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### Authors

Cohen, Susannah
Masyn, Katherine
Mastergeorge, Ann
et al.



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# Psychophysiological Responses to Emotional Stimuli in Children and Adolescents with Autism and Fragile X Syndrome

**Susannah Cohen**[1], **Katherine Masyn**[2], **Ann Mastergeorge**[3], and **David Hessl**[4,5]

Susannah Cohen: susannah.cohen@gmail.com; Katherine Masyn: katherine_masyn@gse.harvard.edu; Ann Mastergeorge: amastergeorge@email.arizona.edu; David Hessl: david.hessl@ucdmc.ucdavis.edu

[1]California Department of Health Care Services 1501 Capitol Ave, MS 0000 Sacramento, California 95814

[2]Harvard Graduate School of Education, Harvard University 14 Appian Way Cambridge, Massachusetts 02138

[3]Norton School of Family and Consumer Sciences, University of Arizona 650 N Park Ave Tucson, Arizona 85721-0078

[4]Medical Investigation of Neurodevelopmental Disorders (MIND) Institute, University of California, Davis Medical Center 2825 50th St. Sacramento, California 95817

[5]Department of Psychiatry and Behavioral Sciences University of California, Davis School of Medicine

## Abstract

**Objective—**Individuals with autism demonstrate atypical and variable responses to social and emotional stimuli, perhaps reflecting heterogeneity of the disorder. The goal of this study was to determine whether unique profiles of psychophysiological responses to such stimuli could be identified in individuals diagnosed with autism, with fragile X syndrome, with comorbid autism and fragile X syndrome, and typically developing males.

**Method—**This study included 52 boys (ages 10–17): idiopathic autism (ASD; *n*=12), fragile X syndrome (FXS; *n*=12), comorbid autism and fragile X syndrome (ASD+FXS; *n*=17), typically developing (TYP; *n*=11). Physiological responses, including potentiated startle, electrodermal response, heart rate variability, and vagal tone were collected concurrently while participants viewed emotionally evocative pictures of human faces or non-social images. While some of these measures have been utilized separately for investigations on these diagnostic groups, they have not been considered together.

**Results—**Results of Kruskal-Wallis one-way analysis of variance by ranks indicate statistically significant differences in distributions of autonomic regulation responses between groups. The most notable differences were between the ASD group and both the fragile X groups on measures of sympathetic activity, with fragile X groups evincing increased activity. Also, both the autism and ASD+FXS groups showed significantly decreased parasympathetic activity compared with

Correspondence to: David Hessl, PhD, Associate Professor, MIND Institute, UC Davis Medical Center, 2825 50th Street, Sacramento, CA 95817, Phone: 916-703-0249, david.hessl@ucdmc.ucdavis.edu.

FXS and TYP groups. Additionally, the ASD+FXS group demonstrated a unique distribution of startle potentiation and arousal modulation.

**Conclusion—**This study provides evidence that autonomic arousal and regulation profiles could be useful to distinguishing subgroups of autism and shed light on the variability underlying emotional responsivity.

## Keywords

startle potentiation; vagal tone; emotion regulation; autonomic arousal

Autism is a broad diagnosis based on a prescribed social deficit (Rogers, Ozonoff, & Hansen, 2013). The diagnosis, however, is primarily determined by behavioral evaluation and application of the autism criteria in the Diagnostic Manual of Mental Disorders (DSM), and not by genetic investigation. As such, it likely encompasses a broad range of etiologies, and we are still lacking a detailed understanding of the relationships between etiological causes, pathophysiology and clinical symptoms. The National Institute of Mental Health (NIMH) Research Domain Criteria (RDoC; Insel et al, 2010) project is beginning to address the need to improve the categorization of mental illness based on genomics and neuroscience in order to improve clinical diagnosis, targeted treatments and outcomes. Within the framework of autism spectrum disorders, a potentially powerful approach is to compare individuals with idiopathic autism to those with similar social deficits, repetitive and stereotyped behaviors, and communication problems caused by a specific, and well-understood genetic etiology. For the present study, we chose to compare children with idiopathic autism spectrum disorder (ASD) and children with autism caused by fragile X syndrome (FXS), with a focus on psychophysiological responses to emotional and social stimuli.

