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Chapter 5

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

A cascade of processing deficiencies

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Abstract

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

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

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

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

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

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

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

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

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

5.2 Method

5.2.1 Participants

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

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

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

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

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

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

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

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

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

5.3 Results

5.3.1 Performance data

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

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

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

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

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

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

5.3.2.1 Auditory N1 amplitude.

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

5.3.2.2 Auditory N1 latency.

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

5.3.2.3 Auditory P2 amplitude.

In both groups, P2 amplitudes showed their maximum at FCz, \(F(4, 124) = 21.18, p < .0001, \eta^2 = .41\). The main effect of Stimulus, \(F(1, 31) = 4.69, p < .05, \eta^2 = .13,\)

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

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

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

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

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

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

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

<table>
<thead>
<tr>
<th>Modality</th>
<th>Standard Stimuli</th>
<th>Deviant Stimuli</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls Site</td>
<td>FXS Site</td>
</tr>
<tr>
<td></td>
<td>ERP Site</td>
<td>ERP Site</td>
</tr>
<tr>
<td>Auditory</td>
<td>N1 FCz -5.4 (0.6)</td>
<td>FCz -8.5 (0.9)</td>
</tr>
<tr>
<td>Latency</td>
<td>125 (1.1)</td>
<td>126 (1.4)</td>
</tr>
<tr>
<td>Amplitude</td>
<td>P2 FCz 5.6 (0.8)</td>
<td>FCz 8.1 (1.1)</td>
</tr>
<tr>
<td>Latency</td>
<td>233 (6.0)</td>
<td>217 (8.5)</td>
</tr>
<tr>
<td>Amplitude</td>
<td>N2b Fz 1.5 (0.6)</td>
<td>Fz -3.9 (0.9)</td>
</tr>
<tr>
<td>Latency</td>
<td>291 (11.8)</td>
<td>337 (17.1)</td>
</tr>
<tr>
<td>Amplitude</td>
<td>P3b Cz 2.9 (0.6)</td>
<td>Fz 1.7 (0.4)</td>
</tr>
<tr>
<td>Latency</td>
<td>339 (8.1)</td>
<td>311 (9.2)</td>
</tr>
</tbody>
</table>

| Visual   | N1 Oz -1.4 (0.7) | Oz -3.6 (0.8) | FCz -0.9 (0.8) | FCz -4.5 (0.7) |
| Latency  | 98 (6.1)         | 113 (5.1)     | 94 (5.0)       | 116 (5.1)       |
| Amplitude| P2 Oz 6.7 (0.8)  | Oz 9.0 (1.1)  | Oz 7.5 (0.8)   | Oz 10.0 (1.2)   |
| Latency  | 167 (8.6)        | 155 (12.1)    | 162 (9.2)      | 159 (13.1)      |
| Amplitude| N2b F3 -0.8 (0.5) | Fz -4.5 (1.0) | F4 -0.8 (0.6)  | F4 -5.7 (1.4)   |
| Latency  | 225 (8.0)        | 286 (11.5)    | 251 (6.9)      | 270 (11.9)      |
| Amplitude| P3b Cz 7.3 (0.7) | Pz 3.9 (1.1)  | FCz 16.8 (0.8) | Oz 9.9 (1.2)    |
| Latency  | 339 (8.3)        | 378 (9.4)     | 349 (5.2)      | 363 (8.2)       |

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

5.3.3  *Visual ERP data*

5.3.3.1  *Visual N1 amplitude.*

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

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

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

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

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

5.3.3.6 **Visual N2b latency.**
The main effect of Group, $F(1, 31) = 19.31$, $p < .0001$, $\eta^2 = .38$, was included in a significant Stimulus by Group interaction, $F(1, 31) = 14.10$, $p < .01$, $\eta^2 = .31$. For both stimuli, N2b latencies were longer in FXS males than in control participants ($p < .05$). In control participants, N2b latencies were significantly longer for deviant than for standard stimuli ($p < .05$). In FXS males, N2b latencies were somewhat shorter for deviant than for standard stimuli, but this difference failed to reach significance ($p > .05$).
5.3.3.7 *Visual P3b amplitude.*

The ANOVA yielded significant interactions of Laterality by Group, $F(2, 62) = 5.58, p < .05, \eta^2 = .15$, and of Stimulus by Site by Group, $F(4, 124) = 10.75, p < .0001, \eta^2 = .28$, which showed that P3b amplitudes for standard and deviant stimuli peaked, respectively, at Cz and Pz in control participants and at FCz and Oz in FXS males. For both stimuli, P3b amplitudes were significantly smaller in FXS males than in control participants ($p’s < .05$). In both groups, P3b amplitudes were significantly larger for deviant than for standard stimuli ($p’s < .05$).

5.3.3.7 *Visual P3b amplitude.*

The ANOVA yielded significant interactions of Laterality by Group, $F(2, 62) = 5.58, p < .05, \eta^2 = .15$, and of Stimulus by Site by Group, $F(4, 124) = 10.75, p < .0001, \eta^2 = .28$, which showed that P3b amplitudes for standard and deviant stimuli peaked, respectively, at Cz and Pz in control participants and at FCz and Oz in FXS males. For both stimuli, P3b amplitudes were significantly smaller in FXS males than in control participants ($p’s < .05$). In both groups, P3b amplitudes were significantly larger for deviant than for standard stimuli ($p’s < .05$).

5.3.3.8 *Visual P3b latency.*

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

5.3.4 *Auditory vs. Visual P3b comparison*

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

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

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

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

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

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

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

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

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

<table>
<thead>
<tr>
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<tr>
<td>N2b</td>
<td>.28</td>
<td>-.19</td>
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<tr>
<td>P3b</td>
<td>-.36</td>
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</table>

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

5.4 Discussion

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

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

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

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

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

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

Finally, we examined the relation between ERP components and performance measures. Our results showed that P3b amplitude to deviant auditory stimuli predicted the speed of responding to these stimuli in both FXS males and controls. In FXS males, P3b amplitude to deviant stimuli predicted also hit rate to deviant stimuli and the proportion of false alarms to standard stimuli. This pattern of findings was absent for the visual oddball task. However, both N2b and P3b difference scores best explained the variance in RT to visual deviant stimuli in FXS males. The latter finding may be interpreted to suggest that visual deviant stimuli (i.e., smiley faces) resulted in increased triggering of attention (N2b; Näätänen, Kujala, & Winkler, 2011) necessary for efficient network-level decision-making (P3b; Nieuwenhuis et al., 2005) in FXS males. Although our experimental design precludes conclusions in terms of causative relations, the correlations between the P3b and performance in auditory and visual modalities, suggest that information processing deficiencies manifested by the P3b may underlie FXS task performance at the behavioral level. Importantly, to accurately determine whether the observed behavioral
and electrocortical findings are specific to the FXS etiology, as opposed to more general developmental changes affecting intellectual ability, future investigations should preferably include an additional neurodevelopmental disorder matched on both chronological age and intellectual ability. Future investigations may also address whether similar findings can be observed for FXS individuals with lower intellectual performance levels.

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