habituation of N1 to trains of four identical standard tones. Van der Molen and colleagues compared N1 response to late standard tones with N1 response to early standards. In both studies, controls showed a reduction of N1 amplitude after several presentations of the same tone, whereas no N1 habituation could be found in FXS patients.

Regarding behavioral results, Van der Molen's group (Van der Molen et al., 2012b) reported less accuracy, more false alarms and an increase in reaction time in FXS patients in both auditory and visual task compared to controls.

Summarizing the N1 findings in FXS, no differences in N1 latency were found, whereas all studies reported larger N1 amplitudes and a lack of N1 amplitude habituation in FXS compared to controls.

Maturation of N1

The complexity of data concerning maturational changes of N1 makes it difficult to determine if the results obtained in FXS are due to brain alterations specifically underlying FXS, or if they are, at least partially, a phenomenon of delayed brain maturation. Moreover, whereas the auditory N1 characteristics are known to change with brain maturation, studies investigating these changes obtained inconsistent results (Mueller et al., 2008). Already the time point from which an N1 response can be consistently evoked is a matter of controversy. While some researchers found a clear N1 response in children at the age of 9 (Ruhnau et al., 2011), others only obtained a visible N1 in 9-year olds by filtering out slow activity (Ceponiene et al., 2002). In children younger than 9, some researchers managed to evoke an N1 response with longer inter-stimulus-intervals (Paetau et al., 1995), but others could not identify it reliably before 5 years of age (Lippé et al., 2009). The difficulties in detecting an N1 component in children might be due to an overlap of slow P1 and N2 waves, and also to a refractoriness of N1 generators in toddlers, which decreases with age (Ceponiene et al., 2002). According to peak location, the auditory N1 was found at temporal sites in children under six (Bruneau et al., 1997) and thereupon shifted to central sites (Tonnquist-Uhlen et al., 1995), as is prominently found in adults. The results regarding auditory N1 latency are more uniform, indicating a general decrease in latency with maturation (Ladish and Polich, 1989; Gomes et al., 2001; Ceponiene et al., 2002). The visual N1 shows a U-shaped pattern in amplitude from one month to 5 years of age (Lippe et al., 2007), and then a fairly uniform decrease in amplitude (Johnson, 1989; Brecelj et al., 2002) and latency (Johnson, 1989; Lippe et al., 2007). Finally, results concerning N1 amplitude again are somehow inconsistent. Whereas some researchers found an increase in auditory N1 amplitude from 5 to 19 years (Ladish and Polich, 1989), others found an N1 decrease for target tones from 8 to 17 years (Johnstone et al., 1996), while again others could not find any differences in auditory N1 amplitude from one month to 5 years of age (Lippé et al., 2009) nor from 7 to 20 (Johnson, 1989). Gomes and

colleagues (Gomes et al., 2001) explained this inconsistency regarding auditory N1 amplitude by appeal to differences in maturation of the N1 subcomponents described above. They found no auditory N1 amplitude differences across age in what they call the central N1, which corresponds to the frontocentral N1 subcomponent described above. On the contrary, they found a lateral N1 amplitude decrease from childhood to adulthood at temporal electrodes, which corresponds to the T-complex subcomponent. This explanation is similar to Ceponiene and colleagues' account that proposed differently weighted N1 subcomponents in children and adults (Ceponiene et al., 2002).

With such controversy in N1 amplitude developmental characteristics, it is not appropriate to conclude of a delay of maturation in FXS. In fact, larger amplitude and the absence of differences in latencies do not fit the early developmental pattern of N1.

N1 in ID and autism

Since N1 maturation results are mixed, they should be considered in other clinical populations that share some of the symptoms with FXS in order to determine if the results obtained in FXS are a more general phenomenon or if they are specific to it. Patients with ID show relatively consistently prolonged auditory N1 latencies in comparison with healthy controls. This was found by Yamamori et al. (2002) in 30% of young ID patients (1–19 years) in response to randomly presented fixed and enlarged tones. Similary, Ikeada et al. (2009) found longer N1 latencies in response to simple tones in a passive auditory oddball paradigm in their adult cultural-familial type and organic (no chromosomal abnormalities) ID patients. Prolonged N1 latencies in response to an active auditory oddball paradigm have also been found in adolescents (Seidl et al., 1997) and young adults (César et al., 2010) with DS. Using a visual active discrimination task, Henderson et al. (2000) observed prolonged N1 latencies in children with phenylketonuria. These results fit well with the developmental changes of N1 latency reduction described above, suggesting that prolonged N1 latency in comparison to age-matched controls displays retardation in the development of early sensory processing. It is therefore surprising that none of the studies investigating ERP in FXS found a prolonged N1 latency, while several other forms of ID show this characteristic. We might assume differences in the cerebral perturbations underlying some forms of ID and FXS. Results obtained in

patients with autism and ID again shows a different picture. Ferri et al. (2003) investigated ERPs in subjects diagnosed with low-functioning autism and found significantly shorter N1 latencies in response to standard tones in a passive auditory oddball paradigm. The finding of normal N1 latency in FXS, whereas latency is prolonged in ID and shortened in autism, might offer a possibility to differentiate between these disorders even if we cannot yet concretely determine the underlying cerebral mechanisms.

The increase in N1 amplitude found in FXS might also be somehow specific for FXS, since the studies investigating N1 in ID (Yamamori et al., 2002; Ikeda et al., 2009), DS (Seidl et al., 1997; César et al., 2010), and phenylketonuria (Henderson et al., 2000) did not find differences in N1 amplitude between patient population and healthy controls. On the contrary, Henderson and colleagues found a smaller visual N1 in children with phenylketonuria in active discrimination tasks. Moyle et al. (2006) also found reduced visual N1 amplitudes in adults with phenylketonuria compared to healthy controls in a Go-Nogo task. In autism, the results concerning N1 amplitude are fairly inconsistent, which Bomba and colleagues (Bomba and Pang, 2004) explained in their review on auditory evoked potentials through the fact that older ERP studies did not take the developmental changes of N1 into account. More recent studies found either a reduced auditory N1 in response to randomly presented tones of varying intensity in autistic pre-school children with ID, compared to children only diagnosed with ID and healthy controls (Bruneau et al., 1999), or no difference between auditory N1 in response to a passive oddball paradigm in children and adolescents with low-functioning autism and healthy controls (Ferri et al., 2003).

Hyperarousal in FXS as possible factor influencing N1

The auditory N1 complex is supposed to be determined by physical characteristics of the stimulus, such as onset, intensity, frequency, threshold, stimulus rate, and ear of stimulation. Similarly, the appearance of the visual N1 complex is influenced by physical stimulus characteristics, such as luminance (Johannes et al., 1995). Since physical characteristics of the stimulus are always held constant between clinical population and control group, it is unlikely that they are responsible for the increased N1 amplitude found in FXS compared to control groups (St. Clair et al., 1987; Rojas et al., 2001; Castrèn et al., 2003;

Van der Molen et al., 2012a,b). However, the auditory N1 is known to be sensitive to subject factors, states of arousal, and level of performance (Näätänen and Picton, 1987). Näätänen and Picton reported several studies that found an increase in auditory N1 amplitude with higher levels of arousal and alertness. Considering the hyperarousal to sensory stimulation frequently found in FXS (Schneider et al., 2009), it seems probable that this generally higher state of arousal is reflected in an increased N1 amplitude in FXS.

The positive association between levels of performance and N1 amplitude (Näätänen and Picton, 1987) should be closely examined in this context. However, the only study reporting behavioral results and N1 characteristics in FXS is the second study by Van der Molen et al. (2012b), indicating that controls outperformed FXS patients on all behavioral measurements. Comparing the performance of FXS patients with healthy controls might not be appropriate to investigate this association. An ERP study comparing the N1 characteristics in a simple vs. a difficult task in FXS would therefore be interesting.

Habituation of ERP components in ID and autism

Habituation of the N1 component, characterized by a decrease in N1 amplitude with stimulus repetition in controls (Karhu et al., 1997), is found to be attenuated in FXS. Habituation may be based on two mechanisms. First, the unspecific arousal response to the appearance of a new stimulus, which is part of the orienting reflex (Sokolov, 1963), is decreased after repetition (Karhu et al., 1997). Second, a strengthening of selective cortical connections occurs, which is expected to reflect the neural representation of the stimulus characteristics, and thus the memory trace. Surprisingly few studies investigated habituation of ERP components in populations with intellectual disabilities besides FXS and most of them are fairly old. Psatta (1981) investigated habituation of visual-evoked potentials in response to flashes in three groups of children with ID (idiopathic, exogenous, DS) and in age-matched healthy controls. In DS, which was the most impaired group, they found an inversed pattern of habituation, characterized through an increase in amplitude instead of a decrease. The other two groups with ID showed a reduction in amplitude in the later compared with earlier trials that, however, in contrast to the control group did not reach statistical significance. Thus, even though habituation of ERP components was visible in two groups with ID, it did not occur in

the same extent as in normal controls. However, it should be kept in mind that Psatta compared the ID groups only to healthy individuals matched regarding their chronological, not their mental age. Schafer and Peeke (1982) found no habituation in auditory-evoked potentials in patients with DS in response to regularly presented clicks at electrode Cz, whereas healthy controls showed rapid habituation in the N1-P2-N2 complex. Karrer et al. (1995) investigated habituation of visual ERPs in DS using a passive oddball task with colored slides of two adult female faces serving as stimuli. They concluded that infants with DS habituate to repeated stimuli, indicated by a smaller N1 amplitude in response to frequent compared to novel trials. However, this habituation effect could only be found centrally (Cz), but not frontally (Fz). The authors explained this finding through either a different neural organization of visual discrimination in DS or a lack of habituation over the frontal cortex. As for autism, using a visual habituation and recovery paradigm, Verbaten et al. (1991) found no differences in decrease of negativity/positivity for N1, P1, N2, P3, N4, and P4 between autistic children without ID and healthy children, children with conduct disorder and children with emotional disorder. Thus, the autistic group showed no impairment in neuronal repetition suppression. A more recent study by Guiraud et al. (2011) investigated auditory habituation in infants at high-risk for autism (defined by having at least one full older sibling diagnosed with autism). They found poor habituation of P150 in response to standards in an auditory oddball paradigm in the high-risk, but not in the control group. The discrepancy between the two studies could be explained by differences between the visual and auditory modality in autism, whereas Courchesne et al. (1985) found less impairment in the processing of simple visual than auditory information in autism. However, studies targeting a high-risk group of infants should be treated with caution, since it is not clear if they will really develop an autistic spectrum disorder. Moreover, no information about the developmental stage of the subjects was given, which might have accounted more for the lack of habituation than a possible diagnosis of autism. With some qualifications, lack of neuronal habituation seems to be common in ID. Thus, the findings of an absence of N1 habituation in FXS support the hypothesis of neural adaptation being generally impaired in ID. Given that habituation is considered to be the most elementary form of learning, which occurs as early as the fetal stage (Morokuma et al., 2004), impairments in the underlying synaptic mechanisms may contribute to learning difficulties found in ID.

Alterations in brain anatomy and deficient synaptic pruning in FXS as possible basis for deviances displayed in N1

On the neuronal level, this increased N1/N100 m amplitude in FXS patients suggests that more neurons are synchronously active in response to the stimulus presentation than in healthy controls (Rojas et al., 2001). Alternatively, sensory gain control mechanisms, which have been investigated in the context of selective attention (Hillyard et al., 1995), could account for the increased N1 amplitude. Gain control could be altered in FXS, in a way that signals get amplified constantly instead of only when stimuli are expected. These alterations might be related to either early, possibly even prenatal, alteration of neurodevelopment or delayed or otherwise disrupted synaptic pruning occurring postnatally. Comparing anatomic brain alterations that are found very early in FXS to cerebral alterations occuring later in life helps differentiate between these mechanisms (Hoeft et al., 2010). Volumetric, voxel-based, and surface-based modeling approaches in magnetic resonance imagery showed among other alterations a smaller superior temporal gyri in children and adults with FXS full mutation (Gothelf et al., 2008) compared to healthy subjects. In addition, greater gray matter volumes in occipito- temporal areas have been found in infants with FXS compared to normally developed and children with non-syndromic delay (Hoeft et al., 2010). As described above, these two regions are believed to be involved in auditory/visual N1 generation. Further, FXS toddlers showed a greater gray matter increase over time in temporal and occipital areas, which was interpreted as a possible indication of deficient synaptic pruning in FXS (Hoeft et al., 2007), fitting observations in animal models (Weiler and Greenough, 1999; Pfeiffer and Huber, 2007, 2009). According to these models, reduction of unnecessary neurons and synapses and strengthening of neuronal connections in order to compensate by tempting more efficient synaptic configurations are believed to be impaired in FXS.

However, assumptions regarding the underlying brain mechanisms remain hypothetical and should be addressed by combining EEG with other brain imaging techniques.

P2

Description of P2

Similar to N1, P2 is studied in FXS in order to reveal alterations in early sensory processing. The auditory P2 is the second ERP with positive polarity, occurring after N1 with a latency of approximately 50–250ms (Crowley and Colrain, 2004). Especially in older ERP-studies, the P2 was mainly referred to in combination with the N1 component, as the N1-P2 complex or "vertex potential," but recent research suggested the potential of the P2 as a component on its own, which is the result of independent processes (Crowley and Colrain, 2004). In contrast to other components, the P2 has a similar scalp topography across auditory, somatosensory, and visual modalities, being maximal over the vertex (Crowley and Colrain, 2004). Previously, the auditory P2 sources were assumed to be located in the auditory cortex, but recent studies indicated more distributed sources, most likely in the mesencephalitic reticular activating system (Crowley and Colrain, 2004), the planum temporale, as well as the auditory association cortex (Godey et al., 2001). For the visual P2, source analyses suggested a generator in the parieto–occipital and temporal regions (Freunberger et al., 2007). Appearance of auditory P2 is influenced by stimulus characteristics like tone intensity, pitch, and inter-stimulus interval, as well as subject factors including attention and age (Crowley and Colrain, 2004). The visual P2 seems to be larger for animals than for non-animal nature scenes or simple visual patterns (Antal et al., 2000) and is also influenced by attention (Luck and Hillyard, 1994).

P2 findings in FXS

St. Clair's group (St. Clair et al., 1987) reported no differences in auditory P2 latency between FXS patients, DS patients, and healthy controls. As for amplitude, they found the P2 amplitude to be significantly larger in FXS compared to DS and healthy controls. Rojas and colleagues did not investigate the P200m responses, because they were only measureable in nine of 11 subjects in each group (Rojas et al., 2001). Similarly to St. Clair, Van der Molen and colleagues found no differences between FXS and controls concerning P2 latency. This

was the case in the passive auditory oddball paradigm (Van der Molen et al., 2012a) as well as in the active auditory and visual oddball paradigms (Van der Molen et al., 2012b). However, the latency in the active auditory oddball paradigm was found to be significantly shorter in both groups following deviant stimuli, in comparison to standard stimuli. In the passive auditory oddball paradigm, the P2 amplitude following both standard and deviant stimuli was larger at all sites in FXS than in controls (Van der Molen et al., 2012b). In controls, P2 amplitude in response to deviant stimuli was significantly smaller than in response to standard stimuli. This difference in P2 amplitude according to the probability of the stimulus could not be found in FXS. In contrast to this finding, FXS patients showed smaller P2 amplitudes following deviant stimuli in the active auditory oddball paradigm, as did controls, and P2 amplitudes were not found to be larger in FXS (Van der Molen et al., 2012b). In the visual modality, there was neither a difference between amplitudes in FXS and controls, nor did the probability of the stimulus have an effect. Consequently, the obtained P2 results are somehow inconsistent with an increased P2 amplitude in FXS only in the auditory modality, once in an active (St. Clair et al., 1987) and once in a passive paradigm (Van der Molen et al., 2012a), whereas in another study no differences in the active paradigm were found (Van der Molen et al., 2012b). Moreover, the FXS patients showed a difference in P2 amplitude between standard and deviant stimuli, but only in the active auditory paradigm, whereas controls showed this difference also in the passive paradigm. The lack of differences between visual P2 in FXS patients and controls is not discussed by the authors, but could reflect modality differences in stimulus processing, suggesting that visual processing in FXS is less impaired than auditory processing. This would be in line with the modality differences found in P3 amplitude discussed below. The most investigated influence on P2 amplitude is attention, with a decrease in P2 amplitude in response to an increase in level of attentiveness (Crowley and Colrain, 2004). Both groups show this effect in the active auditory but not in the visual oddball paradigm, in such a way that the P2 amplitude is decreased in response to deviant tones which require a behavioral response. Additionally, the controls show this difference in the passive oddball paradigm. It is possible that the controls paid more attention to the deviant tones even though no response was required, whereas the FXS patients might have been distracted by the silent movie which they watched during the task. However, this is only a hypothesis, and

factors influencing the P2 in FXS patients need further research since the results obtained so far do not allow clear conclusions.

Maturation of P2

The auditory P2 becomes a clearly distinguishable wave at all central sites at about age 10. The maximum peak shifts from Pz in younger children to Fz and Cz in older children and adults (Ponton et al., 2000). Changes in auditory P2 latency with maturation were not found by Johnstone and colleagues in children from 8 to 17 years (Johnstone et al., 1996), neither by Ponton's group in subjects from 5 to 20 (Ponton et al., 2000) or Mueller's group in different age groups between 9 and 74 (Mueller et al., 2008). However, it seems as though auditory P2 latency decreases with age between one month and 5 years of age (Lippé et al., 2009) and increases with age in adulthood (Picton et al., 1984; Anderer et al., 1996). The results concerning auditory P2 amplitude are more controversial. Johnstone and colleagues reported a P2 amplitude increase from 8 to 17 (Johnstone et al., 1996) and Mueller and colleagues found greater P2 amplitudes in the adult than in the child population (Mueller et al., 2008). Conversely, Ponton's group (Ponton et al., 2000) and Lippé's group (Lippé et al., 2009) observed a decrease in P2 amplitude across age. This controversy makes it difficult to determine if the alterations found in P2 amplitude in FXS might be caused by developmental delay.

P2 in ID and autism

Three of the reviewed investigators who studied N1 reported similarly prolonged auditory P2 latencies in subjects with ID (Yamamori et al., 2002) and DS (Seidl et al., 1997; César et al., 2010), whereas no differences in amplitude were found. In one of the few studies mentioning P2 results, Lincoln and colleagues report no differences between subjects with autism, subjects with receptive developmental language disorder and healthy controls regarding P2 amplitude or latency in response to randomly presented tones differing in frequency and intensity (Lincoln et al., 1995). Thus, the increased auditory P2 amplitude partially found in FXS might be specific to FXS, since it is not found in other forms of ID or

autism, whereas the prolonged P2 latency commonly found in ID is not observed in FXS. Congenital and developmental aberrations in the temporal lobe that affect areas believed to be involved in P2 generation (Gothelf et al., 2008; Hoeft et al., 2010) might be related to the P2 amplitude alterations found in FXS, as discussed for N1 above. Van der Molen and colleagues emphasize the influence that these early sensory processing deficits probably have on the generation of memory templates required for stimulus discrimination (Van der Molen et al., 2012a).

Mismatch Negativity – MMN

Description of MMN

In contrast to the components discussed so far, which predominantly reflect early sensory processing, the MMN is the first cognitive component. The MMN has mainly been investigated in the auditory modality and describes a negative-deflecting wave that peaks maximally at central midline scalp sites between 160 and 220ms in response to a mismatching stimulus occurring in a repetitive train of identical stimuli (Luck, 2005). Thus, the MMN reflects the brain mechanisms underlying the classification and differentiation of perceived stimuli. Two approaches explain the generation of MMN differently. Some authors describe the MMN as the outcome of a relatively automatic process not specifically requiring attention to compare incoming stimuli with a sensory memory trace of preceding stimuli (Alho, 1995). Sources are believed to lie in the auditory cortex, differing with stimulus characteristics, with supplementary sources in the frontal lobe and possibly in the hippocampus and the thalamus (Alho, 1995). In this approach, the MMN is seen as a process independent from the N1 with distinct source generators in the auditory cortex (Korzyukov et al., 1999). Other authors presented evidence suggesting a competing theory, namely, that MMN is not generated by separate auditory cortex sources, but rather arises from stimulus-specific adaptation of N1 activity (Jääskeläinen et al., 2004).

MMN findings in FXS

Castrèn and colleagues only analyzed the ERPs in response to the standard stimulus in their oddball paradigm and therefore could not report MMN results (Castrèn et al., 2003). Van der Molen and colleagues investigated the MMN with a classical passive auditory oddball paradigm (Van der Molen et al., 2012b). They found a trend for longer MMN latency in controls compared to FXS, which did not reach significance. MMN was found to maximally peak at Cz in controls and Fz in FXS patients, with significantly smaller amplitude in FXS at Cz, Pz and Oz.