FXS, an X-linked single gene disorder, has a strong association with autism. FXS occurs when there is a substantially abnormal expansion of the *FMR1* gene, causing a reduction in translation of the protein FMRP, which is involved in the structure of neurons and synaptic transmission (Hagerman, 2002). At least one-third of boys with FXS meet diagnostic criteria for Autistic Disorder (Rogers, Wehner, & Hagerman, 2001), however up to two-thirds demonstrate symptoms consistent with the more broadly defined autism spectrum disorder (Clifford et al., 2007). Given this association as well as the substantial overlap in symptoms, such as problems with social gaze, repetitive and stereotyped behaviors, and abnormalities of communication, FXS provides a relatively homogeneous model disorder for gaining insight into autism.

Individuals with ASD show deficits in social and emotional processing (e.g., Dawson, Meltzoff, Osterling, Rinaldi, & Brown, 1998; Hobson & Lee, 1998; Teunisse & De Gelder, 1994). There is a growing body of evidence that individuals with ASD exhibit noticeable impairments in face perception (e.g., Deruelle, Rondan, Gepner, & Tardif, 2004; Grelotti, Gauthier, & Schultz, 2002; Teunisse & De Gelder, 1994). These impairments may be observable very early in development (Osterling & Dawson, 1994) and such deficits in face processing have been found to be most pronounced during tasks with increased complexity or with an emotional component present (Bormann-Kischkel, Vilsmeier, & Baude, 1995;

Capps, Yirmiya, & Sigman, 1992). Although not as widely studied, there are some indications that individuals with FXS also have abnormalities in the face processing system, such as discerning direction of gaze (Garrett, Menon, MacKenzie, & Reiss, 2004; Watson et al, 2008).

Neuroimaging studies indicate that compared with individuals who are typically developing, individuals with ASD or FXS evince structural abnormalities and functional differences in many of the brain regions associated with face processing tasks, including the prefrontal cortex and the amygdala (e.g., Dalton et al., 2008; Gothelf, et al., 2008; Kim, et al., 2012; Schultz, 2005; Wang, Dapretto, Hariri, Sigman, & Bookheimer, 2004). Recently, abnormal amygdala activity (Kim, Burris, Bassal et al., 2012) and increased eye pupil reactivity (Farzin et al., 2009, 2011) in response to viewing fearful faces has been reported in FXS compared with healthy controls. Although autistic-like features, such as gaze-aversion and perseveration, are widely reported in individuals with FXS, so are affectionate and socially engaging behaviors (Bregman, Leckman, & Ort, 1988). Thus, it has been proposed that social deficits seen in FXS are a manifestation of underlying social anxiety, rather than autistic social dysfunction (Bailey et al., 1998; Bregman et al., 1988; Hagerman, 2002), suggesting dysregulation of the arousal system, as opposed to a deficit in social attention or comprehension.

One way to consider social emotional processing is to measure activity in the autonomic nervous system (ANS) related to arousal and amygdala function, and in response to emotionally evocative events. The ANS is made up of the sympathetic nervous system (SNS) and the parasympathetic nervous system (PNS). The SNS is responsible for the “fight/flight” reactions such as reducing digestive secretions, increasing heart rate (HR), and contracting blood vessels. Among the most effective tools available for measuring sympathetic tone is electrodermal response (EDR). EDR refers to changes in skin conductance due to eccrine sweat gland activity following an emotional event. Electrodermal activity changes in the presence of startling or threatening stimuli, aggressive or defensive feelings, and during positive or negative emotional events (Bradley et al., 1996; Lang et al., 1998). In neurotypical individuals, there is an increased sympathetic response to images of faces compared to non-social images, as indexed by skin conductance levels (Hirstein, Iversen, & Ramachandran, 2001).

Several researchers have reported an association between autistic behaviors and persistent hyperactivity of the SNS (e.g., Ming, Julu, Brimacombe, Connor, & Daniels, 2005; Murphy et al., 2000, Hirstein et al., 2001). Although the evidence is limited, individuals with autism appear to index atypical patterns of sympathetic arousal in response to social versus non-social visual stimuli (Hirstein et al., 2001, Ben Shalom et al., 2006). Like children with autism, children with FXS have been reported as having chronically high sympathetic activity (e.g., Belser & Sudhalter, 1995; Keysor, Mazzocco, McLeod, & Hoehn-Saric, 2002; Roberts, Boccia, Bailey, Hatton, & Skinner, 2001).

