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Alterations of visual and auditory evoked potentials in fragile X syndrome

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ABSTRACT

Background: Fragile X Syndrome (FXS) is the most common monogenic form of intellectual disability and one of the few known monogenic causes 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 electrophysiology.
Methods: We compared auditory (AEP) and visual (VEP) evoked potentials in twelve adolescents and young adults (10–22 years) affected by FXS to healthy controls matched by chronological age ($N = 12$) and developmental age of cognitive functioning ($N = 9$; 5–7 years), using analysis of variance.
Results: In the visual modality, the N70 and N2 amplitude have been found increased in FXS in comparison to the chronological, but not the developmental control group at occipital sites, whereas in the auditory modality N1, P2 and N2 amplitude as well as N2 latency have been found increased in FXS, relative to both chronological and developmental control groups at mid-central sites.
Conclusions: The AEP/VEP profile suggests disruptions in sensory processing specific to FXS that exceed immaturity of physiological activity. In addition, 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 hyperreactive nervous system in FXS.

1. Background

Fragile X Syndrome (FXS) is the most common monogenic form of intellectual disability (ID) and affects about 2% of male patients with ID (Ropers and 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. Women can also be affected but the penetrance of the mutation is reduced and its expressivity more variable in them (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 et al., 2004). FMRP directly targets approximately 5% of all mRNAs (Darnell and Klann, 2013). However, changes in protein synthesis observed in the absence of FMRP affect about 20% of pre-synaptic protein synthesis (Darnell and Klann, 2013). Thus, secondary alterations are believed to additionally account for the changes in protein synthesis observed in the absence of FMRP (Darnell and Klann, 2013). Alterations in protein synthesis result in a loss of synaptic plasticity in FXS (Bassell and Warren, 2008). Structurally, dendritic spines are increased in number and appear elongated whereas synapses appear immature in FXS patients and fragile X knockout mice (Comery et al., 1997). Thus, the absence of FMRP is likely to prevent activity-based synapse maturation and synaptic pruning, which are essential for normal brain development (Weiler and Greenough, 1999) and cognitive development (Schneider et al., 2009).

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Table 1
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)

Patients affected by FXS frequently show deficits in language, executive functions, visuo-spatial and social cognition. Further, they tend to show aberrant 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. FXS is thus considered one of the few known monogenic causes of autism (Rogers et al., 2001). Further, reduction of FMRP levels have been found in the cerebrellar vermis of adult subjects with autism who were not diagnosed with FXS, suggesting that common neurobiological mechanisms might account for the shared symptoms between non-syndromic autism and FXS (Fatemi et al., 2011). However, autistic symptoms vary considerably in their intensity between patients affected by FXS (Schneider et al., 2009).

Disrupted pathways in synaptic plasticity, the potential link between the *FMR1* mutation and the learning disability often found in FXS, are likely to be associated with impairments in mechanisms of information processing (Belmonte and Bourgeron, 2006). Early sensory processing can be non-invasively investigated using the AEP/VEP technique that records local field potentials, which are summarized postsynaptic potentials from large groups of neurons (Luck, 2005). Studies investigating AEP/VEPs in FXS so far exclusively used oddball paradigms and mostly studied AEPs (St Clair et al., 1987; Castrèn et al., 2003; Van Der Molen et al., 2012a,b). The most consistent findings were enhanced N1 and decreased P3 amplitudes, as well as prolonged N2 latencies in FXS compared to healthy age-matched controls, whereas findings concerning other components tended to be more variable. Thus, some AEPs appeared to be specifically altered in FXS, revealing disruptions in early sensory processing. In this study we aim to investigate to which extent the altered AEP/VEPs in FXS can be explained by immature as opposed to otherwise disrupted sensory processing. Since parameters of AEP/VEPs specifically change with brain development (Lippé et al., 2007, 2009), the altered AEP/VEPs in FXS might reflect immature physiological activity due to deficits in synaptic pruning. In this case, the AEP/VEPs in FXS would resemble those of individuals on the same level of cognitive functioning. However, given that the absence of FMRP has been found to interfere with functional and structural brain development, we hypothesize that the disruptions in sensory processing reflected by AEP/VEPs exceed immaturity. In order to distinguish between immaturity and specific alterations of the AEP/VEPs, we compared the FXS patients to an additional control group matched to the developmental age of cognitive functioning, assessed by Intelligence Quotient (IQ).

Further, we aimed to investigate differences in the extent of impairments in sensory processing between auditory and visual modality in FXS. The only previous study investigating VEPs in FXS found the auditory modality to be more affected than the visual modality (Van Der Molen et al., 2012a), which matches modality differences in performance found in FXS (Schneider et al., 2009; Van Der Molen et al., 2010). This indicates that FMRP absence might affect sensory processing differently depending on modality. We hypothesize that the VEPs appear less altered in FXS than the AEPs. We thereby examine whether the extent to which the AEP/VEP alterations can be explained by physiological immaturity varies between modalities.

2. Methods

2.1. 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 1). 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 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 at the Ste-Justine Hospital, the University of Montreal, kindergartens and summer day camps. Four of the twelve FXS patients had also been diagnosed with autistic disorder; eight FXS patients showed language delay and nine 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 (5× methylphenidate, 2× atomoxetine, 1× amphetamine mixed salts) and/or antidepressant (1× 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 and 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 questionnaire (Lam and Aman, 2007) and the aberrant behavior checklist (Aman et al., 1985), 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 before the experiment from participants and parents or legal caregivers following a full explanation of the procedures undertaken.

2.2. 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 50 ms broadband noise presented 150 times in a randomly distributed interstimulus interval varying from 1200 to 1400 ms at 79 dB SPL intensity and 16-bit resolution as in a developmental study previously conducted in our laboratory (Lippé et al., 2009). 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 500 ms, and subtending a visual angle of 2°. The original and reversed checkerboard stimulus were presented 200 times each. Stimuli had a luminance of 40 cd/m^2 and were displayed on a 40.5 cm × 30.5 cm ViewSonic monitor (ViewSonic, Canada) at 114 cm distance from the participant's eyes. This visual paradigm has been created for a developmental study previously conducted in our laboratory (Lippé et al., 2007). 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 40 kΩ (Tucker, 1993). Signals were acquired and processed by a G4 Macintosh computer using NetStation EEG