Maturation of MMN

Auditory MMN is considered a developmentally stable ERP component that is already present in preterm infants (Cheour- Luhtanen et al., 1996) and thus might reflect information processing mechanisms developing very early in ontogenesis (Csepe, 1995; Cheour et al., 2000; Mueller et al., 2008). However, differences between MMN in infants and adults have been reported, including a decrease in latency with age (Cheour et al., 2000; Shafer et al., 2000; Mueller et al., 2008). Concerning MMN amplitude, smaller amplitudes in infants than in school-aged children and adults have been found (Oades et al., 1997; Cheour et al., 2000). In contrast, general (Shafer et al., 2000) or local decreases in amplitude with age (Gomot et al., 2000; Mueller et al., 2008) have also been reported. Thus, it has been suggested that the frontal system matures earlier than the sensory- specific temporal system (Gomot et al., 2000). According to these results, it might be possible that the reduced MMN amplitude found in FXS does reflect some sort of delay in brain maturation, but it cannot be said with certainty since the results concerning MMN amplitude maturation are not clear cut.

MMN in ID and autism

Attenuated MMN amplitudes are frequently found in ID. Ikeda and colleagues conducted three studies investigating MMN in adult subjects with ID, using a passive oddball paradigm with synthetic vowels and pure tones. An attenuated MMN amplitude to both kinds

of stimuli was found in patients with ID in all three studies (Ikeda et al., 2000, 2004, 2009), whereas greater MMN latencies in ID were only observed in the first study (Ikeda et al., 2000). Holopainen's group also found attenuated MMN amplitudes in a passive oddball paradigm at the individual maximal electrode for children with ID and children with dysphasia in comparison to a group with healthy control children, but no differences in latency (Holopainen et al., 1998). Nakagawa and colleagues found a smaller MMN amplitude in adults with ID compared to healthy controls in a passive oddball paradigm with fixed inter-stimulus intervals, whereas in conditions with random inter-stimulus-intervals the ID patients did not show any MMN (Nakagawa et al., 2002). By contrast, children with low-functioning autism tend to show shorter MMN latencies (Gomot et al., 2002; Ferri et al., 2003) and higher MMN amplitudes in response to novel, but not to deviant stimuli in comparison with healthy controls (Ferri et al., 2003). Therefore, it seems that MMN amplitude is generally reduced in several forms of ID, including FXS, whereas other forms of MMN alterations are found in autistic subjects with ID. In line with Jääskeläinen's theory (Jääskeläinen et al., 2004), perturbations in brain mechanisms underlying N1 would also account for alterations in MMN appearance. This may gain some support through the fact that FXS patients also show alterations in N1 amplitude. However, if the MMN is a component on its own with distinct sources in the temporal lobe, congenital, and developmental aberrations in the temporal lobe, such as those mentioned above under N1 and P2 (Gothelf et al., 2008; Hoeft et al., 2010), might contribute to MMN alterations in FXS.

N2

Description of N2

As mentioned above, N2 is one of the first cognitive components that have been studied in FXS. Several components are identified in the N2 time range. Luck differentiates between three types of N2: first, a basic N2, probably consisting of different subcomponents, and elicited by a repetitive, non-target stimulus (Näätänen and Picton, 1986); second, the MMN evoked by deviant, but task-irrelevant stimuli (sometimes also referred to as N2a); and

finally a N2b that responds to deviant target stimuli and thus is expected to reflect stimulus categorization processes (Luck, 2005). This section will primarily discuss this last type of N2, the N2b. For auditory deviant stimuli, the N2b is largest over central sites, whereas it is maximal at posterior sites for visual stimuli (Simson et al., 1977). However, it is not clear if auditory and visual N2b reflect homologous neural processes (Luck, 2005). Sources for auditory N2 in response to target and novel stimuli were suggested in the temporal lobe, in the narrow area of the auditory cortex close to N1 generators (Albrecht et al., 2000), more specifically in the superior/middle temporal gyrus (Kiehl et al., 2001). Visual N2 generators were suggested to lie in the inferior temporal cortex (Wijers et al., 1997).

N2 findings in FXS

The extent to which the N2 results of St. Clair and colleagues can be interpreted is limited. Even though they stated that they have averaged responses to frequent and rare tones separately, they only reported general N2 results, and it is not clear for which kind of stimulus the average is shown. Moreover, they did not report any behavioral results obtained through their active oddball paradigm task, since they only used it to control for whether participants were able to perceive the difference between the stimuli. Nevertheless, they found significantly longer N2 latencies in FXS and DS patients relative to healthy controls, but no differences in N2 amplitude between the three groups (St. Clair et al., 1987). Van der Molen and colleagues found that N2b maximally peaks at Oz in controls and at FCz in FXS patients in their passive auditory oddball paradigm (Van der Molen et al., 2012a). N2b latency was found to be shorter in response to deviant tones in both groups. Moreover, N2b latency was found to be longer in FXS patients in response to both stimuli compared to controls. N2b amplitude was larger in FXS than in controls, but only in response to standard stimuli. In controls, N2b amplitude differed between deviant and standard stimuli, with larger amplitude in response to deviant stimuli. This probability-based difference was not found in FXS patients. In the auditory active oddball paradigm, N2b peaked at Fz in controls and at FCz in FXS patients (Van der Molen et al., 2012b). N2b latencies were shorter in response to deviant tones in both groups, whereas FXS patients showed generally longer latencies. Larger auditory N2b amplitudes were found in FXS in response to both kinds of stimuli. In the visual

modality, N2b peaked at F3 in controls for standard stimuli and at Fz for deviant stimuli, whereas it peaked at F4 in FXS patients for both stimuli. In controls, N2b latencies were shorter in response to deviants than to standards, whereas FXS patients tended to show an inversed pattern. FXS patients showed longer N2b latencies and larger N2b amplitudes than controls. In the active auditory and visual oddball paradigms, FXS patients were generally less accurate, slower, and showed more false alarms than the control group, and they committed significantly more false alarms in the auditory compared to the visual task (Van der Molen et al., 2012b). However, ERP results were not presented separately for correct vs. incorrect answers.

Controversy in N2 results obtained in FXS

It is worth mentioning again that Van der Molen's group did not find differences in N2b amplitude in the active oddball paradigms. This absence of result is puzzling, since the N2b is supposed to be larger in response to task-relevant deviants. On the other hand, this larger N2b amplitude for deviants is observed in the passive oddball paradigm, which is normally known to elicit MMN rather than N2b. In the P2 section, it has been discussed that controls might have paid more attention to the deviant stimuli in the passive auditory paradigm, even though there were no task requirements, which could also account for the differences in N2b amplitude found in the passive oddball paradigm. Since the authors did not address this topic, it is difficult to determine which part of the paradigm might account for the missing differences between N2b amplitude in response to standards and deviants in the active paradigms. Further, this makes it more difficult to interpret alterations observed in FXS patients. Since St. Clair and colleagues also found the enhanced N2 amplitude, it could be a general phenomenon in FXS. On the other hand, Castrèn's group (Castrèn et al., 2003) found smaller N2 amplitudes in response to standard tones in FXS, which is in contrast to St. Clair and Van der Molen's findings. However, unlike St. Clair and Van der Molen's groups, Castrèn and colleagues investigated children with FXS and not adults. Further, they used a passive oddball paradigm and only report ERPs in response to standard tones. Thus, their N2 is most likely a basic N2, whereas the active paradigms of St. Clair and Van der Molen might also evoke N2b responses. St. Clair's group also found a more frontal N2 scalp distribution in

patients with FXS compared to healthy controls, which is not reported in Van der Molen's active paradigms (Van der Molen et al., 2012b), but in the passive auditory paradigm with N2b peaking at Fz in FXS and Oz in controls (Van der Molen et al., 2012a). Thus, it seems that the more frontal distribution in FXS mainly appears in passive paradigms. The data for N2 in FXS is more controversial than for other components discussed so far, which might be partially due to the fact that it is more sensitive to changes in task parameters, making it more difficult to compare studies with different experimental designs. Supplementary differences between the study designs, which might have contributed to the inconsistent results, can be consulted in *tables II, III* and *IV*.

Maturation of N2

In their review, Patel and Azzam suggest a maturation effect on N2b latency, which decreases with age and is directly associated with decreasing reaction times (Patel and Azzam, 2005). On the contrary, Mueller's group did not find differences in N2 latency between different age groups (Mueller et al., 2008). As for amplitude, a decrease in auditory N2 amplitude across age is reported (Johnstone et al., 1996; Mueller et al., 2008). Maximal N2 peak amplitude is known to move from posterior sites in infants to frontal sites in adults, beginning at approximately 14 years of age (Oades et al., 1997). Thus, the more frontal distribution of N2 in FXS children compared to controls found in passive paradigms by Castrèn's and Van der Molen's group is puzzling and cannot be explained through a delay in brain maturation. Only in Van der Molen's active auditory oddball paradigm do FXS patients show a more parietal N2b peak than controls, which would be in line with a delayed development of topography.

N2 in ID

Findings in ID fit largely with the observed decrease in N2 latency with maturation, in a way that patients with ID (Yamamori et al., 2002) and DS (Seidl et al., 1997; César et al., 2010) showed prolonged N2 latencies compared with healthy controls, and thus showed a delayed maturation of N2. This is also supported by the fact that, in patients with DS, St. Clair and

colleagues found the same N2 latency prolongation as in FXS (St. Clair et al., 1987). Thus, it seems as if the prolonged N2 latency in FXS is indeed a general phenomenon in ID, reflecting delayed brain maturation. Alterations in N2 amplitude were only reported by César et al. (2010), who found smaller N2 amplitudes in patients with DS. N2 amplitude findings are not only controversial in FXS, but also in other forms of ID, as is also the case for observations regarding the effect of brain maturation on N2 amplitude. Further research investigating N2 in well-controlled paradigms is therefore needed.

P3

Description of P3

Similar to N2, the components in the P3 time range of about 250–500ms after stimulus onset can be broken down to several distinguishable ERPs. The main distinction is made between a frontal-central maximal P3a and a parietal maximal P3b component (Squires et al., 1975), which occur after unpredictable, infrequent deviances in stimulus characteristics. The P3a component is believed to be somewhat more automatic (Squires et al., 1975) and is elicited by truly unexpected or surprising stimuli (Verleger et al., 1994). The literature focuses mainly on the P3b component, which is often simply referred to as P3. The P3b occurs in response to task-relevant shifts and is sensitive to target probability, not to physical stimuli characteristics (Picton, 1992). The P3b is generated after the stimulus categorization process, but before response selection and execution (Luck, 1998). P3b amplitude increases in proportion to the effort devoted to the task (Isreal et al., 1980), but is also decreased by task difficulty (Luck, 1998), which complicates the interpretation of the component. There is no consensus in the field about the cognitive process reflected by P3b, but one frequently discussed hypothesis is the "context updating" process suggested by Donchin (1981), according to which the P3b reflects the updating of one's representation of the environment. In Polich's theoretical framework, P3a reflects focal attention processing which facilitates context maintenance, which itself is reflected by P3b and involves working memory operations. P3 is believed to be generated through frontal and temporal/parietal brain

activation, suggesting a circuit pathway between these areas (Polich, 2007). The P3 is of particular interest in FXS, since it is known to be strongly determined by genetic factors and biological determinants (Polich, 2007).

P3 findings in FXS

St. Clair and colleagues found a longer P3 latency in FXS and DS patients in comparison to the healthy control group (St. Clair et al., 1987). Additionally, the P3 amplitude in FXS and DS was found to be significantly smaller than in healthy controls. This was consistently found in FXS patients, independently of variables such as age, percentage of fragility, and intellectual functioning. St. Clair and colleagues found the P3 to be split in different components in some of their FXS patients. Seven out of 28 FXS patients showed a P3 clearly separated into two parts and several others showed partial P3 separation. It is not clear if the separation simply goes back to the P3a and P3b components or if it is caused by a genetic factor determining the ERP profile. They explored the relation between physical dysmorphysm, i.e., facial and testicular abnormalities, and complete separation of P3, since subjects without physical dysmorphism never showed completely separated P3 components. However, the correlation failed to reach significance. Another striking feature of the waveforms was that most of the FXS and some of the DS patients generated P3 in response to both frequent and infrequent stimuli. This lack of differentiation in P3 amplitude could not be traced back to an insufficient comprehension of the two-tone discrimination task, since the 28 patients chosen for analysis were all able to identify the deviant tones.

Van der Molen and colleagues (Van der Molen et al., 2012a,b) did not report an FXS specific separation of the P3 component, but, in contrast to St. Clair and colleagues, they investigated the P3a (Van der Molen et al., 2012a) and P3b (Van der Molen et al., 2012b) component separately, since they used a passive oddball paradigm in the first and an active oddball paradigm in the second study. Van der Molen's group found a prolonged P3a latency in FXS in response to standard and deviant tones in the passive auditory oddball paradigm. They also found differences in lateralization of P3a generation. Whereas the peak amplitudes for P3a were observed at the central midline in controls, the P3a peaked maximally at the left central electrode leads in FXS patients. Controls and FXS patients both showed larger P3a

amplitudes in response to deviant in comparison to standard tones, whereas the amplitudes in response to deviant tones were larger in controls than in FXS. In the active auditory oddball paradigm, P3b latency was longer at Cz than at Oz in both groups. Additionally, P3b latency was longer in FXS patients than in controls at Cz. P3b amplitude peaked at Cz in controls for both kinds of stimuli, whereas it peaked at Oz in FXS for standard stimuli, and at Pz for deviant stimuli. FXS patients showed smaller P3b amplitudes in comparison to controls for standard and deviant tones. In the visual modality, FXS patients also showed longer P3b latencies than controls, which was significant for standard stimuli. Visual P3b peaked at Cz (standard stimuli) and Pz (deviant stimuli) in controls, and FCz (standard stimuli) and Oz (deviant stimuli) in FXS. Similar to the auditory conditions, visual P3b amplitude was significantly smaller in FXS in response to both stimuli, but was generally larger in response to deviant stimuli in both groups. Van der Molen and colleagues also found an interesting modality specific difference in FXS patients: P3b amplitude to auditory stimuli was significantly reduced in comparison to visual stimuli. The behavioral results matched the modality differences found in P3b amplitude, showing that FXS patients made fewer errors in the visual than in the auditory task. This difference was not found in controls. To assess if ERP components can predict behavioral performance, Van der Molen and colleagues carried out a regression analysis (Van der Molen et al., 2012b). The P3b amplitude relating to deviant auditory stimuli was the only ERP that could predict performance in the active oddball paradigm task. It predicted reaction time to deviant tones in FXS patients and controls, as well as the hit rate to deviant stimuli and the proportion of false alarms to standard stimuli in FXS patients. In the visual paradigm, this pattern could not be found, even though the P3b difference scores were considered to be the best explanation for the variance in reaction times to deviant stimuli in FXS patients. Since both MMN and P3a seem to be attenuated in FXS, the authors expected factors affecting the MMN component to also have an influence on the P3a component. However, linear regression analysis did not reveal a significant direct association between MMN and P3a latency or amplitude (Van der Molen et al., 2012a). To summarize, both groups investigating P3 in FXS found prolonged P3 latencies in active and passive auditory and active visual oddball paradigms (St. Clair et al., 1987; Van der Molen et al., 2012a,b). The most striking difference were the deviances in P3 amplitude observed in FXS. Even though Van der Molen and colleagues reported larger P3a and P3b amplitudes for

deviant stimuli compared to standard stimuli in both controls and FXS patients (Van der Molen et al., 2012a,b), FXS patients still showed significantly reduced P3b amplitudes in both modalities. Given that FXS patients already showed delays in N2, it is not surprising that P3 latency is also prolonged in comparison to controls. Moreover, P3 latency is known to be proportional to stimulus evaluating time and varies with individual differences in cognitive capability (Polich, 2007).

Maturation of P3 – P3 in autism

Results concerning auditory P3 maturation consistently show a decrease in latency (Goodin et al., 1978; Johnson, 1989; Ladish and Polich, 1989; Pearce et al., 1989; Fuchigami et al., 1993; Johnstone et al., 1996) and an increase in amplitude (Ladish and Polich, 1989; Johnstone et al., 1996; Mueller et al., 2008) from childhood to adolescence. The same pattern is found in visual P3 maturation (Pfueller et al., 2011). Studies investigating P3 in ID are predominantly in line with these findings, suggesting a delayed maturation of the P3 component. Ikeda and colleagues found a decrease in auditory P3 latency with an increase in IQ in response to a passive oddball paradigm in their adult ID patients (Ikeda et al., 2009). Consistently with St. Clair and colleagues' findings (St. Clair et al., 1987), a prolonged auditory P3 latency has been observed in DS (Seidl et al., 1997; César et al., 2010). Additionally, patients with DS showed no P3 habituation to repeated stimulus presentation (Seidl et al., 1997) and a lower P3 amplitude (César et al., 2010). In contrast, Henderson et al. (2000) did not find differences between children with phenylketonuria and healthy controls in visual P3 latency and amplitude in an active oddball paradigm. The authors explained this absence of differences by appeal to the good dietary phenylalanine control of the patients, which limits the severity of ID, as well as the simplicity of the task. It may also arise from modality differences in the impairments found in ID, as suggested by the modality differences found by Van der Molen's group in FXS (Van der Molen et al., 2012b). Regarding autism, Bomba and Pang summarized the most common auditory P3 findings, indicating an unaffected latency and an attenuation in amplitude (Bomba and Pang, 2004). Thus, the P3 alterations found in FXS largely fit into general P3 latency prolongation in ID and amplitude reduction in autism.

Associations between alterations in early stages of information processing and later stages of stimulus categorization in FXS

The observed P3 alterations in FXS have been explained by St. Clair and colleagues through a general malformation of limbic and associated medial temporal regions of the brain, in which P3 generators are assumed to be located (Smith et al., 1986). Part of this general malformation would be abnormal pyramidal neuronal functioning (Opitz et al., 1984). On the synaptic level, initial impairment in early stimulus processing, as reflected in N1 and P2 deviations, is likely to impair the formation of stimulus memory that is needed for the later N2, MMN, and P3 components. Thus, it is in line with these deviations that P3 latency is prolonged in FXS, since the latency of P3 is believed to reflect the duration of stimulus evaluation (Donchin, 1981). This assumed relation between the underlying synaptic impairments in the early stages of information processing, reflected through N1 and P2 enhancement, which compromises pre-attentive change detection (MMN) and stimulus categorization (N2), involuntary triggering of attention (P3a), and context updating (P3b), may also account for the deviations found in P3 amplitude. If the building of a memory trace for a stimulus was impaired, as suggested by the findings for the ERP components discussed so far, the stimulus categorization would be more difficult. This would have an influence on P3 latency and amplitude (Luck, 1998). Van der Molen and colleagues calculated the correlation between early sensory change detection components (N1 and P2) and active attentional components (N2b and P3) and found these to be positively associated in the auditory paradigms in controls, but not in FXS (Van der Molen et al., 2012b). Since there is no direct association between N1-P2 and N2b-P3, it seems probable that additional factors contribute to P3 alterations in FXS, despite the alterations found in previous components. Surprisingly, no direct association between MMN and P3a was found (Van der Molen et al., 2012a), even though it would seem plausible that pre-attentive change detection reflected by the MMN would have an effect on the mechanism detecting unattended stimuli, the cognitive process reflected by P3a. The authors contended that the missing association results from differences in the neuronal mechanisms generating the MMN and P3a (Van der Molen et al., 2012a). The MMN is believed to be generated in auditory and frontal cortices, and the P3a through frontocentral neuronal mechanisms, which also reflects assumed bottom-up (MMN) vs. top-

down (P3a) information processing (Escera and Corral, 2007). Thus, the MMN activity is not a prerequisite for P3a generation. Moreover the two components are differently affected by changes in stimulus characteristics and contextual demands (Sussman, 2007; Wetzel and Schroger, 2007).

Associations between P3b and behavioural performance in FXS

Even though only correlative and thus not necessarily causative, the association between P3b and behavioral performance measures found by Van der Molen and his group suggests that the impairments reflected by deviances in P3b characteristics may underlie the deficits in behavioral performance found in FXS (Van der Molen et al., 2012b). This is an important notion for the significance of ERP measures, since they reflect underlying neural mechanisms on the one hand and enable prediction of behavioral outcomes on the other. The differences between auditory and visual P3b amplitudes in FXS also manifested themselves in the behavioral results, since FXS patients performed significantly worse in the auditory than the visual oddball task (Van der Molen et al., 2012b). The authors discussed the possibility of a difference in the meaning of the stimuli, which might elicit more attention in the visual task (smiley faces vs. pure tones). However, this is not reflected in reaction times, which do not differ between the two tasks. Thus, Van der Molen and colleagues saw the explanation in poor auditory discrimination abilities rather than in poor task engagement (Van der Molen et al., 2012b). This is supported by findings indicating modality differences in FXS performance impairments (Sullivan et al., 2007; Van der Molen et al., 2010) and fits with the FXS modality differences found in the P2 and P3b components (Van der Molen et al., 2012b). Therefore, it can be assumed that the severity of stimulus processing impairments found in FXS varies across modalities, with the auditory modality being more affected than the visual modality. The authors matched lateralization differences found for P3a in FXS (Van der Molen et al., 2012a) to similar left lateralized brain activity during working memory tasks observed through neuroimaging studies (Hoeft et al., 2007). This could be interpreted as compensatory brain activity required for recruitment of attentional resources (Van der Molen et al., 2012a).