Porges’ polyvagal theory (Porges, 2007) emphasizes that physiological states regulated by the vagus nerve action on the heart support distinct types of behavior. According to the theory, vagal withdrawal facilitates mobilization and fight or flight responses, whereas

increased vagal activity supports spontaneous social engagement. Also, the vagus inhibits the sympathetic influences to the heart and reduces the hypothalamic pituitary adrenal (HPA) axis activity. This latter point has particular relevance to FXS, a group already known to demonstrate impaired vagal tone and elevated HPA activity (Hessl et al., 2002; Roberts et al., 2001). Functionally, the vagal brake, by modulating visceral state, enables the individual to rapidly engage and disengage with objects and other individuals and to promote self-soothing behaviors and calm states. Thus, it is hypothesized that vagal disturbances may play a role in the social reciprocity deficits, social anxiety symptoms and arousal regulation problems observed in individuals with ASD and FXS. There is evidence that some children with ASD may have low parasympathetic activity, including in response to viewing human emotions (Ming et al, 2005; Bal et al., 2010; Vaughan Van Hecke et al. 2009), as well as deficits in PNS modulation across tasks (Althaus et al., 1999; 2004). Like children with autism, children with FXS have chronically low PNS activity, as well as impaired PNS modulationand high and stable HR (Roberts et al., 2001; Hall et al, 2009; Roberts & Levenson, 2006). There is also evidence that those with comorbid ASD+FXS have even lower PNS activity and worse PNS modulation than individuals with FXS without ASD (Roberts et al., 2001).

The amygdala also plays a central role in emotional regulation, the physiology of arousal, and is well known for its role in detecting threat to the organism in the environment. The amygdala receives sensory input from several adjacent brain structures, and interfaces with several areas of the motor system that provide specific types of emotional response, including SNS and PNS regulation, somatic responses, and modulation of the startle response (e.g., Davis, 1989; Davis, Walker, & Lee, 1997; LeDoux, Farb, & Ruggiero, 1990). The potentiated startle reflex is a bio-behavioral probe of amygdala activity in response to intense stimuli. The greatest potentiation occurs when the startle probe is paired with novel negative stimuli. Conversely, the greatest attenuation occurs when the probe is paired with a positively valenced stimulus (Bradley, Cuthbert, & Lang, 1990; Vrana, Spence, & Lang, 1988). The physical movements involved in this response, apparently evolved to protect the individual from harm, include eye blink, abdominal contraction, as well as movement of the head, shoulders, and limbs (Davis & Astrachan, 1978).

Few studies have made direct, multidimensional comparisons between individuals with ASD and FXS to gain insights into convergent or divergent mechanisms associated with similar behavioral phenotypes. The present study addresses this issue by comparing groups of boys who had comorbid diagnoses of autism and FXS (ASD+FXS) with groups of boys who had either idiopathic autism (ASD) or FXS without comorbid autism, as well as those who were developing typically (TYP).

Consistent with prior studies (Ming et al., 2005; Bal et al., 2010), we expected that the ASD group would have elevated tonic sympathetic activity compared to TYP controls, as evinced by EDR, HR, and startle, but somewhat lower activity than the FXS and the ASD+FXS groups, expected to have the highest sympathetic activity. In addition, we anticipated an increase in SNS activity to non-social compared with social pictures as evinced by increased EDR activity and faster HR. Vagal tone was expected to be lower than the TYP group, but higher than both the FXS and ASD+FXS groups.

Those with FXS were hypothesized to have a greater general sympathetic response, as evinced by longer, faster, and more numerous EDR's, and faster HR, and larger magnitude startle responses than the TYP or ASD groups. As it is expected that those with FXS are typically able to recognize and respond to faces, we anticipated increased EDRs and shorter interbeat intervals (IBIs) to social pictures compared with non-social pictures for this group. We anticipated attenuation of startle to pleasant, non-social pictures, and potentiation to unpleasant ones, consistent with prior theory and imaging studies implicating the amygdala in mediating social anxiety and gaze avoidance in FXS (Hessl, Rivera & Reiss, 2004; Kim et al., 2012). Vagal tone was expected to be lower in ASD+FXS and ASD groups than TYP, consistent with the Porges' theory regarding the role of the vagus in social behavior as well as previous studies demonstrating reduced vagal tone in boys with FXS (Roberts et al., 2001).