General discussion and conclusion

ERP alterations found in FXS: common in ID vs. specific for FXS

The ERP findings obtained in the five studies discussed above and summarized in *table V,* show that ERP is a useful measure to investigate impaired mechanisms of information processing in FXS, since several components showed a different profile in FXS patients compared to healthy controls.

Table V. Main ERP component findings in FXS patients compared with healthy controls.

Component	Latency	Amplitude
N1	**No difference** (Castrèn, Paakkonen, Tarkka, Ryynanen, & Partanen, 2003; Rojas et al., 2001; St Clair, Blackwood, Oliver, & Dickens, 1987; M. J. Van der Molen et al., 2011, 2012a)	**Increased** (Castrèn et al., 2003; Rojas et al., 2001; St Clair et al., 1987; M. J. Van der Molen et al., 2011, 2012a) **No habituation** (Castrèn et al., 2003; M. J. Van der Molen et al., 2011)
P2	**No difference** (St Clair et al., 1987; Van der Molen et al., 2011; 2012)	**Inconsistent** *Increased* (St Clair et al., 1987; Van der Molen et al., 2011) *No difference* (Van der Molen, 2012)
MMN	**No difference, Trend: prolonged, n.s.** (Van der Molen et al., 2011)	**Decreased** (Van der Molen et al., 2011)
N2	**Prolonged** (St. Clair et al., 1987; Van der Molen, 2011; 2012)	**Inconsistent** *No difference* (St. Clair et al., 1987) *Increased* (Van der Molen, 2011; 2012)
P3	**Prolonged** (St Clair et al., 1987; Van der Molen, 2011; 2012)	**Decreased** (St. Clair et al., 1987. Van der Molen, 20122; 2012)

However, reported results were not always consistent, especially in N2, for which two groups found enhanced amplitudes and one group reduced amplitudes, which might have been due to differences in study design. However, comparisons with studies investigating the development of ERPs with age suggest that some of the alterations might be caused by a general delay of brain maturation. According to the findings presented in this review, this could particularly concern MMN, N2, and P3. Further, some of the alterations might be

common in clinical populations sharing symptoms with FXS, like other forms of ID or autism. Indications for general alterations in ID are found for N1 habituation, MMN amplitude, N2 and P3 latency and P3 amplitude. In contrast to that, N1 and P2 amplitude alterations seem more FXS specific. It is possible that distinct syndrome-specific perturbations in early sensory processes influence later components in similar ways. To address this topic, it would be advisable to consider supplementary control groups, matching the FXS patients' stage of mental development. This could be done using either patients with other forms of ID or chronologically younger healthy controls. Furthermore, it would be interesting to study different age groups with FXS to investigate the developmental course of ERP components in FXS.

Cascade of impaired neuronal mechanisms as a basis for symptoms in FXS

ERP results obtained so far in FXS consistently show a cascade of impaired mechanisms in electrical summation necessary for basic stimulus processing, attentional processing, and memory formation. This is consistent with some of the symptoms found in FXS, as attentional problems might be explained through synaptic processes probably also underlying the ERP deviances. Further, the described cascade of impaired mechanisms could be the basis for other symptoms found in FXS. For example, hyperarousal, hyperactivity, and anxiety in FXS might be related to neural hyperreactivity in response to sensory stimuli. Moreover, the formation of a cerebral stimulus representation might be impaired through synaptic dysfunction. It is likely that this difficulty in memory formation affects further learning, which then results in cognitive deficits.

Future directions

All in all, the ERP results fit with the symptoms found in FXS, as well as the anatomical findings obtained through brain imaging studies and assumptions concerning underlying neuronal mechanisms gained in animal models. Until now only a few FXS ERP studies have been published, so much remains to be discovered. Since existing ERP studies mainly focused on the auditory modality, other modalities should be investigated. The results

obtained so far suggest that processing impairments vary across modalities. Moreover, deficits in the domain of social cognition could be addressed by using stimuli with more social relevance, like human voices or faces. It would be of particular interest to study other ERP components that are related to cognitive processes known to be impaired in FXS. For example, the face-specific N170 would be a promising candidate, since some evidence for impaired face recognition in FXS is reported (Turk and Cornish, 1998). Further, language-related ERPs like the N400, which occurs in response to violations of semantic expectations (Luck, 2005), or the P600, which is evoked by syntactic violations, would be interesting, since language is among the most impaired cognitive functions in FXS. Additionally, habituation of more ERP components besides N1 could be investigated. Finally, ERP studies might be helpful as outcome measures in clinical trials to assess the influence of medical treatment on the synaptic mechanisms reflected by ERP components.

References

Albrecht, R., Suchodoletz, W., & Uwer, R. (2000). The development of auditory evoked dipole source activity from childhood to adulthood. *Clinical Neurophysiology, 111*(12), 2268-2276.

Alho, K. (1995). Cerebral generators of mismatch negativity (MMN) and its magnetic counterpart (MMNm) elicited by sound changes. *Ear and Hearing, 16*(1), 38-51.

Anderer, P., Semlitsch, H. V., & Saletu, B. (1996). Multichannel auditory event-related brain potentials: effects of normal aging on the scalp distribution of N1, P2, N2 and P300 latencies and amplitudes. *Electroencephalography and Clinical Neurophysiology, 99*(5), 458-472.

Antal, A., Keri, S., Kovacs, G., Janka, Z., & Benedek, G. (2000). Early and late components of visual categorization: an event-related potential study. *Cognitive Brain Research, 9*(1), 117-119.

Bassell, G. J., & Warren, S. T. (2008). Fragile X syndrome: loss of local mRNA regulation alters synaptic development and function. *Neuron, 60*(2), 201-214.

Baumgardner, T. L., Reiss, A. L., Freund, L. S., & Abrams, M. T. (1995). Specification of the neurobehavioral phenotype in males with fragile X syndrome. *Pediatrics, 95*(5), 744-752.

Bear, M. F., Huber, K. M., & Warren, S. T. (2004). The mGluR theory of fragile X mental retardation. *Trends in Neuroscience, 27*(7), 370-377.

Bennetto, L., Pennington, B. F., Porter, D., Taylor, A. K., & Hagerman, R. J. (2001). Profile of cognitive functioning in women with the fragile X mutation. *Neuropsychology, 15*(2), 290-299.

Berry-Kravis, E., Hessl, D., Coffey, S., Hervey, C., Schneider, A., Yuhas, J., . . . Hagerman, R. (2009). A pilot open label, single dose trial of fenobam in adults with fragile X syndrome. *Journal of Medical Genetics, 46*(4), 266-271.

Bomba, M. D., & Pang, E. W. (2004). Cortical auditory evoked potentials in autism: a review. *International Journal of Psychophysiology, 53*(3), 161-169.

Brecelj, J., Strucl, M., Zidar, I., & Tekavcic-Pompe, M. (2002). Pattern ERG and VEP maturation in schoolchildren. *Clinical Neurophysiology, 113*(11), 1764-1770.

Bregman, J. D., Leckman, J. F., & Ort, S. I. (1988). Fragile X syndrome: genetic predisposition to psychopathology. *Journal of Autism and Developmental Disorders, 18*(3), 343-354.

Bruneau, N., Roux, S., Adrien, J. L., & Barthelemy, C. (1999). Auditory associative cortex dysfunction in children with autism: evidence from late auditory evoked potentials (N1 wave-T complex). *Clinical Neurophysiology, 110*(11), 1927-1934.

Bruneau, N., Roux, S., Guerin, P., Barthelemy, C., & Lelord, G. (1997). Temporal prominence of auditory evoked potentials (N1 wave) in 4-8-year-old children. *Psychophysiology, 34*(1), 32-38.

Budimirovic, D. B., Bukelis, I., Cox, C., Gray, R. M., Tierney, E., & Kaufmann, W. E. (2006). Autism spectrum disorder in Fragile X syndrome: differential contribution of adaptive socialization and social withdrawal. *American Journal of Medical Genetics Part A, 140A*(17), 1814-1826.

Castrèn, M., Paakkonen, A., Tarkka, I. M., Ryynanen, M., & Partanen, J. (2003). Augmentation of auditory N1 in children with fragile X syndrome. *Brain Topography, 15*(3), 165-171.

Ceponiene, R., Rinne, T., & Näätänen, R. (2002). Maturation of cortical sound processing as indexed by event-related potentials. *Clinical Neurophysiology, 113*(6), 870-882.

César, C. P., Caovilla, H. H., Munhoz, M. S., & Gananca, M. M. (2010). Late auditory event-related evoked potential (P300) in Down's syndrome patients. *Brazilian Journal of Otorhinolaryngology, 76*(2), 206-212.

Cheour, M., Leppanen, P. H., & Kraus, N. (2000). Mismatch negativity (MMN) as a tool for investigating auditory discrimination and sensory memory in infants and children. *Clinical Neurophysiology, 111*(1), 4-16.

Cheour-Luhtanen, M., Alho, K., Sainio, K., Rinne, T., Reinikainen, K., Pohjavuori, M., . . . Naatanen, R. (1996). The ontogenetically earliest discriminative response of the human brain. *Psychophysiology, 33*(4), 478-481.

Comery, T. A., Harris, J. B., Willems, P. J., Oostra, B. A., Irwin, S. A., Weiler, I. J., & Greenough, W. T. (1997). Abnormal dendritic spines in fragile X knockout mice: maturation and pruning deficits. *Proceedings of the National Academy of Sciences United States of America, 94*(10), 5401-5404.

Cornish, K., Kogan, C., Turk, J., Manly, T., James, N., Mills, A., & Dalton, A. (2005). The emerging fragile X premutation phenotype: evidence from the domain of social cognition. *Brain and Cognition, 57*(1), 53-60.

Cornish, K. M., Li, L., Kogan, C. S., Jacquemont, S., Turk, J., Dalton, A., . . . Hagerman, P. J. (2008). Age-dependent cognitive changes in carriers of the fragile X syndrome. *Cortex, 44*(6), 628-636.

Courchesne, E. (1990). Chronology of postnatal human brain development: Event-related potential, positron emission tomography, myelinogenesis, and synaptogenesis studies. In J. W. Rohrbaugh, R. Parasuraman & R. Johnson, Jr. (Eds.), *Event-related brain potentials: Basic issues and applications* (pp. 210-241). New York, NY, US: Oxford University Press.

Courchesne, E., Lincoln, A. J., Kilman, B. A., & Galambos, R. (1985). Event-related brain potential correlates of the processing of novel visual and auditory information in autism. *Journal of Autism and Developmental Disorders, 15*(1), 55-76.

Crowley, K. E., & Colrain, I. M. (2004). A review of the evidence for P2 being an independent component process: age, sleep and modality. *Clinical Neurophysiology, 115*(4), 732-744.

Csepe, V. (1995). On the origin and development of the mismatch negativity. *Ear and Hearing, 16*(1), 91-104.

Daily, D. K., Ardinger, H. H., & Holmes, G. E. (2000). Identification and evaluation of mental retardation. *American Family Physician, 61*(4), 1059-1067, 1070.

Di Russo, F., Martínez, A., Sereno, M. I., Pitzalis, S., & Hillyard, S. A. (2002). Cortical sources of the early components of the visual evoked potential. *Human Brain Mapping, 15*(2), 95-111.

Donchin, E. (1981). Surprise!...Surprise? *Psychophysiology, 18*(5), 493-513.

Escera, C., & Corral, M. J. (2007). Role of mismatch negativity and novelty-P3 in involuntary auditory attention. *Journal of Psychophysiology, 21*(3-4), 251-264.

Farzin, F., & Rivera, S. M. (2010). Dynamic Object Representations in Infants with and without Fragile X Syndrome. *Frontiers in Human Neuroscience, 4*, 12.

Ferri, R., Elia, M., Agarwal, N., Lanuzza, B., Musumeci, S. A., & Pennisi, G. (2003). The mismatch negativity and the P3a components of the auditory event-related potentials in autistic low-functioning subjects. *Clinical Neurophysiology, 114*(9), 1671-1680.

Freunberger, R., Klimesch, W., Doppelmayr, M., & Holler, Y. (2007). Visual P2 component is related to theta phase-locking. *Neuroscience Letters, 426*(3), 181-186.

Fuchigami, T., Okubo, O., Fujita, Y., Okuni, M., Noguchi, Y., & Yamada, T. (1993). Auditory event-related potentials and reaction time in children: evaluation of cognitive development. *Developmental Medicine & Child Neurology, 35*(3), 230-237.

Garner, C., Callias, M., & Turk, J. (1999). Executive function and theory of mind performance of boys with fragile-X syndrome. *Journal of Intellectual Disability Research, 43 (Pt 6)*, 466-474.

Godey, B., Schwartz, D., de Graaf, J. B., Chauvel, P., & Liegeois-Chauvel, C. (2001). Neuromagnetic source localization of auditory evoked fields and intracerebral evoked potentials: a comparison of data in the same patients. *Clinical Neurophysiology, 112*(10), 1850-1859.

Gomes, H., Dunn, M., Ritter, W., Kurtzberg, D., Brattson, A., Kreuzer, J. A., & Vaughan, H. G., Jr. (2001). Spatiotemporal maturation of the central and lateral N1 components to tones. *Developmental Brain Research, 129*(2), 147-155.

Gomot, M., Giard, M. H., Adrien, J. L., Barthelemy, C., & Bruneau, N. (2002). Hypersensitivity to acoustic change in children with autism: electrophysiological evidence of left frontal cortex dysfunctioning. *Psychophysiology, 39*(5), 577-584.

Gomot, M., Giard, M. H., Roux, S., Barthelemy, C., & Bruneau, N. (2000). Maturation of frontal and temporal components of mismatch negativity (MMN) in children. *Neuroreport, 11*(14), 3109-3112.

Goodin, D. S., Squires, K. C., Henderson, B. H., & Starr, A. (1978). Age-related variations in evoked potentials to auditory stimuli in normal human subjects. *Electroencephalography and Clinical Neurophysiology, 44*(4), 447-458.

Goodrich-Hunsaker, N. J., Wong, L. M., McLennan, Y., Tassone, F., Harvey, D., Rivera, S. M., & Simon, T. J. (2011). Adult Female Fragile X Premutation Carriers Exhibit Age- and CGG Repeat Length-Related Impairments on an Attentionally Based Enumeration Task. *Frontiers in Human Neuroscience, 5*, 63.

Gothelf, D., Furfaro, J. A., Hoeft, F., Eckert, M. A., Hall, S. S., O'Hara, R., . . . Reiss, A. L. (2008). Neuroanatomy of fragile X syndrome is associated with aberrant behavior and the fragile X mental retardation protein (FMRP). *Annals of Neurology, 63*(1), 40-51.

Guiraud, J. A., Kushnerenko, E., Tomalski, P., Davies, K., Ribeiro, H., & Johnson, M. H. (2011). Differential habituation to repeated sounds in infants at high risk for autism. *Neuroreport, 22*(16), 845-849.

Henderson, R. M., McCulloch, D. L., Herbert, A. M., Robinson, P. H., & Taylor, M. J. (2000). Visual event-related potentials in children with phenylketonuria. *Acta Paediatrica, 89*(1), 52-57.

Hillyard, S. A., Mangun, G. R., Woldorff, M. G., & Luck, S. J. (1995). Neural Systems Mediating Selective Attention. In M. S. Gazzaniga (Ed.), *The Cognitive Neurosciences* (pp. 665-681). Cambridge, MA: MIT Press.

Hoeft, F., Carter, J. C., Lightbody, A. A., Cody Hazlett, H., Piven, J., & Reiss, A. L. (2010). Region-specific alterations in brain development in one- to three-year-old boys with fragile X syndrome. *Proceedings of the National Academy of Sciences of the United States of America, 107*(20), 9335-9339.

Hoeft, F., Hernandez, A., Parthasarathy, S., Watson, C. L., Hall, S. S., & Reiss, A. L. (2007). Fronto-striatal dysfunction and potential compensatory mechanisms in male adolescents with fragile X syndrome. *Human Brain Mapping, 28*(6), 543-554.

Holopainen, I. E., Korpilahti, P., Juottonen, K., Lang, H., & Sillanpaa, M. (1998). Abnormal frequency mismatch negativity in mentally retarded children and in children with developmental dysphasia. *Journal of Child Neurology, 13*(4), 178-183.

Ikeda, K., Hashimoto, S., Hayashi, A., & Kanno, A. (2009). ERP evaluation of auditory sensory memory systems in adults with intellectual disability. *International Journal of Neuroscience, 119*(6), 778-791.

Ikeda, K., Hayashi, A., Hashimoto, S., & Kanno, A. (2004). Distinctive MMN relative to sound types in adults with intellectual disability. *Neuroreport, 15*(6), 1053-1056.

Ikeda, K., Okuzumi, H., Hayashi, A., Hashimoto, S., & Kanno, A. (2000). Automatic auditory processing and event-related brain potentials in persons with mental retardation. *Perceptual Motor Skills, 91*(3 Pt 2), 1145-1150.

Isreal, J. B., Chesney, G. L., Wickens, C. D., & Donchin, E. (1980). P300 and tracking difficulty: evidence for multiple resources in dual-task performance. *Psychophysiology, 17*(3), 259-273.

Jääskeläinen, I. P., Ahveninen, J., Bonmassar, G., Dale, A. M., Ilmoniemi, R. J., Levanen, S., . . . Belliveau, J. W. (2004). Human posterior auditory cortex gates novel sounds to consciousness. *Proceedings of the National Academy of Sciences of the United States of America, 101*(17), 6809-6814.

Johannes, S., Munte, T. F., Heinze, H. J., & Mangun, G. R. (1995). Luminance and spatial attention effects on early visual processing. *Brain Res Cogn Brain Res, 2*(3), 189-205.

Johnson, R., Jr. (1989). Developmental evidence for modality-dependent P300 generators: a normative study. *Psychophysiology, 26*(6), 651-667.

Johnstone, S. J., Barry, R. J., Anderson, J. W., & Coyle, S. F. (1996). Age-related changes in child and adolescent event-related potential component morphology, amplitude and latency to standard and target stimuli in an auditory oddball task. *International Journal of Psychophysiology, 24*(3), 223-238.

Karhu, J., Herrgard, E., Paakkonen, A., Luoma, L., Airaksinen, E., & Partanen, J. (1997). Dual cerebral processing of elementary auditory input in children. *Neuroreport, 8*(6), 1327-1330.

Karrer, R., Wojtascek, Z., & Davis, M. G. (1995). Event-related potentials and information processing in infants with and without Down syndrome. *American Journal of Mental Retardation, 100*(2), 146-159.

Kiehl, K. A., Laurens, K. R., Duty, T. L., Forster, B. B., & Liddle, P. F. (2001). Neural sources involved in auditory target detection and novelty processing: an event-related fMRI study. *Psychophysiology, 38*(1), 133-142.

Korzyukov, O., Alho, K., Kujala, A., Gumenyuk, V., Ilmoniemi, R. J., Virtanen, J., . . . Naatanen, R. (1999). Electromagnetic responses of the human auditory cortex generated by sensory-memory based processing of tone-frequency changes. *Neuroscencei Letters, 276*(3), 169-172.

Krueger, D. D., & Bear, M. F. (2011). Toward fulfilling the promise of molecular medicine in fragile X syndrome. *Annual Review of Medicine, 62*, 411-429.

Ladish, C., & Polich, J. (1989). P300 and probability in children. *Journal of Experimental Child Psychology, 48*(2), 212-223.

Lincoln, A. J., Courchesne, E., Harms, L., & Allen, M. (1995). Sensory modulation of auditory stimuli in children with autism and receptive developmental language disorder: event-related brain potential evidence. *Journal of Autism and Developmental Disorders, 25*(5), 521-539.

Lippé, S., Martinez-Montes, E., Arcand, C., & Lassonde, M. (2009). Electrophysiological study of auditory development. *Neuroscience, 164*(3), 1108-1118.

Lippé, S., Roy, M. S., Perchet, C., & Lassonde, M. (2007). Electrophysiological markers of visuocortical development. *Cerebral Cortex, 17*(1), 100-107.

Loesch, D. Z., Bui, Q. M., Grigsby, J., Butler, E., Epstein, J., Huggins, R. M., . . . Hagerman, R. J. (2003). Effect of the fragile X status categories and the fragile X mental retardation protein levels on executive functioning in males and females with fragile X. *Neuropsychology, 17*(4), 646-657.

Luck, S. J. (1998). Sources of dual-task interference: Evidence From Human Electrophysiology. *Psychological Science, 9*(3), 223-227.

Luck, S. J. (2005). *An introduction to the event-related potential technique*. Cambridge, Ma: MIT Press.

Luck, S. J., & Hillyard, S. A. (1994). Electrophysiological correlates of feature analysis during visual search. *Psychophysiology, 31*(3), 291-308.

Luck, S. J., Woodman, G. F., & Vogel, E. K. (2000). Event-related potential studies of attention. *Trends in Cognitive Science, 4*(11), 432-440.

Meguid, N. A., Fahim, C., Sami, R., Nashaat, N. H., Yoon, U., Anwar, M., . . . Evans, A. C. (2012). Cognition and lobar morphology in full mutation boys with fragile X syndrome. *Brain and Cognition, 78*(1), 74-84.

Morokuma, S., Fukushima, K., Kawai, N., Tomonaga, M., Satoh, S., & Nakano, H. (2004). Fetal habituation correlates with functional brain development. *Behaviuoral Brain Research, 153*(2), 459-463.

Moyle, J. J., Fox, A. M., Bynevelt, M., Arthur, M., & Burnett, J. R. (2006). Event-related potentials elicited during a visual Go-Nogo task in adults with phenylketonuria. *Clinical Neurophysiology, 117*(10), 2154-2160.

Mueller, V., Brehmer, Y., von Oertzen, T., Li, S. C., & Lindenberger, U. (2008). Electrophysiological correlates of selective attention: a lifespan comparison. *BMC Neuroscience, 9*, 18.

Näätänen, R., & Picton, T. (1987). The N1 wave of the human electric and magnetic response to sound: a review and an analysis of the component structure. *Psychophysiology, 24*(4), 375-425.

Näätänen, R., & Picton, T. W. (1986). N2 and automatic versus controlled processes. *Electroencephalography and clinical neurophysiology. Supplement, 38*, 169-186.

Nakagawa, K., Shoji, H., Katada, A., & Ozaki, H. (2002). How regularities in stimulus series and in ISI affect on MMN in persons with intellectual disabilities. *International Congress Series, 1232*(0), 363-366.

Oades, R. D., Dittmann-Balcar, A., & Zerbin, D. (1997). Development and topography of auditory event-related potentials (ERPs): mismatch and processing negativity in individuals 8-22 years of age. *Psychophysiology, 34*(6), 677-693.

Opitz, J. M., Westphal, J. M., & Daniel, A. (1984). Discovery of a connective tissue dysplasia in the Martin-Bell syndrome. *American Journal of Medical Genetics, 17*(1), 101-109.

Paetau, R., Ahonen, A., Salonen, O., & Sams, M. (1995). Auditory evoked magnetic fields to tones and pseudowords in healthy children and adults. *Journal of Clinical Neurophysiology, 12*(2), 177-185.

Patel, S. H., & Azzam, P. N. (2005). Characterization of N200 and P300: selected studies of the Event-Related Potential. *International Journal of Medical Science, 2*(4), 147-154.

Pearce, J. W., Crowell, D. H., Tokioka, A., & Pacheco, G. P. (1989). Childhood developmental changes in the auditory P300. *Journal of Child Neurology, 4*(2), 100-106.

Pfeiffer, B. E., & Huber, K. M. (2007). Fragile X mental retardation protein induces synapse loss through acute postsynaptic translational regulation. *Journal of Neuroscience 27*(12), 3120-3130.

Pfeiffer, B. E., & Huber, K. M. (2009). The state of synapses in fragile X syndrome. *Neuroscientist, 15*(5), 549-567.

Pfueller, U., Oelkers-Ax, R., Gmehlin, D., Parzer, P., Roesch-Ely, D., Weisbrod, M., & Bender, S. (2011). Maturation of P300 amplitude and short-term learning as reflected

by P300 habituation between trial blocks in children. *International Journal of Psychophysiology, 79*(2), 184-194.

Picton, T. W. (1992). The P300 wave of the human event-related potential. *Journal of Clinical Neurophysiology, 9*(4), 456-479.

Picton, T. W., Stuss, D. T., Champagne, S. C., & Nelson, R. F. (1984). The effects of age on human event-related potentials. *Psychophysiology, 21*(3), 312-325.

Polich, J. (2007). Updating P300: an integrative theory of P3a and P3b. *Clinical Neurophysiology, 118*(10), 2128-2148.

Ponton, C. W., Eggermont, J. J., Kwong, B., & Don, M. (2000). Maturation of human central auditory system activity: evidence from multi-channel evoked potentials. *Clinical Neurophysiology, 111*(2), 220-236.

Primrose, D. A., el-Matmati, R., Boyd, E., Gosden, C., & Newton, M. (1986). Prevalence of the fragile X syndrome in an institution for the mentally handicapped. *British Journal of Psychiarty, 148*, 655-657.

Psatta, D. M. (1981). Visual evoked potential habituation in mental deficiency. *Biological Psychiatry, 16*(8), 729-740.

Reiss, A. L., Lee, J., & Freund, L. (1994). Neuroanatomy of fragile X syndrome: the temporal lobe. *Neurology, 44*(7), 1317-1324.

Reite, M., Sheeder, J., Teale, P., Adams, M., Richardson, D., Simon, J., . . . Rojas, D. C. (1997). Magnetic source imaging evidence of sex differences in cerebral lateralization in schizophrenia. *Archives of General Psychiatry, 54*(5), 433-440.

Reite, M., Teale, P., Goldstein, L., Whalen, J., & Linnville, S. (1989). Late auditory magnetic sources may differ in the left hemisphere of schizophrenic patients. A preliminary report. *Archives of General Psychiatry, 46*(6), 565-572.

Rogers, S. J., Wehner, D. E., & Hagerman, R. (2001). The behavioral phenotype in fragile X: symptoms of autism in very young children with fragile X syndrome, idiopathic autism, and other developmental disorders. *Journal of Developmental & Behavioral Pediatrics, 22*(6), 409-417.

Rojas, D. C., Benkers, T. L., Rogers, S. J., Teale, P. D., Reite, M. L., & Hagerman, R. J. (2001). Auditory evoked magnetic fields in adults with fragile X syndrome. *Neuroreport, 12*(11), 2573-2576.

Ropers, H. H. (2010). Genetics of early onset cognitive impairment. *Annual Review of Genomics and Human Genetics, 11*, 161-187.

Ropers, H. H., & Hamel, B. C. (2005). X-linked mental retardation. *Nature Review Genetics, 6*(1), 46-57.

Rowe, D. C., Vesterdal, W. J., & Rodgers, J. L. (1998). Herrnstein's syllogism: genetic and shared environmental influences on IQ, education, and income. *Intelligence, 26*(4), 405-423.

Ruhnau, P., Herrmann, B., Maess, B., & Schroger, E. (2011). Maturation of obligatory auditory responses and their neural sources: evidence from EEG and MEG. *Neuroimage, 58*(2), 630-639.

Schafer, E. W., & Peeke, H. V. (1982). Down syndrome individuals fail to habituate cortical evoked potentials. *American Journal of Mental Deficiency, 87*(3), 332-337.

Schneider, A., Hagerman, R. J., & Hessl, D. (2009). Fragile X syndrome -- from genes to cognition. *Developmental disabilities research reviews 15*(4), 333-342.

Seidl, R., Hauser, E., Bernert, G., Marx, M., Freilinger, M., & Lubec, G. (1997). Auditory evoked potentials in young patients with Down syndrome. Event-related potentials (P3) and histaminergic system. *Cognitive Brain Research, 5*(4), 301-309.

Shafer, V. L., Morr, M. L., Kreuzer, J. A., & Kurtzberg, D. (2000). Maturation of mismatch negativity in school-age children. *Ear and Hearing, 21*(3), 242-251.

Simson, R., Vaughn, H. G., Jr., & Ritter, W. (1977). The scalp topography of potentials in auditory and visual discrimination tasks. *Electroencephalography and Clinical Neurophysiology, 42*(4), 528-535.

Smith, M. E., Stapleton, J. M., & Halgren, E. (1986). Human medial temporal lobe potentials evoked in memory and language tasks. *Electroencephalography and Clinical Neurophysiology, 63*(2), 145-159.

Sokolov, E. N. (1963). Higher nervous functions; the orienting reflex. *Annual Review of Physiology, 25*, 545-580.

Squires, N. K., Squires, K. C., & Hillyard, S. A. (1975). Two varieties of long-latency positive waves evoked by unpredictable auditory stimuli in man. *Electroencephalography and Clinical Neurophysiology, 38*(4), 387-401.

St Clair, D. M., & Blackwood, D. H. (1985). Premature Senility in Down's Syndrome. *The Lancet, 326*(8445), 34.

St Clair, D. M., Blackwood, D. H., Oliver, C. J., & Dickens, P. (1987). P3 abnormality in fragile X syndrome. *Biological Psychiatry, 22*(3), 303-312.

Sudhalter, V., & Belser, R. C. (2001). Conversational characteristics of children with fragile X syndrome: tangential language. *American Journal on Mental Retardation, 106*(5), 389-400.

Sullivan, K., Hatton, D. D., Hammer, J., Sideris, J., Hooper, S., Ornstein, P. A., & Bailey, D. B., Jr. (2007). Sustained attention and response inhibition in boys with fragile X syndrome: measures of continuous performance. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics, 144B*(4), 517-532.

Sussman, E. S. (2007). A New View on the MMN and Attention Debate

The Role of Context in Processing Auditory Events. *Journal of Psychophysiology, 21*(3-4), 164-175.

Tonnquist-Uhlen, I., Borg, E., & Spens, K. E. (1995). Topography of auditory evoked long-latency potentials in normal children, with particular reference to the N1 component. *Electroencephalography and Clinical Neurophysiology, 95*(1), 34-41.

Turk, J., & Cornish, K. (1998). Face recognition and emotion perception in boys with fragile-X syndrome. *Journal of Intellectual Disability Research, 42 (Pt 6)*, 490-499.

Van der Molen, M. J., Van der Molen, M. W., Ridderinkhof, K. R., Hamel, B. C., Curfs, L. M., & Ramakers, G. J. (2012a). Auditory and visual cortical activity during selective attention in fragile X syndrome: A cascade of processing deficiencies. *Clinical Neurophysiology, 123*(4), 720-729.

Van der Molen, M. J., Van der Molen, M. W., Ridderinkhof, K. R., Hamel, B. C., Curfs, L. M., & Ramakers, G. J. (2012b). Auditory change detection in fragile X syndrome males: a brain potential study. *Clinical Neurophysiology, 123*(7), 1309-1318.

Van der Molen, M. J. W., Huizinga, M., Huizenga, H. M., Ridderinkhof, K. R., Van der Molen, M. W., Hamel, B. J., . . . Ramakers, G. J. A. (2010). Profiling Fragile X Syndrome in males: strengths and weaknesses in cognitive abilities. *Research in Developmental Disabilities, 31*, 426-439.

Verbaten, M. N., Roelofs, J. W., van Engeland, H., Kenemans, J. K., & Slangen, J. L. (1991). Abnormal visual event-related potentials of autistic children. *Journal of Autism and Developmental Disord, 21*(4), 449-470.

Verleger, R., Jaskowski, P., & Wauschkuhn, B. (1994). Suspense and surprise: on the relationship between expectancies and P3. *Psychophysiology, 31*(4), 359-369.

Vogel, E. K., & Luck, S. J. (2000). The visual N1 component as an index of a discrimination process. *Psychophysiology, 37*(2), 190-203.

Weiler, I. J., & Greenough, W. T. (1999). Synaptic synthesis of the Fragile X protein: possible involvement in synapse maturation and elimination. *American Journal of Medical Genetics, 83*(4), 248-252.

Wetzel, N., & Schroger, E. (2007). Cognitive control of involuntary attention and distraction in children and adolescents. *Brain Research, 1155*, 134-146.

Wijers, A. A., Lange, J. J., Mulder, G., & Mulder, L. J. (1997). An ERP study of visual spatial attention and letter target detection for isoluminant and nonisoluminant stimuli. *Psychophysiology, 34*(5), 553-565.

World Health Organization. (2004). *ICD-10 : international statistical classification of diseases and related health problems* (10th revision, 2nd ed.). Geneva: World Health Organization.

Yamamori, H., Okubo, O., & Harada, K. (2002). Brain evoked potentials of 3-D auditory stimuli. *Pediatrics International, 44*(4), 420-426.

Second article

Alterations of Visual and Auditory Event-Related Potentials in Fragile X Syndrome

Ready for submission to the *Journal of Neurodevelopmental Disorders*, August 2013

Inga Sophia Knoth[1,2] , Phetsamone Vannasing[1], Jacques Michaud[1] & Sarah Lippé[1,2]

[1]*Centre de Recherche CHU Ste-Justine, University of Montreal, Montreal, QC, Canada*

[2]*Centre de Recherche en Neuropsychologie et Cognition, University of Montreal, Montreal, QC, Canada*

Abstract

Fragile X Syndrome (FXS) is the most common form of X-linked Intellectual Disability (ID) and the only known mono-genetic cause of autism. It is caused by a trinucleotide repeat expansion in the FMR1 (‘Fragile X mental retardation 1’) gene, which prevents expression of the ‘fragile X mental retardation protein’ (FMRP). In FXS, the absence of FMRP leads to altered structural and functional development of the synapse, while preventing activity-based synapse maturation and synaptic pruning, which are essential for normal brain development and cognitive development. Possible impairments in information processing can be non-invasively investigated using electrophysiological methods. We examined event-related potentials (ERPs) evoked by basic auditory and visual stimulation in twelve adolescents and young adults (10-22) affected by FXS, as well as healthy controls matched by chronological age and developmental age of cognitive functioning. We found an increased auditory and visual N1 amplitude in FXS, relative to both control groups, as well as an increased auditory P2 and N2 amplitude and an increased auditory N2 latency. The ERP profile suggests disruptions in sensory processing specific to FXS that exceed immaturity of physiological activity. Thereby, the auditory modality seems to be more affected than the visual modality. Results are discussed in light of possible underlying neuronal mechanisms, including deficits in synaptic pruning and neuronal inhibition that might account for a hyperexcitable nervous system in FXS.

Introduction

Fragile X Syndrome (FXS) is the most common form of X-linked Intellectual Disability (ID), which affects about 2% of male ID patients (Ropers & Hamel, 2005). It is caused by a trinucleotide repeat expansion in the FMR1 ('Fragile X mental retardation 1') gene, which is located on the X-chromosome. Despite their non-mutated X-chromosome women can also be affected (approximately half of the prevalence found in men), but with greater variation in the phenotype (Bennetto et al., 2001). The FMR1 mutation prevents expression of the 'fragile X mental retardation protein' (FMRP), which is known to repress the translation of specific mRNAs in response to the activation of metabotropic Glutamate Receptors (mGluRs) (Bear, Huber, & Warren, 2004). In FXS, the absence of FMRP leads to altered structural and functional development of the synapse. Structurally, altered dendritic development, such as increased density of dendritic spines, weak, elongated dendritic spines and immature synaptic connections are found in FXS patients and fragile X knockout mice (Comery et al., 1997). Functionally, the exaggerated mRNA translation caused by the FMRP deficit results in continuous enhanced mGluR-dependent synaptic long-term depression. In consequence, protein-synthesis in the synapses is not modified specifically to stimuli induction, which results in a loss of protein synthesis dependent plasticity (Bassell & Warren, 2008). Thus, the FMRP absence is likely to prevent activity-based synapse maturation and synaptic pruning, which are essential for normal brain development (Weiler & Greenough, 1999) and cognitive development (Schneider et al., 2009).

Patients affected by FXS frequently show deficits in language, executive functions, visuo-spatial and social cognition. Further, they tend to show abnormal behavior, emotional instability and hyperarousal to sensory stimulation (Schneider et al., 2009). Most of the symptoms found in FXS are typical of the autistic spectrum; about 30% of male individuals with FXS meet the full diagnostic criteria for autism, which explains why FXS is considered the only known monogenetic cause of autism (Rogers et al., 2001). However, symptoms and their intensity vary considerably between different patients affected by FXS (Schneider et al., 2009).

Disrupted pathways in synaptic plasticity, the potential link between the genetic

mutation of FMR1 and the learning disability often found in FXS, are likely to be associated with impairments in mechanisms of information processing (Belmonte & Bourgeron, 2006). Early sensory and cognitive processing can be non-invasively investigated using the Event Related Potential (ERP) technique that records local field potentials, which are summarized postsynaptic potentials from large groups of neurons (Luck, 2005). Studies investigating ERPs in FXS so far exclusively used oddball paradigms and mostly studied auditory ERPs (Castrèn et al., 2003; St Clair et al., 1987; M. J. Van der Molen et al., 2012a, 2012b). Auditory N1 amplitude has been found to be enhanced in FXS (Castrèn et al., 2003; Rojas et al., 2001; St Clair et al., 1987; M. J. Van der Molen et al., 2012a, 2012b). Results concerning auditory P2 amplitude are inconsistent; in two studies an enhanced P2 amplitude was reported (St Clair et al., 1987; M. J. Van der Molen et al., 2012b), whereas in another study no difference between FXS patients and control group was detected (M. J. Van der Molen et al., 2012a). Mismatch-negativity (MMN) amplitude was found to be decreased (M. J. Van der Molen et al., 2012b), whereas results for auditory N2 amplitude were again inconsistent, stating an increase in amplitude in FXS (M. J. Van der Molen et al., 2012a, 2012b) or no difference between FXS and controls (St Clair et al., 1987). Finally, auditory P3 amplitude was consistently found to be decreased in FXS compared to healthy controls (St Clair et al., 1987; M. J. Van der Molen et al., 2012a, 2012b). Concerning latency, auditory N2 and P3 latency appeared to be increased in FXS patients compared to healthy controls (St Clair et al., 1987; M. J. Van der Molen et al., 2012a, 2012b), whereas no differences were detected in auditory N1, P2 and MMN latency (Castrèn et al., 2003; Rojas et al., 2001; St Clair et al., 1987; M. J. Van der Molen et al., 2012a, 2012b). Only one study investigated visual ERPs in FXS thus far, also using an oddball paradigm and showing an increase in N1 and N2 amplitude and a decrease in P3 amplitude, but no differences in latencies and P2 amplitude (M. J. Van der Molen et al., 2012a). According to this study, the alterations found in stimulus processing in FXS vary across modalities, with the auditory modality being more affected than the visual modality. This seems to match modality differences in performance found in FXS (Sullivan et al., 2007; M. J. W. Van der Molen et al., 2010).

Further, alterations in the sensory components N1 and P2 seem to be more specific to FXS, whereas the latter three merely cognitive components are frequently found to be altered

in most types of ID (Knoth & Lippé, 2012).

Parameters of ERPs like amplitude and latency have also been found to specifically change with brain development, which makes them valuable instruments in the investigation of brain maturation (Lippé et al., 2007). However, ERP studies conducted with FXS patients so far mostly compared the ERPs of the FXS population only to healthy age matched controls (Knoth & Lippé, 2012). Thus, it is not clear if brain development in FXS remains immature due to deficient synaptic pruning, or if sensory processing is further disrupted, leading to an ERP profile specific to FXS. So far the possibility of immature physiological activity as cause for the aberrant ERP profile in FXS has not been taken into account.

In this study we aimed to investigate both basic auditory and visual processing, since the early sensory components appear to be specifically altered in FXS. Therefore, we chose basic auditory and visual stimulation paradigms. In order to distinguish between immaturity and specific alterations of the ERPs, we compared the FXS patients not only to healthy age matched controls, but also to healthy controls with their developmental age of cognitive functioning, assessed by Intelligence Quotient (IQ).

Method

Participants

Twelve FXS patients aged from 10 to 22 years diagnosed with full mutation of the FMR1 gene were compared to 21 healthy controls matched by chronological age or developmental age and gender (*Table I*). The developmental control group contains children whose chronologic age matches the developmental age of patients with intellectual disability (IQ<70). Note that not all patients meet the criteria for an intellectual disability. A total of 18 FXS patients had been tested; six patients were excluded from data analysis due to epileptic activity, difficulties in testing and extensive movement artifacts.

Patients were recruited on the basis of DNA analysis previously conducted by geneticists at the CHU Sainte-Justine Mother and Child University Hospital Center in Montreal. Healthy controls were recruited by posters and pamphlets displayed in the Ste-Justine Hospital, the University of Montreal and kindergartens around the hospital. In addition, parents were directly approached in kindergartens and summer day camps and ads were placed on classified websites. Seven of the 12 FXS patients had also been diagnosed with autistic disorder; eight FXS patients showed language delay and eight FXS patients were also diagnosed with Attention Deficit Hyperactivity Disorder (ADHD). Five of the tested patients did not take any medication, while seven patients were medicated with psychostimulant (5x methylphenidate, 2x atomoxetine, 1x amphetamine mixed salts) and/or antidepressant (1x citalopram) drugs to treat symptoms of autism, attention deficit hyperactivity disorder, depression and anxiety. All patients underwent detailed physical examinations in the developmental clinic of the hospital following their diagnosis. None of the patients has been diagnosed with hearing deficits within the scope of these evaluations. Parents reported normal hearing and normal or corrected-to-normal vision in all patients and control participants upon specific request. Healthy controls had no history of brain injuries, psychiatric or neurological illnesses and did not take any medication. All participants were born at term and right-handed.