The ASD+FXS group was expected to show general sympathetic hyperarousal, consistent with earlier studies showing heightened sympathetic reactivity to sensory, emotional and other stimuli in FXS (Miller et al., 1999; Farzin et al., 2009; Roberts et al., 2001). Therefore, we expected that this group would have larger, faster, and more numerous EDRs, and faster HR than ASD or TYP group (see Cohen, 1995). Startle response was similarly expected to be of highest magnitude of all the comparison groups. Vagal tone was expected to be lower than TYP and ASD groups, consistent with prior studies.

## Method

### Participants

All study participants were seen at the UC Davis MIND Institute. Most eligible participants had been previously referred for clinical assessment and treatment, and had agreed to be contacted for additional research studies. The remaining participants were recruited from families who responded to flyers placed at various regional centers and education facilities. Only boys were included in this study. Girls were excluded because females with FXS show less impairment because of their second, normally functioning X chromosome. Participants in the autism spectrum groups (ASD, ASD+FXS) all met criteria for Autistic Disorder or Pervasive Developmental Disorder, Not Otherwise Specified (PDD-NOS) and all met criteria for autism spectrum disorder on the Autism Diagnostic Observation Schedule (ADOS) and DSM-IV (American Psychiatric Association, 1994) according to Harris and colleagues (2008), completed by the clinical psychologist (DH) or another staff member with research reliability ADOS training. Only 2 of the 12 ADOS measures for the ASD group were completed by our laboratory, the remainder through outside clinical evaluations or by other MIND investigators, with diagnosis confirmed at the institute using the DSM-IV criteria by the lead clinical psychologist and behavioral pediatrician. The presence or absence of the *FMR1* gene mutation was confirmed by evaluation of CGG repeat length using standard methods (Tassone et al., 2004). IQ assessments included the Wechsler Intelligence Scales (WISC-III for children 6–16, and WAIS-III for adults 17 and older). However, recent IQ scores from trained professionals not associated with this study were also included when available if a center-based IQ was not completed. The total sample consisted of 52 boys between the ages of 10 and 17 years (ASD, $n$=12, $M_{age}$ =12.3 years;

FXS, $n$=12, $M_{age}$ =12.6; ASD+FXS, $n$=17, $M_{age}$ =13.7; TYP, $n$=11, $M_{age}$=12.1). Typically developing boys were chronological age matched to the clinical samples, and there were no significant group differences in age for any of the physiological comparisons.

## Procedure

Participants were seated in a room specifically designed for psychophysiological research. The participant was directed to sit in a chair next to a desk, on which there was a computer monitor for presentation of the visual stimuli. Electrodes were attached to the face, fingers, chest, and ankle. Headphones, through which the sound stimulus (SS) was presented, were placed over the ears. An observation mirror between the two rooms allowed researchers to monitor the study remotely and two cameras allowed researchers additional perspectives. Two researchers worked together to monitor and record in real-time whether or not the participant was looking at the screen.

**Stimulus Materials—**All participants viewed a series of 36 emotionally evocative pictures, 24 of which were paired with a brief acoustic startle stimulus. It was necessary to include some pictures without an acoustic probe for the cardiac analyses, as detailed below. Prior to start of the picture presentation protocol, there was a 30 second rest period without acoustic probes, and a 30 second period with two acoustic probes. These periods without visual stimuli represented the baseline, and allowed for measurement of tonic states of arousal. The full protocol was 12 minutes long.

The 18 pictures representing non-social (NS) images came from the International Affective Picture System (Center for the Study of Emotion and Attention, 1998):6 unpleasant (U-NS: i.e., snarling dog), 6 pleasant (P-NS: i.e., basket of puppies), and 6 neutral (N-NS: i.e., light bulb). The 18 pictures of facial expressions representing social (S) images came from the NimStim facial affect set: 6 unpleasant/fearful (U-S), 6 pleasant/happy (P-S), and 6 neutral (N-S) faces (Tottenham, Borscheid, Ellersten, Marcus, & Nelson, 2002). Each picture was presented for 6 seconds, and followed by an 8 second inter-stimulus interval (black screen) (Figure 1). Acoustic startle stimuli (SS; 50 msec white noise burst at 95 decibels) were delivered through headphones and distributed evenly across emotion valence (unpleasant, neutral, or pleasant) and social/non-social picture categories. Onset of the SS was randomized within given parameters. In order to allow time for the pictures to be attended to, and response to be captured in the given time frame, the SS did not occur during the first or last 0.5 seconds of the picture presentation.