Intelligence in patients and controls was examined using the completely non-verbal Leiter-R International Performance Scale (Roid & Miller, 1997) for children and adolescents

and the Wechsler Abbreviated Scale of Intelligence (Wechsler, 1999) for adults. The non-verbal scale was chosen in order to reduce the impact of language deficits in patients on the global IQ result. Developmental age of patients was calculated on the basis of IQ in order to match them with healthy controls. Autistic behavior was quantified using the repetitive behavior scale and the abnormal behavior questionnaire, which were completed by parents of patients and minor control participants. The study protocol was reviewed and approved by the ethics, administrative, and scientific committees at the Ste-Justine's Hospital Research Center. Informed consent was obtained from participants and parents or legal caregivers before the experiment.

Table I. Demographics of the study population.

Variable	FXS Patients	Chronological age matched controls	Developmental age matched controls
N	12, 4 ♀	12, 3 ♀	9, 3 ♀
Age range	10-22 years	11-32 years	5-7 years
Mean age (SD)	14.7 (3.75)	16.9 (± 6.02)	5.8 (± 0.83)
IQ range	32-93	87-129	97-118
Mean IQ (SD)	51 (± 16.57)	113 (± 14.05)	108 (± 7.25)

Apparatus and Stimuli

Auditory and visual stimuli were generated by a Dell GX150 PC using E-Prime 1.0 (Psychology Software Tools Inc. Pittsburgh, PA, USA). The EEG recording took place in a dark soundproof experimental chamber. Auditory stimulation consisted of 50ms broadband noise presented in a randomly distributed inter-stimulus interval varying from 1200 to 1400 ms at 79 dB SPL intensity and 16-bit resolution. The two speakers (Optimus XTS 24, Boston, MA, USA) were located laterally at 30 cm distance from the subject's ears. During auditory stimulation all subjects watched a silent movie. Following this, visual stimulation consisted of a black and white checkerboard stimulus presented at a reversal rate of 1 Hz, meaning that the checkerboard changed every 500ms, and subtending a visual angle of 2 degrees. Stimuli had a luminance of 40 cd/m^2 and were displayed on a 40.5 X 30.5 cm ViewSonic monitor (ViewSonic, Canada) at 114 cm distance from the participant's eyes. An assistant observed

whether the participant looked at the screen at all times and gave a signal whenever the participant looked elsewhere, in order to exclude these EEG segments from analyses. The assistant likewise directed the attention of participants to the screen by holding small objects in the lower middle part of the screen and talking to them if necessary. A dense array EEG system containing 128 electrodes was used for recording (Electrical Geodesics System Inc., Eugene, OR, USA). The vertex was used as the reference electrode during recording and impedances were maintained below 40kΩ (Tucker, 1993). Signals were acquired and processed by a G4 Macintosh computer using NetStation EEG Software (Version 2.0). Digitalization of EEG data was carried out at a sampling rate of 250Hz in 1024 ms epochs and an analog 0.01-100Hz bandpass filter was applied.

Off-line analyses were carried out with BrainVision Analyser software, version 2.0 (Brain Products, Munich, Germany). Data were digitally filtered with a 1-50 Hz filter for the visual and a 1-30 Hz filter for the auditory experiment and re-referenced to an average reference. Eye movement artifacts were corrected using semi-automatic Ocular Correction ICA as implemented in BrainVision Analyser. Algorithmic artifact rejection of voltage exceeding ± 100μV was followed by visual data inspection of segmented data in which segments with artifacts were manually rejected. In the patient group, an average of 4 of 150 segments were rejected in the auditory and an average of 8 of 200 segments were rejected in the visual paradigm. For the control groups, rejection rates were 1/150 in the auditory and 1/200 in the visual paradigm for the chronological control group and 1/150 in the auditory and 8/200 in the visual paradigm for the developmental control group. Rejection rates did not differ significantly between the three groups (auditory condition: (χ^2 (2) = 1.63, p = .43), visual condition: (χ^2 (2) = 5.79, p = .06)).

Auditory and Visual Event-related Potential Analysis

Artifact-free segments were averaged and baseline corrected. In the auditory paradigm, an average of 146 artifact-free segments were available for the patient group and an average of 149 segments for the chronological and developmental control group. In the visual paradigm, an average of 191 segments were available for patients, 198 for chronological controls and 191 for developmental controls. Number of presented segments did not differ

between the three groups (auditory condition: (χ^2 (2) = 1.27, p = .53), visual condition: (χ^2 (2) = 5.79, p = .06)). Amplitudes and latencies were measured at electrodes FCz and Cz for auditory ERPs since they showed the clearest N1, P2 and N2 amplitudes in all groups, and at electrode Oz for visual ERPs, since they showed the clearest N70, P1 and N2 amplitudes. Approximate time windows for each component were defined by visual inspection of group averages. Components were then individually selected for each subject. Amplitudes were defined from baseline (200ms pre-stimulus) to the highest amplitude of each component and latencies were defined from stimulus onset to the highest point of each component.

Statistical analysis

Statistical analyses were performed using SPSS statistics, version 20 (IBM Corp., Armonk, NY, USA). Firstly, we compared the following variables between male and female FXS patients: IQ; N1, N2 and P2 amplitudes and latencies at electrode Cz and FCz for auditory ERPs and N70, P1 and N2 amplitudes and latencies at electrode Oz for visual ERPs. Since female patients are known to be less affected, due to their intact X-chromosome, which mitigates the outcome of the FMR1 mutation, we verified whether male and female patients differed from each other in respect of ERPs or can be considered as a single group. Student's t-test has been used for normally distributed variables and the Mann-Whitney U test for not normally distributed variables. Analysis was then carried out between FXS patients, chronological and developmental age matched controls for the variables listed above. Normally distributed variables for which homogeneity of variance could be assumed were tested using analysis of variance (ANOVA) and if significant differences between groups were detected, Tukey's test was carried out for post-hoc analysis. Not normally distributed variables were tested using the non-parametric Kruskal–Wallis one-way analysis of variance (K-W ANOVA) and if significant differences between groups were detected, pairwise comparisons were carried out using the Mann-Whitney U test controlling for the familywise error rate with a Bonferroni correction. Effect sizes are provided for significant ANOVAs and K-W ANOVAs. Data obtained from the abnormal behaviour questionnaire and the repetitive behaviour scale were compared between FXS patients and chronological control group, using student's t-test or Mann-Whitney U test. Significance level for all statistical tests was set to 5% (α = .05).

Results

Male vs. female FXS patients

With a mean of 68.25 (±17.11), female patients had higher IQs than male patients (M = 43.38, ± 8.38) (p = .006). However, no significant difference between male and female FXS patients was found in the visual ERP components N70, P1 and N2 at electrode Oz, as well as in the auditory components N1, P2 and N2 at electrode FCz and Cz (p > .05). Thus, FXS patients of both genders were combined into one patient group.

IQ, abnormal and repetitive behaviour

One-way ANOVA shows a difference in IQ between FXS patients, chronological and developmental age matched controls ($F(2,28) = 67.12$, p = .00, $R^2 = 0.83$). FXS patients differ from the chronologic age matched control group (p = .00) and the developmental age matched control group (p = .00), while both control groups do not significantly differ from each other. *Table II* shows mean values of abnormal and repetitive behaviour in FXS patients and chronological controls as reported by parents/care givers in the abnormal behaviour questionnaire and the repetitive behaviour scale.

Table II. Mean values (SD) of abnormal and repetitive behaviour in participants as reported by their parents/caregivers.

Scale	FXS patients	Chronological controls	p-value
Irritability	4 (4.54)*	0.78 (1.09)	0.04
Lethargy	4.13 (5.11)*	0.33 (0.52)	0.04
Stereotypical Behaviour	1.75 (3.24)	0 (.00)	0.08
Hyperactivity	3.35 (4.34)*	1.52 (0.51)	0.02
Inappropriate Speech	3.25 (3.45)*	0.33 (0.52)	0.02
Self Mutilation	1.78 (2.22)*	0.17 (0.41)	0.03
Compulsive Behaviour	1.78 (2.49)	0.33 (0.82)	0.06
Ritualized Behaviour	1.89 (1.96)*	0 (0.00)	0.01
Immutable Behaviour	4.22 (4.92)*	0.17 (0.41)	0.02
Restrictive Behaviour	1.22 (1.64)*	0 (0.00)	0.03

* significant difference between FXS and chronological control group (p<.05)

Event-related potentials

Auditory ERPs

Group averages of the auditory ERP responses at electrode FCz and Cz are presented in *figure 1* and *2*. *Table III* shows mean amplitudes and latencies at electrode FCz and Cz for every group.

Table III. Mean amplitudes in μV and latencies in ms (SD) for auditory ERP components in FXS patients, chronological control group and developmental control group.

Component	Parameter	Electrode	FXS patients	Chronological control group	Developmental control group
N1	Amplitude	FCz	-.92 (1.25) ŦŦ	-0.34 (0.93)	0.66 (0.69)
		Cz	-2.62 (1.7) *Ŧ	-0.74 (0.75)	1.07 (1.36)
	Latency	FCz	100 (16)	96.67 (20.6)	88 (22.54)
		Cz	94 (10.85) Ŧ	94.33 (12.47)	78.22 (16.38)
P2	Amplitude	FCz	3.63 (1.91) *Ŧ	0.95 (1.00)	1.72 (1.13)
		Cz	6.08 (2.29) *Ŧ	1.93 (1.09)	3.74 (1.46)
	Latency	FCz	180.67 (30.22) Ŧ	178.67 (26.22)	134.22 (20.99)
		Cz	185 (28.85) Ŧ	172 (21.57)	142.22 (24.59)
N2	Amplitude	FCz	-1.76 (0.97)	-0.99 (1.05)	-1.08 (0.70)
		Cz	-3.07 (1.36) *Ŧ	-1.04 (0.65)	-1.12 (0.86)
	Latency	FCz	328.83 (27.47) *Ŧ	269 (24.67)	245.78 (59.47)
		Cz	319.67 (26.67) Ŧ	288.67 (24.17)	245.78 (61.32)

* significant difference between FXS and chronological control group (p<.05)
Ŧ significant difference between FXS and developmental control group (p<.05)

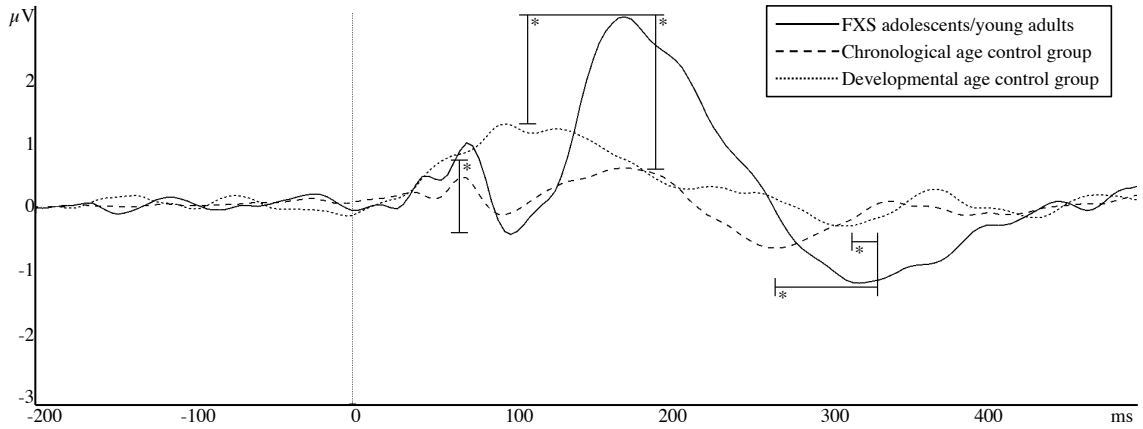

Figure 1. Group Averages of Auditory ERPs at electrode FCz for the FXS group and the two control groups. 0ms marks stimulus onset. Significant differences are only marked for differences between FXS and control groups.

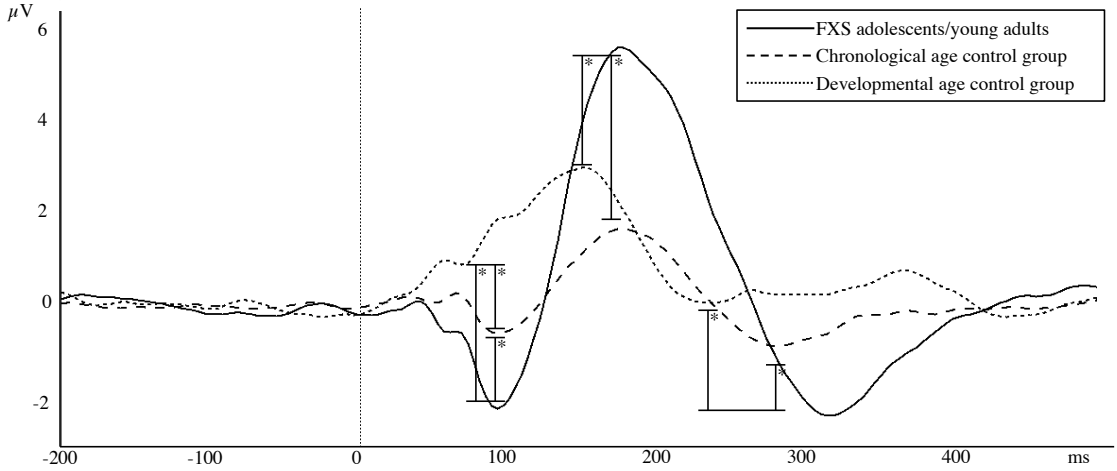

Figure 2. Group Averages of Auditory ERPs at electrode Cz for the FXS group and the two control groups. 0ms marks stimulus onset. Significant differences are only marked for differences between FXS and control groups.

Auditory N1

Auditory N1 amplitude differed between groups at electrode FCz ($F(2,30) = 6.41, p = .005, R^2 = .30$), with post hoc analysis revealing that FXS patients had a higher N1 amplitude than developmental controls ($p = .004$). A difference between groups in N1 was also found at electrode Cz (χ^2 (2) = 23.87, p = .00, $R^2 = .57$). According to post hoc analysis, the differences between all three groups were significant ($p = .00$). FXS patients showed the highest N1 amplitude, followed by the chronological controls and finally developmental controls, which showed the smallest N1 amplitude. At electrode FCz there was no difference in N1 latency between groups ($F(2,30) = .99, p = .38$), whereas a difference in latency was found at electrode Cz ($F(2,30) = 4.85, p = .015, R^2 = .24$). Post hoc analysis showed that N1 latency at electrode Cz was shorter in developmental controls, compared to both other groups ($p = .024$).

Auditory P2

A difference in auditory P2 between the three subgroups was found at electrode FCz ($F(2,30) = 11.07, p = .005, R^2 = .42$) and Cz ($F(2,30) = 17.72, p = .00, R^2 = .54$). Post hoc analysis revealed that auditory P2 amplitude was higher in FXS patients than in chronological controls ($p = .00$ for both electrodes) and developmental controls ($p = .013$ for electrode FCz

and $p = .011$ for electrode Cz). Auditory P2 latency also differed between groups at electrode FCz ($F(2,30) = 9.61, p = .001, R^2 = .39$) and Cz ($F(2,30) = 7.56, p = .002, R^2 = .34$). P2 latency was smaller in developmental controls, compared to FXS patients ($p = .001$ for electrode FCz and $p = .002$ for electrode Cz) and chronological controls ($p = .002$ for electrode FCz and $p = .031$ for electrode Cz).

Auditory N2

At electrode FCz, there was no difference found in auditory N2 amplitude between FXS patients and both control groups ($F(2,30) = 2.39, p = .108$), whereas a difference between groups was detected at electrode Cz ($F(2,30) = 14.81, p = .00, R^2 = .50$). N2 amplitude appeared to be larger in FXS patients than in chronological controls and developmental controls at electrode Cz ($p = .00$ for both groups). N2 latency differed between groups at electrode FCz ($F(2,30) = 6.41, p = .005, R^2 = .48$), being longer in FXS patients than in chronological controls ($p = .002$) and developmental controls ($p = .00$). At electrode Cz, N2 latency also differed between groups ($F(2,30) = 9.5, p = .001, R^2 = .39$), being smaller in developmental controls than in FXS patients ($p = .00$) and chronological controls ($p = .043$).

Visual ERPs

Mean amplitudes and latencies at electrode Oz for every subgroup are presented in *table IV*. *Figure 3* shows the group averages of the visual ERP response at electrode Oz.

Table IV. Mean amplitudes in μV and latencies in ms (SD) for visual ERP components in FXS patients, chronological control group and developmental control group.

Component	Parameter	FXS patients	Chronological control group	Developmental control group
N70	Amplitude	-2.56 (1.63)*	-1.09 (0.73)	-2.21 (1.66)
	Latency	74 (11.63)	78.67 (5.74)	78.22 (4.06)
P1	Amplitude	4.30 (3.62)	3.94 (2.73)	5.73 (2.40)
	Latency	109 (11.71)	111.67 (3.17)	113.33 (4.00)
N2	Amplitude	-2.25 (1.89)*	-0.75 (0.83)	-1.36 (1.43)
	Latency	309.33 (35.91)	275.5 (37.25)	348 (30.20)

* significant difference between FXS and chronological control group (p<.05)
∓ significant difference between FXS and developmental control group (p<.05)

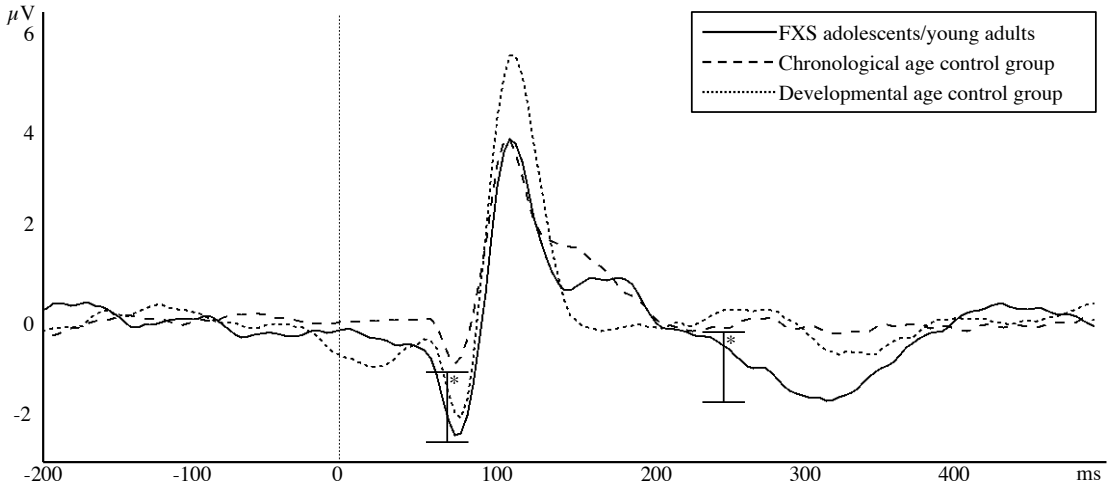

Figure 3. Group averages of visual ERPs at electrode Oz for the FXS group and the two control groups. 0ms marks stimulus onset. Significant differences are only marked for differences between FXS and control groups.

Visual N70

A difference in N70 amplitude between the three groups was detected at electrode Oz ($F(2,30) = 3.68, p = .037, R^2 = .19$). Post-hoc analysis revealed that the N70 amplitude was larger in FXS patients than in the chronological control group ($p = 0.036$), whereas there was no difference between developmental control group and the two other groups. No difference between the groups has been found in N70 latency ($\chi^2 (2) = 0.64$, p = .727).

Visual P1

No difference was detected in visual P1 amplitude between the three groups ($F(2,30) = 0.30, p = .747$). Visual P1 latency was found to differ between groups ($F(2,30) = 9.14, p = .001, R^2 = .38$). According to post hoc analysis developmental controls appeared to have a longer visual P1 latency than chronological controls ($p = .001$). P1 latency in FXS patients appeared longer than in chronological controls, but this difference failed to reach significance ($p = .057$). FXS patients and developmental controls also did not differ in P1 latency.

Visual N2

Visual N2 amplitude differed between groups ($\chi^2 (2) = 7.00$, p = .03, $R^2 = .18$). Post hoc analysis revealed that N2 amplitude is higher in FXS patients than in chronological controls ($p = 0.01$), while it does not differ from the N2 amplitude in developmental controls ($p = .277$). Chronological and developmental control group did not differ in N2 amplitude ($p = .148$). A difference between groups was also found in N2 latency ($F(2,30) = 11.06, p = .00, R^2 = .42$). N2 latency appeared to be longer in developmental controls than in FXS patients ($p = .046$) and chronological controls ($p = .00$), whereas the difference between FXS patients and chronological controls failed to reach significance ($p = .062$).

Discussion

FXS patients showed alterations in ERPs evoked by basic auditory and visual stimulation. However, we found that auditory processing is more impaired in FXS patients than visual processing. Furthermore, FXS patients are not only impaired compared to their age-matched peers, but also compared to children with the same developmental age. Auditory information processing in FXS patients does not resemble immature information processing, but has its own particularities. A summary of ERP alterations in FXS found in our study is given in *table V*.

Table V. Alterations in auditory and visual ERPs in FXS obtained in this study.

Component	Modality	Amplitude	Latency
N1	Auditory	*Increased* in FXS compared to developmental controls at FCz *Increased* in FXS compared to both control groups at CZ	No difference
N70	Visual	*Increased* in FXS compared to chronological control group	No difference
P2	Auditory	*Increased* in FXS compared to both control groups at FCz and Cz	No difference
P1	Visual	No difference	No difference
N2	Auditory	*Increased* in FXS compared to both control groups at Cz	*Increased* Latency in FXS compared to both control groups at FCz
	Visual	*Increased* in FXS compared to chronological control group	No difference

Specifically, an increased auditory N1 amplitude and no difference in N1 latency has been found in our patient population, replicating the findings of every relevant ERP study conducted with FXS patients so far (Castrèn et al., 2003; St Clair et al., 1987; M. J. Van der Molen et al., 2012a, 2012b). We also found an increased visual N70 amplitude in FXS, but only compared to the chronological control group, while no difference in latency was found. This resembles the findings of Van der Molen's group (M. J. Van der Molen et al., 2012a), who published the only study investigating visual ERPs in FXS to this point and who found the first negative component (N1) to be increased in FXS, compared to healthy controls. Thus,

increases in the first negative auditory and visual component seem to be fairly consistently found in FXS. Since our findings revealed a larger auditory N1 amplitude in FXS compared not only to the chronological but also to the developmental age matched control group, it is unlikely that the processes involved in the generation of the auditory N1 are simply immature functioning in FXS. Further, the increase in N1 amplitude seems to be somewhat specific to FXS when compared to other forms of ID and autism that often present a difference in N1 latency and no difference (or even a reduction) in N1 amplitude (Knoth & Lippé, 2012). An increased state of arousal is believed to increase auditory N1 amplitude (Näätänen & Picton, 1987). Since hyperarousal to sensory stimuli is often found in FXS (Schneider et al., 2009), the increased N1 amplitude might reflect this state of hyperarousal and there might be a common underlying mechanism for both. Regarding the neural mechanisms, Rojas and colleagues, who conducted a Magnetoencephalography (MEG) study with FXS patients and found a higher N100m (the N1 equivalent in MEG) amplitude in FXS suggest that more neurons are synchronously active in response to the sensory stimulation in FXS than in healthy controls (Rojas et al., 2001). This could mean that FXS patients have a surplus of neurons in brain regions that are involved in N1 generation. Alternatively, neuronal activation could be less inhibited in FXS than in healthy controls.

An explanation supposing a surplus of neurons is supported by neuroanatomical aberrations in FXS. Among other alterations, greater gray matter volume in the occipital cortex has been found in infants with FXS compared to normally developed children and children with non-syndromic delay (Hoeft et al., 2008). The occipital cortex contains most of the visual cortex, which is believed to be involved in visual N70 generation (Shigeto, Tobimatsu, Yamamoto, Kobayashi, & Kato, 1998). In contrast, the superior temporal gyrus, which contains the primary auditory cortex and is believed to be involved in auditory N1 generation (Näätänen & Picton, 1987), is found to be smaller in FXS, relative to healthy controls (Gothelf et al., 2008). However, over a course of two years FXS children showed a greater gray matter increase in several brain structures, including not only temporal-occipital regions, but also the superior temporal gyrus, relative to chronological- and developmentally-age matched controls. This could indicate deficient synaptic pruning in FXS (Hoeft et al., 2010). Synaptic pruning, a neuroregulatory process in which unnecessary neurons and

synapses are reduced in order to strengthen more efficient neuronal configurations, might be impaired in FXS, according to animal model studies (Pfeiffer & Huber, 2007, 2009; Weiler & Greenough, 1999). In consequence, more redundant neurons, which have not been eliminated through synaptic pruning, would respond to sensory stimulation in a sort of non-specific arousal response, accounting for the increased N1 amplitude.

This finds support in studies that investigated N1 habituation in FXS (Castrèn et al., 2003; M. J. Van der Molen et al., 2012b). In both studies, controls showed a reduction of N1 amplitude after several presentations of the same stimulus, while this N1 habituation did not occur in FXS. The reduction of N1 amplitude in controls suggests that less neurons synchronously respond to a stimulus after several repetitions, which could reflect a reduction of the non-specific arousal or novelty response that generally occurs after the first appearance of a sensory stimulus (Karhu et al., 1997). Thus, deficient synaptic pruning might lead to an excess of less adapted synaptic configurations that non-specifically respond to sensory stimulation, while reducing the capacity for more efficient synaptic connections. This mechanism might be reflected in increased auditory N1 amplitude and a lack of N1 habituation.

Impaired neuronal inhibition as an explanation for increased N1 amplitudes finds support in a study that investigated resting state EEG in FXS (M. J. Van der Molen & Van der Molen, 2013). They found an increased relative theta power and a decreased relative upper alpha in FXS, compared to healthy controls. Alpha oscillations in EEG are believed to be involved in neural inhibitory regulation mechanisms that gate information by reducing the processing capabilities of a given area (Jensen & Mazaheri, 2010). A reduced alpha rhythm in FXS could indicate impaired sensory gating mechanisms, which fail to inhibit redundant neural activity. In consequence, there would be more neuronal activation, which might be reflected in increased N1 amplitude. It would be interesting to further investigate the possible relationship between alpha/theta power abnormalities and increased N1 amplitude in FXS.

All hypotheses described above concerning the possible neuronal mechanism underlying the increased N1 amplitude seem to suggest a hyperexcitable nervous system in FXS. These assumptions remain hypothetical, however, and should be further addressed by combining EEG with other brain imaging techniques.

Previous P2 results in FXS were somewhat inconsistent, since an increased auditory P2 amplitude in FXS has been found in two studies (St Clair et al., 1987; M. J. Van der Molen et al., 2012b), whereas no difference in auditory and visual P2 amplitude was found in another study (M. J. Van der Molen et al., 2012a). P2 latency did not differ between FXS patients and controls in any of the studies. Our study is in accordance with the greater part of the literature, having found increased auditory P2 amplitude in FXS patients in comparison with chronological and developmental control groups, but no differences in P2 latency. The increased auditory P2 amplitude seems to be part of the ERP profile specific to FXS, since other IDs show mostly a prolonged P2 latency, but no changes in P2 amplitude (Knoth & Lippé, 2012).

The fact that no alterations are found in the visual P1 in FXS indicates a modality difference in basic sensory information processing in FXS, suggesting more altered components in the auditory modality. This is in accordance with modality differences in performance found in FXS (Sullivan et al., 2007; M. J. W. Van der Molen et al., 2010). Further differences between visual and auditory ERPs have been found in the later P3 component in FXS, suggesting again that there are less processing deficits in the visual modality than in the auditory (M. J. Van der Molen et al., 2012a).

Lastly, we investigated auditory and visual basic N2 in FXS. In the visual modality, no difference in N2 latency was found, which differs from the results reported by Van der Molen and colleagues, who found an increased visual N2 latency in FXS, compared to chronological controls (M. J. Van der Molen et al., 2012a). However, since the mean latency of our developmental group lies between FXS patients and chronological controls, the difference between FXS patients and chronological controls might have been significant in a direct comparison. Further, visual N2 amplitude was only found to be larger in FXS compared to the chronological control group, but did not differ from the developmental control group. Since N2 amplitude is known to decrease with age (Lippé et al., 2007) and the visual N2 amplitude did not differ between FXS and developmental control group, it can be argued that the visual N2 is immature in FXS rather than specifically altered. Moreover, prolonged N2 latency seems to be a general phenomenon in ID (Knoth & Lippé, 2012).

In contrast, we found a prolonged auditory N2 latency at FCz and an increased auditory N2 amplitude at Cz in FXS, compared to both control groups. Again, there seems to be a modality difference in the N2 in FXS, again suggesting that auditory processing is more affected. However, auditory N2 results obtained in FXS so far are quite inconsistent (Knoth & Lippé, 2012). The controversy might be partially due to the fact that the N2 is more sensitive to changes in task parameters than the previously described components, making it more difficult to compare studies with different experimental designs.

Conclusion

The present study presents a profile of altered ERPs in FXS, which likely reflects impairments in basic neural sensory processing. The additional comparison to a control group matched by developmental age of cognitive functioning of patients with ID leads to the conclusion that auditory components in FXS are not simply immature, but appear specifically altered. Conversely, the visual N70 and N2 amplitude does not differ between FXS patients and developmental controls. Notably, information processing seems to be more severely impaired in the auditory than in the visual modality, as suggested by Van der Molen (M. J. Van der Molen et al., 2012a), which could account for language deficits in FXS. The knowledge that visual processing is less affected, as well as indications of a hyperexitable nervous system, could be considered in the design of behavioral treatments for FXS patients.

A longitudinal study with frequent follow-ups investigating ERPs from a very young age in infants affected by FXS could help discriminate early, possibly even prenatal, alterations of neurodevelopment resulting from delayed or otherwise altered synaptic pruning occurring postnatally. However, the implementation of such a study would be very difficult, since FXS is currently diagnosed at an average age of 3 years in boys and 3.4 years in girls (Bailey, Raspa, Bishop, & Holiday, 2009). Routine genetic screenings in newborns have recently been discussed (Bailey, Skinner, Davis, Whitmarsh, & Powell, 2008) and might offer an opportunity to further unravel the mechanisms of neurodevelopment and early information processing in FXS.

Concerning ERPs, it would be interesting to investigate more complex stimulus processing and learning mechanisms in FXS. Of particular interest would be the face-specific N170 ERP, since impaired face recognition has been reported in FXS (Turk & Cornish, 1998). Further, alterations in a cortical region specialized in face processing, the fusiform gyrus, have been found in FXS (Hoeft et al., 2008) and functional studies show that FXS patients recruit brain regions differently from healthy controls during face and gaze processing (Dalton et al., 2008; Garrett et al., 2004; Holsen et al., 2008; Watson et al., 2008). Another field of particular interest in FXS would be language processing, since language is a cognitive domain that is especially impaired in FXS. ERPs in response to words pronounced by a human voice could be interesting as well as the examination of language-related ERPs, like the N400 and the P600.

Acknowledgements

This research was supported by Scottish Rite Charitable Foundation of Canada grant to Sarah Lippé. We thank Domitille Malfait for carrying out the neuropsychological evaluation of most of the patients and Maude Joannette and Patricia Laniel for their help in the acquisition of EEG data.

References

Bailey, D. B., Jr., Raspa, M., Bishop, E., & Holiday, D. (2009). No change in the age of diagnosis for fragile x syndrome: findings from a national parent survey. *Pediatrics, 124*(2), 527-533.

Bailey, D. B., Jr., Skinner, D., Davis, A. M., Whitmarsh, I., & Powell, C. (2008). Ethical, legal, and social concerns about expanded newborn screening: fragile X syndrome as a prototype for emerging issues. *Pediatrics, 121*(3), e693-704.

Bassell, G. J., & Warren, S. T. (2008). Fragile X syndrome: loss of local mRNA regulation alters synaptic development and function. *Neuron, 60*(2), 201-214.

Bear, M. F., Huber, K. M., & Warren, S. T. (2004). The mGluR theory of fragile X mental retardation. *Trends in Neuroscience, 27*(7), 370-377.

Belmonte, M. K., & Bourgeron, T. (2006). Fragile X syndrome and autism at the intersection of genetic and neural networks. *Nature Neuroscience, 9*(10), 1221-1225.

Bennetto, L., Pennington, B. F., Porter, D., Taylor, A. K., & Hagerman, R. J. (2001). Profile of cognitive functioning in women with the fragile X mutation. *Neuropsychology, 15*(2), 290-299.

Castrèn, M., Paakkonen, A., Tarkka, I. M., Ryynanen, M., & Partanen, J. (2003). Augmentation of auditory N1 in children with fragile X syndrome. *Brain Topography, 15*(3), 165-171.

Comery, T. A., Harris, J. B., Willems, P. J., Oostra, B. A., Irwin, S. A., Weiler, I. J., & Greenough, W. T. (1997). Abnormal dendritic spines in fragile X knockout mice: maturation and pruning deficits. *Proceedings of the National Academy of Sciences of the United States of America, 94*(10), 5401-5404.

Dalton, K. M., Holsen, L., Abbeduto, L., & Davidson, R. J. (2008). Brain function and gaze fixation during facial-emotion processing in fragile X and autism. *Autism Research, 1*(4), 231-239.

Garrett, A. S., Menon, V., MacKenzie, K., & Reiss, A. L. (2004). Here's looking at you, kid: neural systems underlying face and gaze processing in fragile X syndrome. *Archives of General Psychiatry, 61*(3), 281-288.

Gothelf, D., Furfaro, J. A., Hoeft, F., Eckert, M. A., Hall, S. S., O'Hara, R., . . . Reiss, A. L. (2008). Neuroanatomy of fragile X syndrome is associated with aberrant behavior and the fragile X mental retardation protein (FMRP). *Annals of Neurology, 63*(1), 40-51.

Hoeft, F., Carter, J. C., Lightbody, A. A., Cody Hazlett, H., Piven, J., & Reiss, A. L. (2010). Region-specific alterations in brain development in one- to three-year-old boys with fragile X syndrome. *Proceedings of the National Academy of Sciences of the United States of America, 107*(20), 9335-9339.

Hoeft, F., Lightbody, A. A., Hazlett, H. C., Patnaik, S., Piven, J., & Reiss, A. L. (2008). Morphometric spatial patterns differentiating boys with fragile X syndrome, typically developing boys, and developmentally delayed boys aged 1 to 3 years. *Archives of General Psychiatry, 65*(9), 1087-1097.

Holsen, L. M., Dalton, K. M., Johnstone, T., & Davidson, R. J. (2008). Prefrontal social cognition network dysfunction underlying face encoding and social anxiety in fragile X syndrome. *Neuroimage, 43*(3), 592-604.

Jensen, O., & Mazaheri, A. (2010). Shaping functional architecture by oscillatory alpha activity: gating by inhibition. *Frontiers in Human Neuroscience, 4*, 186.

Karhu, J., Herrgard, E., Paakkonen, A., Luoma, L., Airaksinen, E., & Partanen, J. (1997). Dual cerebral processing of elementary auditory input in children. *Neuroreport, 8*(6), 1327-1330.

Knoth, I. S., & Lippé, S. (2012). Event-related potential alterations in fragile X syndrome. *Frontiers in Human Neuroscience, 6*, 264.

Lippé, S., Roy, M. S., Perchet, C., & Lassonde, M. (2007). Electrophysiological markers of visuocortical development. *Cerebral Cortex, 17*(1), 100-107.

Luck, S. J. (2005). *An introduction to the event-related potential technique*. Cambridge, Ma: MIT Press.

Näätänen, R., & Picton, T. (1987). The N1 wave of the human electric and magnetic response to sound: a review and an analysis of the component structure. *Psychophysiology, 24*(4), 375-425.

Pfeiffer, B. E., & Huber, K. M. (2007). Fragile X mental retardation protein induces synapse loss through acute postsynaptic translational regulation. *Journal of Neuroscience 27*(12), 3120-3130.

Pfeiffer, B. E., & Huber, K. M. (2009). The state of synapses in fragile X syndrome. *Neuroscientist, 15*(5), 549-567.

Rogers, S. J., Wehner, D. E., & Hagerman, R. (2001). The behavioral phenotype in fragile X: symptoms of autism in very young children with fragile X syndrome, idiopathic autism, and other developmental disorders. *Journal of Developmental & Behavioral Pediatrics, 22*(6), 409-417.

Roid, G. H., & Miller, L. J. (1997). Leiter International Performance Scale – Revised: Examiner's Manual. In G. H. Roid & L. J. Miller (Eds.), *Leiter International Performance Scale – Revised*. Wood Dale, IL: Stoelting Co.

Rojas, D. C., Benkers, T. L., Rogers, S. J., Teale, P. D., Reite, M. L., & Hagerman, R. J. (2001). Auditory evoked magnetic fields in adults with fragile X syndrome. *Neuroreport, 12*(11), 2573-2576.

Ropers, H. H., & Hamel, B. C. (2005). X-linked mental retardation. *Nature Reviews Genetics, 6*(1), 46-57.

Schneider, A., Hagerman, R. J., & Hessl, D. (2009). Fragile X syndrome -- from genes to cognition. *Developmental disabilities research reviews 15*(4), 333-342.

Shigeto, H., Tobimatsu, S., Yamamoto, T., Kobayashi, T., & Kato, M. (1998). Visual evoked cortical magnetic responses to checkerboard pattern reversal stimulation: A study on the neural generators of N75, P100 and N145. *Journal of the Neurological Sciences, 156(2),* 186-194.

St Clair, D. M., Blackwood, D. H., Oliver, C. J., & Dickens, P. (1987). P3 abnormality in fragile X syndrome. *Biological Psychiatry, 22*(3), 303-312.

Sullivan, K., Hatton, D. D., Hammer, J., Sideris, J., Hooper, S., Ornstein, P. A., & Bailey, D. B., Jr. (2007). Sustained attention and response inhibition in boys with fragile X syndrome: measures of continuous performance. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics, 144B*(4), 517-532.

Tucker, D. M. (1993). Spatial sampling of head electrical fields: the geodesic sensor net. *Electroencephalography and Clinical Neurophysiology, 87*(3), 154-163.

Turk, J., & Cornish, K. (1998). Face recognition and emotion perception in boys with fragile-X syndrome. *Journal of Intellectual Disability Research, 42 (Pt 6)*, 490-499.

Van der Molen, M. J., & Van der Molen, M. W. (2013). Reduced alpha and exaggerated theta power during the resting-state EEG in fragile X syndrome. *Biological Psychology, 92*(2), 216-219.

Van der Molen, M. J., Van der Molen, M. W., Ridderinkhof, K. R., Hamel, B. C., Curfs, L. M., & Ramakers, G. J. (2012a). Auditory and visual cortical activity during selective attention in fragile X syndrome: A cascade of processing deficiencies. *Clinical Neurophysiology, 123*(4), 720-729.

Van der Molen, M. J., Van der Molen, M. W., Ridderinkhof, K. R., Hamel, B. C., Curfs, L. M., & Ramakers, G. J. (2012b). Auditory change detection in fragile X syndrome males: a brain potential study. *Clinical Neurophysiology, 123*(7), 1309-1318.

Van der Molen, M. J. W., Huizinga, M., Huizenga, H. M., Ridderinkhof, K. R., Van der Molen, M. W., Hamel, B. J., . . . Ramakers, G. J. A. (2010). Profiling Fragile X Syndrome in males: strengths and weaknesses in cognitive abilities. *Research in Developmental Disabilities, 31*, 426-439.

Watson, C., Hoeft, F., Garrett, A. S., Hall, S. S., & Reiss, A. L. (2008). Aberrant brain activation during gaze processing in boys with fragile X syndrome. *Archives of General Psychiatry, 65*(11), 1315-1323.

Wechsler, D. (1999). *Wechsler Abbreviated Scale of Intelligence*. San Antonio, TX: The Psychological Corporation.

Weiler, I. J., & Greenough, W. T. (1999). Synaptic synthesis of the Fragile X protein: possible involvement in synapse maturation and elimination. *American Journal of Medical Genetics 83*(4), 248-252.

General discussion

The primary objective of this Master's thesis was to investigate basic information processing in a syndromic ID caused by an X-linked genetic mutation – FXS – for which an impaired cascade of biological mechanisms is well defined. We expected impaired information processing, more so in the auditory than the visual modality, to be reflected in an altered ERP profile specific to FXS, rather indicating immature physiological activity.

The first necessary step in designing an ERP study in FXS was to review studies that have been conducted so far, in order to connect our work with the existing literature. This literature review has been presented in the first part of this Master's thesis. The results summarized and discussed in the review lead to the following conclusions. First, FXS patients show an altered ERP profile in response to auditory stimulation. These alterations can most likely be ascribed to altered neurodevelopment and resultant impairments in neuronal information processing. Further, the alterations in ERP profile may be related to the aberrant brain anatomy and performance deficits found in FXS. Alterations in early sensory ERP components appear to be relatively specific to FXS, whereas more cognitive components occurring later seem to be altered in other forms of ID as well. Finally, a modality difference might exist in the extent of impairment in sensory processing, suggesting that auditory processing is more severely impaired than visual processing in FXS.