**Measures—**The physiological measures were collected using an integrated psychophysiological data collection system (Biopac Systems, Inc., Santa Barbara, CA). Data processing was completed with Acknowledge software. Artifacts in the physiological data, such as when participants touched electrodes enough to disrupt the signal, when headphones were removed or not placed correctly, or when participants showed excessive movement, were marked on the record and the corresponding responses were excluded from analyses.

**Potentiated startle—**The eyeblink electromyographic (EMG) startle reflex to the SS was measured from two small electrodes placed below the right over the obicularis oculi muscle.

When processing the startle data, the raw EMG signal was filtered by setting the low frequency to 90Hz, and the high frequency to 250 Hz. The data was then rectified so that all the EMG activity was in the positive direction. The temporal range to measure the EMG response was set from −0.05 to +0.25 seconds following the SS. Finally, the maximum peak of the response, within the given timeframe, was determined by the software program. For each of the 24 SS, this maximum peak of response (mV) was used in the statistical analysis. Two (17%) of the ASD participants, two (12%) of the ASD+FXS participants, and two (17%) of the FXS participants were not able to tolerate the startle electrodes and therefore have missing potentiated startle data.

**Electrodermal Activity—**Electrodes were applied to the palmar surface of the second and third fingers of the left hand. Tonic levels were measured during the baseline period, prior to the acoustic probe, and phasic modulation was measured during the picture presentation task. Frequency, amplitude, and speed of responses were all analyzed. The EDR analyses included all electrodermal fluctuations above the 0.05 MicroMhos threshold that began 0.05 seconds after the onset of the picture stimuli to the end of the subsequent interstimulus interval. One (8%) of the ASD participants, one (6%) of the ASD+FXS participants, and two (17%) of FXS participants were not able to tolerate the EDR electrodes and therefore have missing electrodermal data.

**Cardiovascular Activity—**Cardiovascular estimates of autonomic (parasympathetic and sympathetic) activity was derived from heart inter-beat interval (IBI) data acquired using a digitized electrocardiogram (ECG) collected through disposable electrodes placed on the chest and abdomen. An IBI is the distance (in milliseconds) between one heartbeat and the next, and when averaged over a period of time, is analogous to heart rate.

In the cardiac analyses general HRV, vagal tone, and picture type influence on measures of interest were considered. The frequency domain variables of interest include the absolute value of HF power (in $ms^2$), HF power presented in normalized units (n.u.), and the LF/HF power percent. Heart rate variability (HRV) refers to variations in IBI, and is affected by changes in respiration. Assignment of very low frequency (VLF), low frequency (LF), or high frequency (HF) spectral power allows for assessment of different aspects of HRV. For example, LF power is thought to be mediated by sympathetic activity (e.g., Pagani et al., 1997; Saul, Rea, Eckberg, Berger, & Cohen, 1990; Sloan et al., 1996). It is widely agreed, however, the HF power is an effective measure of vagal tone modulation, a reflection of parasympathetic activity (Platisa & Gal, 2006). For the purposes of the current study, during analysis of HRV data, the HF spectral power was assessed, thus providing an indicator of vagal tone. The LF/HF ratio is an index of relative balance between sympathetic and vagal systems (Task force of the European Society of Cardiology & North American Society of Pacing and Electrophysiology, 1996; Malliani, 1999). Unlike IBI which can be measured within relatively short time windows, HRV calculations are most accurate during windows which are several minutes long. Therefore, both general HRV and vagal tone were assessed over the duration of the visual stimuli presentation (passive task), and then compared across groups. Frequency domain differences were considered during the baseline condition as well as during the picture events. In order to consider the difference in HR response to the onset

of the picture stimuli, the average of the two R-R intervals following the picture onset was subtracted from the average of the two intervals immediately prior to the picture. Analyses were performed for all pictures together, as well as by specific picture type. The average R-R interval was also compared in response to specific picture type. These analyses were performed on the 12 episodes lacking the SS.

Although all participants viewed about five minutes of the social and non-social visual stimuli (the minimum amount needed to accurately assess vagal tone), four individuals did not see the entire slide show due to participant distress. It has been suggested that comparisons of heart rate variability and vagal tone should be done over the same duration of time (Task force of the European Society of Cardiology & North American Society of Pacing and Electrophysiology, 1996). However, because the sample size was so small, we felt it was important to include all available data from all participants (N=48, duration = 290–530 seconds). One (9%) of the TYP participants, one (8%) of the ASD participants, and two (17%) of FXS participants were not able to tolerate the ECD electrodes and therefore have missing cardiovascular activity data.