A general shortcoming of the reviewed studies was the lack of control for immature brain responses as a possible cause for altered ERPs, since they only compare FXS patients to healthy aged matched controls (except for one study in which a third group of patients with Down Syndrome is tested in order to control for effects that rely on intellectual disability (St Clair et al., 1987)). For all other studies, the level of cognitive functioning of FXS patients must be considered as a confounding variable that might influence the results.

On the basis of these conclusions, we decided to investigate basic sensory processing in FXS. In order to control for immaturity of brain responses, we added a control group of healthy children that match the age of cognitive functioning of FXS patients with ID. Since, among other things, the absence of FMRP causes aberrant neurodevelopment rather than

immaturity, we expected at least some of the ERP components in FXS patients to differ not only from the chronological, but also from the developmental control group.

Finally, an earlier ERP study, as well as behavioural findings, suggest a modality difference in the extent of impairment in FXS, with the auditory modality being more affected than the visual modality. Thus, we investigated both basic auditory and visual processing in order to detect possible modality differences in the altered ERP components.

As assumed, we found an altered ERP profile in FXS that largely corresponded to the scarce existing literature. This is an important finding since only a few studies with rather small FXS sample sizes have been published so far. Notably, increased N70/N1 amplitude in response to visual and auditory stimulation, as well as prolonged auditory N2 latency, can be considered fairly stable phenomena in FXS, since they have now been found in six and four studies respectively, using different paradigms. Importantly, our findings of increased auditory P2 and N2 amplitude in FXS help clarify a certain discrepancy that has been found in the literature concerning these components. Since our basic stimulation created less interference with higher cognitive factors that might influence the appearance of these components, such as attention and memory in oddball paradigms, we can assume that the ERP profile obtained in our study uniquely reflects basic sensory processing. Therefore, it is likely that increased auditory P2 and N2 amplitudes are part of the basic ERP profile in FXS, with deviant results in some other studies possibly due to the influence of additional cognitive processes on the components evoked by the paradigm.

The second important finding in our study goes beyond what has been found in the literature up to now, since a comparison between FXS patients and our additional group of developmental controls showed that the auditory ERP profile in FXS does not reflect an immaturity of brain responses, but specific alterations. If cognitive functioning similar to that of the less mature brain were reflected in ERPs, rather than alterations in information processing specific to FXS, the ERP would not differ between developmental controls and FXS patients. However, auditory ERP components in FXS differ not only from those of controls matched by chronological age, but also from those found in controls matched by

developmental age. In contrast, visual N70 and N2 appear similar in FXS and developmental controls and might therefore reflect similar levels of cognitive functioning, rather than alterations in neuronal information processing.

Lastly, a modality difference is found between visual and auditory processing. Visual ERP components seem to be less altered in FXS than auditory components, relative to healthy controls. While auditory N1, P2 and N2 amplitude, as well as N2 latency differ significantly from both control groups, no visual component differs significantly from both control groups. Thus, we found that auditory information processing is more impaired than visual information processing in FXS.

We can thus conclude that our study supports the two main hypotheses that have been formulated in the research objective, i.e. alterations in the ERP profile in FXS relative to chronological as well as to developmental controls and a different extent of ERP alterations between the auditory and the visual modality in FXS.

Impaired mechanisms in FXS

By interpreting the observed ERP alterations in FXS in light of what is known about neurochemical outcomes of FMRP absence, as well as brain activity and anatomy in FXS, we can attempt to understand the neuronal mechanisms underlying deficient information processing in FXS. At this point, the ideas concerning underlying neuronal mechanisms that have been briefly mentioned in the discussion of the research article will be discussed in more detail.

The increased auditory N1 amplitude is of particular interest, since it is the most consistent finding in FXS and reflects early sensory processing. Findings in FXS patients concerning increased N100m (the N1 equivalent in MEG) amplitude, indicate that more neurons are synchronously active in the cerebral cortex during stimulus processing (Rojas et al., 2001). We suggest two explanations for the increased neuronal activation in FXS; a surplus of neurons in brain regions that are involved in generating the N1 component and a deficit in neuronal inhibition. These explanations are not meant to be mutually exclusive or

exhaustive, since most likely a number of factors influence the impairments in information processing found in FXS.

The occipital cortex, which contains most of the visual cortex, has been suggested as a source for the greater part of the visual N70 (Shigeto et al., 1998). The auditory N1 is believed to be generated in the superior temporal gyrus, which includes the primary auditory cortex (Näätänen & Picton, 1987). Greater gray matter volume has been found in the occipital cortex of infants with FXS, relative to normally developed children and children with non-syndromic delay (Hoeft et al., 2008). This supports the hypothesis of a surplus of neurons in FXS, which might contribute to larger visual N70 amplitude. In contrast, a decreased gray matter volume is found in the superior temporal gyrus in children and adolescents with FXS, compared to healthy controls (Gothelf et al., 2008). This seems to contradict the surplus hypothesis upon first sight, since smaller gray matter volume implies fewer neurons. However, findings obtained in a longitudinal study investigating gray matter volume changes with age in FXS and age- and developmentally matched controls offer a possible explanation (Hoeft et al., 2010). Over the course of two years, a greater gray matter increase was found in several brain structures in FXS, including not only temporal-occipital regions, but also the superior temporal gyrus. The authors contrast these findings with early, possibly prenatal, alterations in neurodevelopment, and suggest delayed or otherwise disrupted synaptic pruning occurring postnatally as a cause for the greater volume increase (Hoeft et al., 2010). Synaptic pruning is a regulatory process during neurological development, in which unnecessary neurons and synapses are reduced in order to facilitate changes in structure and strengthen more efficient neuronal configurations. Deficits in synaptic pruning lead to an overabundance of redundant synapses and neurons. Disruptions in synaptic pruning have been found in animal models of FXS (Pfeiffer & Huber, 2007, 2009; Weiler & Greenough, 1999). Further, synaptic plasticity is known to be impaired in FXS (Bassell & Warren, 2008). Synaptic plasticity is the ability of synapses to strengthen or weaken their connections to other synapses in response to increased or decreased common activity (Hebb, 2002). The combination of impaired synaptic pruning and impaired synaptic plasticity in FXS could imply that redundant synapses, which have not been eliminated in synaptic pruning, cannot be efficiently integrated into the neural network.

Thus, sensory stimulation might lead to an increased, non-specific arousal response in these redundant synapses, accounting for the increased N1 amplitude.

This finds further support through studies investigating N1 habituation in FXS. Habituation is considered the most elementary form of learning and occurs as early as the fetal stage of development (Morokuma et al., 2004). Habituation (i.e. repetition suppression) is the brain response to repeated stimuli presentations in any modality. ERPs typically show reduced brain activity amplitude with stimulus repetition (Grill-Spector, Henson, & Martin, 2006), which represents the process of habituation. Both studies investigating N1 habituation in FXS found the expected reduction of N1 amplitude after several presentations of the same stimulus in controls (Castrèn et al., 2003; M. J. Van der Molen et al., 2012b). FXS patients, however, did not show N1 habituation. The reduction of N1 amplitude in controls most likely reflects the reduction of the non-specific arousal or novelty response that generally occurs after the first appearance of a sensory stimulus (Karhu et al., 1997). After a few repetitions, the overall local activity in unspecialized connections is usually suppressed by active inhibition modulated through GABA receptor mechanisms (Disney & Calford, 2001). Given that the GABAergic system appears to be dysfunctional in FXS (Paluszkiewicz et al., 2011), this active inhibition of the arousal response may be impaired in FXS. Further, the second process involved in habituation, the strengthening of selective cortical connections that reflect the neural representation of a stimulus ('memory trace') is also known to be impaired in FXS. As explained earlier, FMRP deficit leads to an exaggerated mRNA translation, which again causes continuous enhanced mGluR-dependent long term depression. In consequence, the protein-synthesis in the synapses is not modified specifically to stimuli induction and a loss of protein synthesis-dependent plasticity occurs (Bassell & Warren, 2008).

Thus, deficient synaptic pruning might lead to an excess of redundant, less adapted synaptic configurations that non-specifically respond to sensory stimulation, while, simultaneously, deficient neuronal inhibition and the loss of synaptic plasticity impair the formation of more efficient synaptic configurations. These mechanisms might be partially reflected in increased N1 amplitude and absence of N1 habituation in FXS, while likely contributing to deficient information processing and learning disability in FXS.

An explanation for increased N1 amplitudes focusing on impaired neuronal inhibition finds support in a study previously mentioned in the electrophysiology section of the introduction that investigated resting state EEG in FXS (M. J. Van der Molen & Van der Molen, 2013). They found an increased relative theta power and a decreased relative upper alpha in FXS, compared to healthy controls. Alpha oscillations in EEG are believed to be involved in neural inhibitory regulation mechanisms that gate information by reducing the processing capabilities of a given area (Jensen & Mazaheri, 2010). Further, evoked alpha and theta oscillations have recently been linked to the generation of the P1-N1 complex (Klimesch et al., 2004). A reduced alpha rhythm in FXS could indicate impaired sensory gating mechanisms, which fail to inhibit redundant neural activity. In consequence, there would be more neuronal activation, which might be reflected in an increased N1 amplitude. As mentioned above, GABAergic feedback from inhibitory interneurons is believed to play a key role in alpha power generation (Jensen & Mazaheri, 2010). A dysfunctional GABAergic system thus appears again as a key mechanism in impaired information processing in FXS.

All of the hypotheses presented above concerning the possible neuronal mechanism underlying increased N70/N1 amplitude seem to suggest a hyperexcitable nervous system in FXS. This would account for some of the symptoms found in FXS, such as hyperarousal and attention deficits. Possible explanations remain hypothetical, however, and should be further explored by combining EEG with other brain imaging techniques.

Findings in children

In the course of our study, we also investigated children between the age of 4.5 and 6 affected by FXS. Unfortunately, some of the children were very anxious and agitated during the testing, forcing us to stop the EEG. Finally, auditory ERP data was only available for three and visual data only for two children. This sample size was not sufficiently large for statistical testing. However, visual inspection of the ERP profiles in FXS children compared to controls matched by chronological and developmental age, suggested an increased visual and auditory N2 amplitude, as well as a prolonged auditory N2 latency. This indicates that at least some of the ERP alterations can already be found in very young FXS patients. In the discussion of the research article, we propose a longitudinal study investigating ERPs at a very young age in infants affected by FXS with frequent follow-ups in order to discriminate early, possibly even prenatal, alterations in neurodevelopment from delayed or otherwise altered synaptic pruning occurring postnatally. The late diagnosis of FXS around the age of 3 years (Bailey et al., 2009) has been mentioned as the main problem in the realization of such a project and routine genetic screenings have been suggested as a possible solution (Bailey et al., 2008). However, the difficulty in testing young children with FXS that we have observed during the course of our study might pose an additional problem.

Implications for treatments of FXS

In the section describing the genetic mechanisms underlying FXS, we mentioned the possibility of treating FXS with mGluR5 inhibitors like fenobam. ERPs could be used as an outcome measure in clinical trials to assess the influence of medical treatments on information processing in FXS. Further, our finding that information processing is less impaired in the visual than in the auditory modality could help justify the design of behavioral trainings for FXS patients that rely more on visual stimulation. For example, pictograms might be more useful than verbal explanations. In addition, indications for a hyperexcitable brain in FXS, especially concerning auditory stimulation, point to the importance of maintaining a calm environment in order to avoid constant hyperarousal.

Limitations

Even though our study provides exciting evidence for information processing deficits in FXS, some issues should be addressed. More than half of our patient population was medicated during the EEG recording with psychostimulant and/or other drugs. Parents or caregivers often decided that the EEG recording would not be possible if they did not administer medication before coming to hospital. As psychoactive drugs are known to have an influence on the parameters of ERPs, medication might have been a confounding variable in our study. However, psychoactive drugs that have been found to influence parameters of ERPs are more likely to reverse the ERP profile of the treated pathology so that it no longer differs from that of controls. For example, children that have been diagnosed with ADHD showed enhanced N1 and P2 amplitudes, as well as reduced N2 amplitudes relative to healthy controls in a cued Go/Nogo task. After being medicated with methylphenidate, a psychostimulant, these components did not differ significantly from controls any more (Broyd et al., 2005). Likewise, reduced P3 amplitude found in non-medicated children with ADHD no longer differed from the control group after they were treated with methylphenidate (Seifert, Scheuerpflug, Zillessen, Fallgatter, & Warnke, 2003). Thus, it seems unlikely that the altered ERP profile, which we detected in our patient population, is caused by drug effects rather than the FXS pathology. On the contrary, the medication would have been more likely to mask ERP alterations in FXS rather than creating alterations. This finds further support in the fact that our results are generally in concordance with the results obtained by Van der Molen's group, who tested non-medicated FXS patients (M. J. Van der Molen et al., 2011, 2012a). Moreover, our results indicate that the medication taken by the patients did not reverse the phenotype in FXS. However, this cannot be said with certainty, since not all patients were medicated and those who were differed in drug type and dosage. A study strategically investigating possible changes in ERP profile induced by psychoactive drugs, including fenobam, would therefore be interesting.

Another shortcoming in our study is the fact that our subjects were not given an audiometric test prior to the EEG testing, to ensure equivalent peripheral sensory integrity of the auditory system. The time and patience of our patient population for our experiment was limited and all of them underwent detailed physical examinations in the developmental clinic

of the hospital upon their diagnosis. In the scope of these evaluations, none of the patients have been diagnosed with hearing deficits. Further, parents reported normal hearing in all patients and control participants upon specific request. However, an audiometric test would have added valuable information to our experiment and will be considered in future studies.

Although the addition of a second control group matching the cognitive functioning of patients with ID has provided us with valuable knowledge and is one of the strongest points in our study, it would have been interesting to have a control group with another kind of ID. Our control group provides evidence that the alterations found in the ERP profile are mostly specific to FXS and do not reflect immature information processing linked to the level of performance. However, a control group with non-syndromic ID or a different kind of syndromic ID would not only have strengthened our conclusion that the ERPs do not simply reflect immature information processing, but also that the ERP profile in FXS might differ from what is generally found in ID. In our literature review, we gathered ERP results in other IDs and autism and compared them to what has been found in FXS. Alterations in N1 and P2 amplitude seemed to be specific to FXS, whereas N1 habituation, MMN and P3 amplitude and N2 and P3 latency seemed to be altered in a similar way in other forms of ID. We suggested the possibility of heterogeneous syndrome-specific perturbations in early sensory processes that lead to homogeneous outcomes in later components. However, it is difficult to compare EEG studies from different research groups, since a number of factors varies between studies, such as paradigm, stimuli parameters, tasks and investigated electrodes, as well as analysis procedures that might all have an influence upon the results. Therefore it is preferable to compare several syndromes, such as different forms of ID, in a single study, in order to investigate information processing.

Perspectives

As mentioned above, it would be interesting to investigate another ID population with the same study parameters in order to discriminate FXS specific alterations from alterations generally found in ID. We are planning to add another ID population to our study, i.e. patients with de novo mutations in the autosomal gene SYNGAP1. Protein-truncating de novo

mutations in the SYNGAP1 gene have recently been identified in 3% of a series of 94 patients with nonsyndromic ID (Hamdan et al., 2009). The gene mutation results in the production of several deficient proteins that are known to be important for synaptic plasticity (Komiyama et al., 2002). Six of these patients have already been identified at the CHU Sainte-Justine Hospital University Center and can be tested for our project. Since both FXS and SYNGAP1 mutations seem to impair synaptic plasticity, it would be of particular interest to compare basic information processing between these IDs.

Additionally, it would be interesting to go beyond basic stimulus processing and investigate more complex information processing in FXS. We are in the course of analysing two more EEG experiments that we carried out with our FXS patient population. Both experiments are habituation paradigms featuring natural stimuli, i.e. human faces and pseudo-words pronounced by a human voice. Since brain anatomy studies found alterations in FXS in the fusiform gyrus, a brain region that seems to be specialized in face processing (Hoeft et al., 2008), and functional studies found altered activity during face and gaze processing in FXS (Dalton et al., 2008; Garrett et al., 2004; Watson et al., 2008), it is of particular interest to investigate face processing in FXS using electrophysiology. We are currently examining habituation of the face-specific ERP N170 in FXS and healthy controls in order to detect possible alterations in face processing and synaptic plasticity, since habituation is a form of learning that can easily be reflected using ERPs. Preliminary results obtained in three patients showed an increased N170 in FXS compared to chronological and developmental age matched controls. While N170 amplitude decreased after the first repetition of a face in controls, the opposite pattern was found in FXS. These preliminary results indicate disturbed face processing and synaptic plasticity in FXS. However, the sample size is as yet too small to draw any firm conclusions. Likewise, we plan to investigate habituation of ERP components evoked by pseudo-words in FXS and controls, since language processing seems to be particularly impaired (Schneider et al., 2009) and alterations are found in language associated brain areas of FXS patients (Gothelf et al., 2008)

Moreover, we plan to carry out additional methods of analysis with our EEG data that go beyond ERPs and might provide further insights into neuronal mechanisms in FXS. On the

one hand, we will calculate the energy of our EEG data, defined as the normalized sum of amplitudes, reflected through sampling points, in a given time window. Previous studies in our laboratory showed that energy in the signal reduces with repetition of a stimulus (Lafontaine et al., in preparation) and is thus likely to reflect habituation processes. On the other hand, we want to investigate complexity in FXS brain signal, relative to healthy controls. Complexity of EEG signals is believed to reflect 'brain noise', which is defined as the variation generated by the deterministic and random components of the brain network process (McIntosh et al., 2010). It can be estimated by multiscale entropy (Costa, Goldberger, & Peng, 2005), which assesses the temporal predictability of the signal in a time series. Complexity of the brain signal is known to increase with maturation (Lippe, Kovacevic, & McIntosh, 2009) and positively predicts performance in behavioural measures of learning tasks (McIntosh, Kovacevic, & Itier, 2008). Furthermore, local complexity decreases with maturation, whereas distributed complexity, which reflects the integration between distributed neuronal populations in the brain, increases (Vakorin, Lippe, & McIntosh, 2011). Thus, a relation between brain noise and the functional variability of the brain can be assumed (McIntosh et al., 2008). Since increased complexity can be observed in a familiar stimulus as compared to an unfamiliar one (Heisz, Shedden, & McIntosh, 2012), habituation is likely reflected in increased brain signal complexity. Finally, reduced signal complexity is found in various pathologies, including autism (Catarino, Churches, Baron-Cohen, Andrade, & Ring, 2011). Thus, since complexity is believed to reveal maturation and functional variability in the brain, and is lower in other pathologies, like autism, we expect it to be reduced in FXS. Further, we expect to find a lessened increase in complexity with stimulus repetition in the habituation paradigm in FXS, relative to controls, reflecting learning disability and impairments in synaptic plasticity.

Conclusion

We found an altered ERP profile in response to basic auditory and visual stimulation in FXS, including increased auditory increased auditory N1, P2 and N2 amplitudes and increased auditory N2 latencies, reflecting impaired neuronal information processing. The auditory ERP profile is not only found to differ from controls matched by chronological age, but also from controls matched to the developmental age of cognitive functioning, indicating aberrations in neurodevelopment specific to FXS rather than immaturity of physiological activity. However, visual N70 and N2 amplitude were only increased in FXS relative to chronological, but not to developmental controls. Thus, we found auditory information processing to be more impaired than visual processing in FXS, reflected in fewer ERP alterations in the visual paradigm. In combination with findings of brain anatomy, biochemistry and behaviour, our results suggest a hyperexcitable nervous system in FXS. Further electrophysiological paradigms and additional methods of analysis will help us to investigate more complex forms of information processing in FXS, including habituation, face and language processing, and compare them with other forms of ID.

General References

Adolphs, R., Tranel, D., & Damasio, A. R. (1998). The human amygdala in social judgment. *Nature, 393*(6684), 470-474.

Bailey, D. B., Jr., Raspa, M., Bishop, E., & Holiday, D. (2009). No change in the age of diagnosis for fragile x syndrome: findings from a national parent survey. *Pediatrics, 124*(2), 527-533.

Bailey, D. B., Jr., Skinner, D., Davis, A. M., Whitmarsh, I., & Powell, C. (2008). Ethical, legal, and social concerns about expanded newborn screening: fragile X syndrome as a prototype for emerging issues. *Pediatrics, 121*(3), e693-704.

Barnea-Goraly, N., Eliez, S., Hedeus, M., Menon, V., White, C. D., Moseley, M., & Reiss, A. L. (2003). White matter tract alterations in fragile X syndrome: preliminary evidence from diffusion tensor imaging. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics, 118B*(1), 81-88.