## Analyses

For analyses pertaining to picture, valence, and social/nonsocial differences, we adjusted measures for baseline measurements of general arousal in order to isolate group differences in response to a specific category of images. The standardized residuals were then used for the remainder of the analyses. We utilized non-parametric tests to evaluate statistical difference in arousal responses across the groups; these tests do not make the same distributional assumptions as their equivalent parametric tests, do not rely on asymptotic sampling distributions for inferences that are not likely to hold for the smaller group sizes in this study, and are more robust to outliers. For overall tests of differences across the groups, we used the Kruskal-Wallis one-way analysis of variance by ranks. For pairwise group comparisons, we used the Mann-Whitney U test of ranks. To test for differences in arousal responses *within* a given group across conditions, we used the Friedman test of ranks for related samples for comparing responses across more than two conditions and we used the Wilcoxon matched-pairs signed-ranks test for pairwise comparisons of within-group responses across two conditions.

Although it is a frequent practice to control for IQ when assessing cognition or behavior in individuals with developmental delay, to unilaterally do so here would have compromised the power of our already small sample to such a degree that it could impact the interpretation of the results. We chose to control for IQ when comparing the groups on physiological measures that were independently associated with IQ in the data; this adjustment was necessary for the number of electrodermal responses in the General and Unpleasant conditions and average R-R interval for the General condition). In these instances, IQ was controlled for by computing the residual coefficient after regressing the measure of interest on IQ. This residual score was then used in place of the raw data for the remaining analyses.

For each family of statistical hypothesis tests (e.g., the associations between group membership and the magnitude of startle response across picture type), we used an adjusted $\alpha$-level for each test such that the overall Type I error rate was kept at $\alpha = .05$. Specifically,

we utilized the Sidak α-level adjustment that, unlike the more commonly-used Bonferroni adjustment, accounts for the correlations between multiple outcomes (Sidak, 1967). We also considered the increased Type II error rate driven by the small group sample sizes and observed associations of practical importance were not fully discounted due of a failure to obtain statistical significance; associations that trended towards significance are also discussed.

## Results

### IQ

The final sample had IQ scores on all of the FXS and ASD+FXS participants, 8 of 11 (73%) individuals in the ASD group, and 6 of 11 individuals (55%) in the TYP group. There were significant differences in the distributions of IQ scores across the four groups (ASD: $\underline{M}_{IQ}$ = 88.4, $\underline{SD}_{IQ}$ = 19.6; FXS: $\underline{M}_{IQ}$= 55.5, $\underline{SD}_{IQ}$ = 11.8; ASD+FXS: $\underline{M}_{IQ}$ =47.22, $\underline{SD}_{IQ}$ = 8.2; TYP: $\underline{M}_{IQ}$ = 102, $\underline{SD}_{IQ}$ =16.0), even when comparing only the clinical groups (excluding the TYP group): Kruskal-Wallis $\chi^2$= 19.0, $\underline{df}$ = 2, $\underline{p}$< 0.001. The TYP group has the highest IQ, on average, followed by the ASD group. The IQ scores for the FXS and ASD+FXS groups were comparable, on average, and notably lower than both the ASD and TYP groups.

### Potentiated Startle

The final sample for the potentiated startle analyses compared forty-six individuals across four diagnostic groups (ASD: $\underline{n}$=10, FXS: $\underline{n}$=10, ASD+FXS: $\underline{n}$=15, TYP: $\underline{n}$=11). The first set of startle analyses considered group differences in overall average startle response without consideration of the type of visual stimuli presented. Differences across groups were not significant ($\chi^2$ = 2.56, $\underline{df}$ = 3, $\underline{ns}$). Comparisons of average baseline magnitude also indicated no significant differences across groups ($\chi^2$ = 2.56, $\underline{df}$ = 3, $\underline{ns}$).

The next set of analyses compared groups on magnitude of startle response to specific picture type (e.g., N-S, P-NS, U-S adjusted for baseline response magnitude. The mean ranks of each group indicate that the TYP and FXS groups consistently demonstrated the smallest adjusted picture type startle response, while the ASD and the ASD+FXS groups consistently evinced the largest. Only the magnitude of response to a pleasant facial expression was significantly associated with group membership, although there was also a trend towards significance in response to the P-NS condition (Table 1). Pairwise comparisons indicate that in response to the P-S condition, the ASD+FXS group had a significantly larger magnitude of response than either the TYP group or the FXS group, and the difference approached significance when compared to the ASD group.