Barry, R. J., Clarke, A. R., & Johnstone, S. J. (2003). A review of electrophysiology in attention-deficit/hyperactivity disorder: I. Qualitative and quantitative electroencephalography. *Clinical Neurophysiology, 114*(2), 171-183.

Bassell, G. J., & Warren, S. T. (2008). Fragile X syndrome: loss of local mRNA regulation alters synaptic development and function. *Neuron, 60*(2), 201-214.

Bennetto, L., Pennington, B. F., Porter, D., Taylor, A. K., & Hagerman, R. J. (2001). Profile of cognitive functioning in women with the fragile X mutation. *Neuropsychology, 15*(2), 290-299.

Berry-Kravis, E. (2002). Epilepsy in fragile X syndrome. *Developmental Medicine & Child Neurology, 44*(11), 724-728.

Berry-Kravis, E., Hessl, D., Coffey, S., Hervey, C., Schneider, A., Yuhas, J., . . . Hagerman, R. (2009). A pilot open label, single dose trial of fenobam in adults with fragile X syndrome. *Journal of Medical Genetics, 46*(4), 266-271.

Bigler, E. D., Mortensen, S., Neeley, E. S., Ozonoff, S., Krasny, L., Johnson, M., . . . Lainhart, J. E. (2007). Superior temporal gyrus, language function, and autism. *Developmental Neuropsychology, 31*(2), 217-238.

Bray, S., Hirt, M., Jo, B., Hall, S. S., Lightbody, A. A., Walter, E., . . . Reiss, A. L. (2011). Aberrant frontal lobe maturation in adolescents with fragile X syndrome is related to delayed cognitive maturation. *Biological Psychiatry 70*(9), 852-858.

Bregman, J. D., Leckman, J. F., & Ort, S. I. (1988). Fragile X syndrome: genetic predisposition to psychopathology. *Journal of Autism and Developmental Disorders, 18*(3), 343-354.

Broyd, S. J., Johnstone, S. J., Barry, R. J., Clarke, A. R., McCarthy, R., Selikowitz, M., & Lawrence, C. A. (2005). The effect of methylphenidate on response inhibition and the event-related potential of children with attention deficit/hyperactivity disorder. *International Journal of Psychophysiology, 58*(1), 47-58.

Budimirovic, D. B., Bukelis, I., Cox, C., Gray, R. M., Tierney, E., & Kaufmann, W. E. (2006). Autism spectrum disorder in Fragile X syndrome: differential contribution of adaptive socialization and social withdrawal. *American Journal of Medical Genetics Part A, 140A*(17), 1814-1826.

Castrèn, M., Paakkonen, A., Tarkka, I. M., Ryynanen, M., & Partanen, J. (2003). Augmentation of auditory N1 in children with fragile X syndrome. *Brain Topography, 15*(3), 165-171.

Catarino, A., Churches, O., Baron-Cohen, S., Andrade, A., & Ring, H. (2011). Atypical EEG complexity in autism spectrum conditions: a multiscale entropy analysis. *Clinical Neurophysiology, 122*(12), 2375-2383.

Chang, B. S., & Lowenstein, D. H. (2003). Epilepsy. *The New England Journal of Medicine, 349*(13), 1257-1266.

Comery, T. A., Harris, J. B., Willems, P. J., Oostra, B. A., Irwin, S. A., Weiler, I. J., & Greenough, W. T. (1997). Abnormal dendritic spines in fragile X knockout mice: maturation and pruning deficits. *Proceedings of the National Academy of Sciences of the United States of America, 94*(10), 5401-5404.

Cornish, K., Kogan, C., Turk, J., Manly, T., James, N., Mills, A., & Dalton, A. (2005). The emerging fragile X premutation phenotype: evidence from the domain of social cognition. *Brain and Cognition, 57*(1), 53-60.

Cornish, K. M., Li, L., Kogan, C. S., Jacquemont, S., Turk, J., Dalton, A., . . . Hagerman, P. J. (2008). Age-dependent cognitive changes in carriers of the fragile X syndrome. *Cortex, 44*(6), 628-636.

Costa, M., Goldberger, A. L., & Peng, C. K. (2005). Multiscale entropy analysis of biological signals. *Physical Review E: Statistical, Nonlinear, and Soft Matter Physics, 71*(2 Pt 1), 021906.

Daily, D. K., Ardinger, H. H., & Holmes, G. E. (2000). Identification and evaluation of mental retardation. *American Family Physician, 61*(4), 1059-1067, 1070.

Dalton, K. M., Holsen, L., Abbeduto, L., & Davidson, R. J. (2008). Brain function and gaze fixation during facial-emotion processing in fragile X and autism. *Autism Research, 1*(4), 231-239.

Disney, A., & Calford, M. B. (2001). Neurosteroids mediate habituation and tonic inhibition in the auditory midbrain. *Journal of Neurophysiology, 86*(2), 1052-1056.

Eliez, S., Blasey, C. M., Freund, L. S., Hastie, T., & Reiss, A. L. (2001). Brain anatomy, gender and IQ in children and adolescents with fragile X syndrome. *Brain, 124*(Pt 8), 1610-1618.

Farzin, F., & Rivera, S. M. (2010). Dynamic Object Representations in Infants with and without Fragile X Syndrome. *Frontiers in Human Neuroscience, 4*, 12.

Garrett, A. S., Menon, V., MacKenzie, K., & Reiss, A. L. (2004). Here's looking at you, kid: neural systems underlying face and gaze processing in fragile X syndrome. *Archives of General Psychiatry, 61*(3), 281-288.

Gothelf, D., Furfaro, J. A., Hoeft, F., Eckert, M. A., Hall, S. S., O'Hara, R., . . . Reiss, A. L. (2008). Neuroanatomy of fragile X syndrome is associated with aberrant behavior and the fragile X mental retardation protein (FMRP). *Annals of Neurology, 63*(1), 40-51.

Grill-Spector, K., Henson, R., & Martin, A. (2006). Repetition and the brain: neural models of stimulus-specific effects. *Trends in Cognitive Sciences, 10*(1), 14-23.

Hall, S. S., Burns, D. D., Lightbody, A. A., & Reiss, A. L. (2008). Longitudinal changes in intellectual development in children with Fragile X syndrome. *Journal of Abnormal Child Psychology, 36*(6), 927-939.

Hamdan, F. F., Gauthier, J., Spiegelman, D., Noreau, A., Yang, Y., Pellerin, S., . . . Michaud, J. L. (2009). Mutations in SYNGAP1 in autosomal nonsyndromic mental retardation. *The New England Journal of Medicine, 360*(6), 599-605.

Hazlett, H. C., Poe, M. D., Lightbody, A. A., Gerig, G., Macfall, J. R., Ross, A. K., . . . Piven, J. (2009). Teasing apart the heterogeneity of autism: Same behavior, different brains in toddlers with fragile X syndrome and autism. *Journal of Neurodevelopmental Disorders, 1*(1), 81-90.

Hebb, D. O. (2002). *The Organization of Behavior: A Neuropsychological Theory*: Taylor & Francis.

Heisz, J. J., Shedden, J. M., & McIntosh, A. R. (2012). Relating brain signal variability to knowledge representation. *Neuroimage, 63*(3), 1384-1392.

Hessl, D., Nguyen, D. V., Green, C., Chavez, A., Tassone, F., Hagerman, R. J., . . . Hall, S. (2009). A solution to limitations of cognitive testing in children with intellectual disabilities: the case of fragile X syndrome. *Journal of Neurodevelopmental Disorders, 1*(1), 33-45.

Hessl, D., Rivera, S. M., & Reiss, A. L. (2004). The neuroanatomy and neuroendocrinology of fragile X syndrome. *Mental Retardation and Developmental Disabilities Research Reviews, 10*(1), 17-24.

Hoeft, F., Carter, J. C., Lightbody, A. A., Cody Hazlett, H., Piven, J., & Reiss, A. L. (2010). Region-specific alterations in brain development in one- to three-year-old boys with fragile X syndrome. *Proceedings of the National Academy of Sciences of the United States of America, 107*(20), 9335-9339.

Hoeft, F., Hernandez, A., Parthasarathy, S., Watson, C. L., Hall, S. S., & Reiss, A. L. (2007). Fronto-striatal dysfunction and potential compensatory mechanisms in male adolescents with fragile X syndrome. *Human Brain Mapping, 28*(6), 543-554.

Hoeft, F., Lightbody, A. A., Hazlett, H. C., Patnaik, S., Piven, J., & Reiss, A. L. (2008). Morphometric spatial patterns differentiating boys with fragile X syndrome, typically developing boys, and developmentally delayed boys aged 1 to 3 years. *Archives of General Psychiatry, 65*(9), 1087-1097.

Holsen, L. M., Dalton, K. M., Johnstone, T., & Davidson, R. J. (2008). Prefrontal social cognition network dysfunction underlying face encoding and social anxiety in fragile X syndrome. *Neuroimage, 43*(3), 592-604.

Hull, C., & Hagerman, R. J. (1993). A study of the physical, behavioral, and medical phenotype, including anthropometric measures, of females with fragile X syndrome. *American Journal of Diseases of Children, 147*(11), 1236-1241.

Jensen, O., & Mazaheri, A. (2010). Shaping functional architecture by oscillatory alpha activity: gating by inhibition. *Frontiers in Human Neuroscience, 4*, 186.

Kanwisher, N., & Yovel, G. (2006). The fusiform face area: a cortical region specialized for the perception of faces. *Philosophical Transactions of the Royal Society B: Biological Sciences, 361*(1476), 2109-2128.

Karhu, J., Herrgard, E., Paakkonen, A., Luoma, L., Airaksinen, E., & Partanen, J. (1997). Dual cerebral processing of elementary auditory input in children. *Neuroreport, 8*(6), 1327-1330.

Kates, W. R., Abrams, M. T., Kaufmann, W. E., Breiter, S. N., & Reiss, A. L. (1997). Reliability and validity of MRI measurement of the amygdala and hippocampus in children with fragile X syndrome. *Psychiatry Research, 75*(1), 31-48.

Klimesch, W. (1999). EEG alpha and theta oscillations reflect cognitive and memory performance: a review and analysis. *Brain Research Reviews, 29*(2-3), 169-195.

Klimesch, W., Schack, B., Schabus, M., Doppelmayr, M., Gruber, W., & Sauseng, P. (2004). Phase-locked alpha and theta oscillations generate the P1-N1 complex and are related to memory performance. *Cognitive Brain Research, 19*(3), 302-316.

Komiyama, N. H., Watabe, A. M., Carlisle, H. J., Porter, K., Charlesworth, P., Monti, J., . . . Grant, S. G. (2002). SynGAP regulates ERK/MAPK signaling, synaptic plasticity, and learning in the complex with postsynaptic density 95 and NMDA receptor. *Journal of Neuroscience, 22*(22), 9721-9732.

Kramer, U. (2008). Atypical presentations of benign childhood epilepsy with centrotemporal spikes: a review. *Journal of Child Neurology, 23*(7), 785-790.

Krueger, D. D., & Bear, M. F. (2011). Toward fulfilling the promise of molecular medicine in fragile X syndrome. *Annual Review of Medicine, 62*, 411-429.

Kwon, H., Menon, V., Eliez, S., Warsofsky, I. S., White, C. D., Dyer-Friedman, J., . . . Reiss, A. L. (2001). Functional neuroanatomy of visuospatial working memory in fragile X syndrome: relation to behavioral and molecular measures. *American Journal of Psychiatry, 158*(7), 1040-1051.

Lafontaine, M. P., Lacourse, K., Théoret, H., Gosselin, F., McIntosh, A. R., Lina, J. M., & Lippé, S. (in preparation). Brain signal complexity increases with repetition suppression during learning of unfamiliar faces.

Laggerbauer, B., Ostareck, D., Keidel, E. M., Ostareck-Lederer, A., & Fischer, U. (2001). Evidence that fragile X mental retardation protein is a negative regulator of translation. *Human Molecular Genetics, 10*(4), 329-338.

LeDoux, J. E. (1995). Emotion: clues from the brain. *Annual Review of Psychology, 46*, 209-235.

Lee, A. D., Leow, A. D., Lu, A., Reiss, A. L., Hall, S., Chiang, M. C., . . . Thompson, P. M. (2007). 3D pattern of brain abnormalities in Fragile X syndrome visualized using tensor-based morphometry. *Neuroimage, 34*(3), 924-938.

Lightbody, A. A., & Reiss, A. L. (2009). Gene, brain, and behavior relationships in fragile X syndrome: evidence from neuroimaging studies. *Developmental Disabilities Research Reviews 15*(4), 343-352.

Lippé, S., Kovacevic, N., & McIntosh, A. R. (2009). Differential maturation of brain signal complexity in the human auditory and visual system. *Frontiers in Human Neuroscience, 3*, 48.

Lippé, S., Martinez-Montes, E., Arcand, C., & Lassonde, M. (2009). Electrophysiological study of auditory development. *Neuroscience, 164*(3), 1108-1118.

Lippé, S., Roy, M. S., Perchet, C., & Lassonde, M. (2007). Electrophysiological markers of visuocortical development. *Cerebral Cortex, 17*(1), 100-107.

Loesch, D. Z., Bui, Q. M., Grigsby, J., Butler, E., Epstein, J., Huggins, R. M., . . . Hagerman, R. J. (2003). Effect of the fragile X status categories and the fragile X mental retardation protein levels on executive functioning in males and females with fragile X. *Neuropsychology, 17*(4), 646-657.

Luck, S. J. (2005). *An introduction to the event-related potential technique*. Cambridge, Ma: MIT Press.

McIntosh, A. R., Kovacevic, N., & Itier, R. J. (2008). Increased brain signal variability accompanies lower behavioral variability in development. *PLOS Computational Biology, 4*(7), e1000106.

McIntosh, A. R., Kovacevic, N., Lippe, S., Garrett, D., Grady, C., & Jirsa, V. (2010). The development of a noisy brain. *Archives Italiennes de Biologie 148*(3), 323-337.

Menon, V., & Uddin, L. Q. (2010). Saliency, switching, attention and control: a network model of insula function. *Brain Structure & Function, 214*(5-6), 655-667.

Morokuma, S., Fukushima, K., Kawai, N., Tomonaga, M., Satoh, S., & Nakano, H. (2004). Fetal habituation correlates with functional brain development. *Behavioural Brain Research, 153*(2), 459-463.

Näätänen, R., & Picton, T. (1987). The N1 wave of the human electric and magnetic response to sound: a review and an analysis of the component structure. *Psychophysiology, 24*(4), 375-425.

Packard, M. G., & Knowlton, B. J. (2002). Learning and memory functions of the Basal Ganglia. *Annual Review of Neuroscience, 25*, 563-593.

Paluszkiewicz, S. M., Martin, B. S., & Huntsman, M. M. (2011). Fragile X syndrome: the GABAergic system and circuit dysfunction. *Developmental Neuroscience, 33*(5), 349-364.

Perry, A., & Factor, D. C. (1989). Psychometric validity and clinical usefulness of the Vineland Adaptive Behavior Scales and the AAMD Adaptive Behavior Scale for an autistic sample. *Journal of Autism and Developmental Disorders, 19*(1), 41-55.

Pfeiffer, B. E., & Huber, K. M. (2007). Fragile X mental retardation protein induces synapse loss through acute postsynaptic translational regulation. *Journal of Neuroscience 27*(12), 3120-3130.

Pfeiffer, B. E., & Huber, K. M. (2009). The state of synapses in fragile X syndrome. *Neuroscientist, 15*(5), 549-567.

Rapoport, M., van Reekum, R., & Mayberg, H. (2000). The role of the cerebellum in cognition and behavior: a selective review. *Journal of Neuropsychiatry & Clinical Neurosciences, 12*(2), 193-198.

Ring, H. A., & Serra-Mestres, J. (2002). Neuropsychiatry of the basal ganglia. *Journal of Neurology, Neurosurgery & Psychiatry, 72*(1), 12-21.

Rivera, S. M., Menon, V., White, C. D., Glaser, B., & Reiss, A. L. (2002). Functional brain activation during arithmetic processing in females with fragile X Syndrome is related to FMR1 protein expression. *Human Brain Mapping, 16*(4), 206-218.

Rogers, S. J., Wehner, D. E., & Hagerman, R. (2001). The behavioral phenotype in fragile X: symptoms of autism in very young children with fragile X syndrome, idiopathic autism, and other developmental disorders. *Journal of Developmental & Behavioral Pediatrics, 22*(6), 409-417.

Rojas, D. C., Benkers, T. L., Rogers, S. J., Teale, P. D., Reite, M. L., & Hagerman, R. J. (2001). Auditory evoked magnetic fields in adults with fragile X syndrome. *Neuroreport, 12*(11), 2573-2576.

Ropers, H. H. (2010). Genetics of early onset cognitive impairment. *Annual Review of Genomics and Human Genetics, 11*, 161-187.

Ropers, H. H., & Hamel, B. C. (2005). X-linked mental retardation. *Nature Reviews Genetics, 6*(1), 46-57.

Schalock, R. L., Luckasson, R. A., Shogren, K. A., Borthwick-Duffy, S., Bradley, V., Buntinx, W. H., . . . Yeager, M. H. (2007). The renaming of mental retardation: understanding the change to the term intellectual disability. *Journal of Intellectual & Developmental Disability, 45*(2), 116-124.

Schneider, A., Hagerman, R. J., & Hessl, D. (2009). Fragile X syndrome -- from genes to cognition. *Developmental disabilities research reviews 15*(4), 333-342.

Seifert, J., Scheuerpflug, P., Zillessen, K. E., Fallgatter, A., & Warnke, A. (2003). Electrophysiological investigation of the effectiveness of methylphenidate in children with and without ADHD. *Journal of Neural Transmission, 110*(7), 821-829.

Shigeto, H., Tobimatsu, S., Yamamoto, T., Kobayashi, T., & Kato, M. (1998). Visual evoked cortical magnetic responses to checkerboard pattern reversal stimulation: A study on the neural generators of N75, P100 and N145. *Journal of the Neurological Sciences, 156(2),* 186-194.

St Clair, D. M., Blackwood, D. H., Oliver, C. J., & Dickens, P. (1987). P3 abnormality in fragile X syndrome. *Biological Psychiatry, 22*(3), 303-312.

Sudhalter, V., & Belser, R. C. (2001). Conversational characteristics of children with fragile X syndrome: tangential language. *American Journal on Mental Retardation, 106*(5), 389-400.

Sullivan, K., Hatton, D., Hammer, J., Sideris, J., Hooper, S., Ornstein, P., & Bailey, D., Jr. (2006). ADHD symptoms in children with FXS. *American Journal of Medical Genetics Part A, 140*(21), 2275-2288.

Tranfaglia, M. R. (2012). Fragile X syndrome: a psychiatric perspective. *Results and Problems in Cell Differentiation, 54*, 281-295.

Vakorin, V. A., Lippe, S., & McIntosh, A. R. (2011). Variability of brain signals processed locally transforms into higher connectivity with brain development. *Journal of Neuroscience, 31*(17), 6405-6413.

Van der Molen, M. J., & Van der Molen, M. W. (2013). Reduced alpha and exaggerated theta power during the resting-state EEG in fragile X syndrome. *Biological Psychology, 92*(2), 216-219.

Van der Molen, M. J., Van der Molen, M. W., Ridderinkhof, K. R., Hamel, B. C., Curfs, L. M., & Ramakers, G. J. (2011). Auditory change detection in fragile X syndrome males: A brain potential study. *Clinical Neurophysiology*.

Van der Molen, M. J., Van der Molen, M. W., Ridderinkhof, K. R., Hamel, B. C., Curfs, L. M., & Ramakers, G. J. (2012a). Auditory and visual cortical activity during selective attention in fragile X syndrome: A cascade of processing deficiencies. *Clinical Neurophysiology, 123*(4), 720-729.

Van der Molen, M. J., Van der Molen, M. W., Ridderinkhof, K. R., Hamel, B. C., Curfs, L. M., & Ramakers, G. J. (2012b). Auditory change detection in fragile X syndrome males: a brain potential study. *Clinical Neurophysiology, 123*(7), 1309-1318.

Verheij, C., Bakker, C. E., de Graaff, E., Keulemans, J., Willemsen, R., Verkerk, A. J., . . . Oostra, B. A. (1993). Characterization and localization of the FMR-1 gene product associated with fragile X syndrome. *Nature, 363*(6431), 722-724.

Watson, C., Hoeft, F., Garrett, A. S., Hall, S. S., & Reiss, A. L. (2008). Aberrant brain activation during gaze processing in boys with fragile X syndrome. *Archives of General Psychiatry, 65*(11), 1315-1323.

Weiler, I. J., & Greenough, W. T. (1999). Synaptic synthesis of the Fragile X protein: possible involvement in synapse maturation and elimination. *American Journal of Medical Genetics 83*(4), 248-252.