Responses to specific valence type (i.e., pleasant, neutral, unpleasant) without consideration of social type were compared across groups. The ASD+FXS group consistently demonstrated the largest adjusted startle response, while the TYP and FXS groups had the smallest for the valence condition. The only statistically significant (based on adjusted α-level of .04) difference in response magnitude across groups occurred in response to the pleasant condition ($\chi^2$ = 12.02, $\underline{df}$= 3, $\underline{p}$ = 0.007). Pairwise comparisons indicated the ASD +FXS group had significantly larger responses to the pleasant condition than either the TYP

or the FXS groups. The ASD group did not differ from the ASD+FXS group in magnitude of responses to the pleasant condition. The ASD group was not significantly different than the TYP group, but approached significant difference with the FXS group.

Responses to social type without consideration of valence were compared across groups (Figure 2). The social condition was significantly different ($\chi^2 = 8.5$, df = 3, p = 0.04) and the non-social condition approached significance across groups ($\chi^2 = 7.51$, df= 3, p = 0.06). Pairwise comparisons indicated that for both social and non-social conditions, the ASD +FXS group had significantly stronger startle responses than either the FXS or the TYP groups. There was also a trend towards significant difference in startle response magnitude to social images between the ASD and the FXS groups, with the ASD group having stronger responses.

Within group comparisons across valence type indicated that the FXS group exhibited a statistically significant difference in response magnitude ($\chi^2 = 8.6$, df = 5, p = 0.01), with the largest average response to the unpleasant condition, and the smallest responses to pleasant condition. The ASD+FXS group also demonstrated a trend towards significant difference in valence type response magnitude ($\chi^2 = 4.93$, df = 5, p = 0.09). However, the pattern of response within the ASD+FXS group appeared to be distinct from the other groups in that the greatest magnitude of response was to the pleasant condition.

## Electrodermal Response

Analyses were conducted on EDRs under both the general response condition without consideration of the presented picture stimuli, as well as during the specific affective and social conditions. For all of the EDR analyses, variability in response was considered on three dimensions: a) number of responses (per second), b) the magnitude of response (MicroMhos), and c) duration of response (seconds).

The final EDR analysis sample consisted of 48 individuals:(ASD: n=11, FXS: n=10, ASD +FXS: n=16, TYP: n=11) with the exception of the General and Unpleasant condition comparisons. For those comparisons, an adjustment for IQ was made, reducing the analysis sample by 8 individuals: (ASD: n=8, FXS: n=10, ASD+FXS: n=16, TYP: n=6)

Comparisons across groups on the EDR dimensions under the general condition indicated significant group differences in EDR response number), magnitude, and duration of response. Both the FXS and the ASD+FXS groups had the strongest and longest responses. The ASD+FXS group also had the greatest average number of responses. However, when the IQ adjusted scores for the number of responses during the general condition were analyzed, the differences across groups were no longer significant although the same patterns were evident with the ASD+FXS group demonstrating the most responses per second and the ASD group showing the fewest.

Prior to adjusting the picture type responses for baseline, characteristics of the average baseline responses were compared across groups. Results indicated a trend towards significant group differences in baseline number of responses ($\chi^2$= 6.98, df = 3, p = 0.07) and magnitude ($\chi^2$= 7.90, df = 3, p = 0.05). Pairwise comparisons indicated the only

difference in number of baseline responses was between the ASD+FXS and TYP groups, with the ASD+FXS group having significantly more. For baseline magnitude, the ASD +FXS group had stronger responses than all the other groups; however, the other groups did not differ from each other. Although the comparisons across all groups did not indicate differences across groups with regards to response duration, pairwise comparisons indicate that the ASD group had significantly shorter duration of response than the ASD+FXS group.

The response patterns across all the presented analyses seemed to indicate very little differentiation by picture, valence, or social type, and appeared more indicative of a general state of arousal (Table 2). In order to explore this further, a series of within group analyses across picture stimuli categories was performed to see if any of the groups evinced differential response patterns. There was little differentiation within groups between any of the specific picture types on any of the response characteristics. The same was true when valence type and social type aggregates were considered. The only exceptions were that the magnitude of response in the ASD group significantly differentiated among specific picture types ($\chi^2$= 11.26, df = 5, p = 0.05), with the smallest response to the N-S condition and the largest response to the P-S condition. Also, the ASD+FXS group had significantly fewer responses to the aggregate of neutral images than to the pleasant or unpleasant images ($\chi^2$ = 6.40, df= 2, p= 0.04).

## Cardiovascular Activity

The final sample consisted of 48 individuals:(ASD: n=11, FXS: n=10, ASD+FXS: n=17, TYP: n=10) with the exception of the General condition. For those comparisons, an adjustment for IQ was made, reducing the analysis sample by 7 individuals: (ASD: n=8, FXS: n=10, ASD+FXS: n=17, TYP: n=6)

The first set of analyses compared time and frequency domains of HRV across groups under the general condition. Time domain analysis revealed that mean differences in R-R trended towards significance across all groups; however, this difference was not present after the adjustment for IQ.

Frequency domain analysis during the general condition indicated statistically significant differences across all groups in LF/HF ratio ($\chi^2$= 8.10, df = 3, p = 0.04), with the ASD and ASD+FXS groups evincing the highest ratios, and the TYP and FXS groups the lowest. A trend towards significance across groups was also seen for the IQ adjusted HF power (n.u.; $\chi^2$= 8.02, df = 3, p = 0.05) with the TYP and FXS groups evincing the highest vagal tones and the lowest LF/HF ratios, the ASD group evincing the lowest vagal tone and highest LF/HF ratio, and ASD+FXS group evinced a mid-range vagal tone and a high LF/HF ratio that was similar to ASD. Pairwise comparisons confirmed that the TYP and FXS groups did not differ from each other on any measure of interest, nor did the ASD and the ASD+FXS groups. Results of frequency domain analysis during the baseline condition were so similar to those of the general condition that they are not presented.

The relative difference in heart rate before and after the onset of the picture stimuli during the general, picture-type, social-type, and valence-type conditions was analyzed. None of the conditions evinced significant differential responses across groups. There was a trend

towards significant difference across groups in response to the neutral social (N-S) conditions (Table 3). Pairwise comparisons indicated significant differences between the TYP and all other groups, with the TYP group displaying a relatively slower heart rate in response to the neutral social (N-S) pre- minus post-stimulus event compared with the other groups. Within-group comparisons indicated no differentiation by picture type for any of the groups of interest. Within-group comparisons of relative change by valence type indicated that only the TYP group had a differential response, with the fastest relative response to the pleasant pictures, and the slowest relative response to the neutral pictures. ($\chi^2$= 7.2, df = 3, p = 0.03). Within-group comparisons of relative change by social type indicated only the FXS group had a differential response, with faster responses to the social condition (Z= −2.4, p = 0.02).

The average R-R in response to the picture stimuli over the entire duration the picture was considered. Between group comparisons of the mean R-R during the baseline condition indicated no meaningful difference across groups ($\chi^2$= 1.22, df = 3, p = 0.75).

Between group comparisons of the adjusted mean R-R during the specific picture conditions indicated significant differences for all specific picture types except for U-NS, which after Sidak adjustment, still trended towards significance (Table 4). Under each condition, the TYP group had the slowest average heart rate and the ASD group had the second slowest heart rate. The ASD+FXS group had the fastest heart rate for the P-S, P-NS, N-S, and U-NS conditions. The FXS group was the fastest for N-NS and U-S conditions. Pairwise comparisons confirmed that the ASD+FXS and FXS groups did not differ with regards to cardiac rate, and tended to be faster than the ASD and TYP groups. The ASD and TYP groups also did not differ, with the exception of the N-NS condition (Z= −2.04, p = 0.04) and P-S condition (Z = −1.69, p = 0.08) where the ASD group had the faster heart rate.

Between group comparisons of the adjusted mean R-R by valence and social types (Table 5) indicated significant differences across all groups for each of the conditions. Under each condition, the TYP group had the slowest average heart rate, followed by the ASD group. The ASD+FXS group had the fastest heart rate for the pleasant, neutral, and non-social conditions, while the FXS group had the fastest for the unpleasant and social conditions.

Pairwise comparisons indicated that the ASD+FXS and the FXS groups did not differ across any of the valence or social conditions. Likewise, the ASD and TYP groups also did not differ. The ASD+FXS group had significantly faster heart rate than both the ASD and the TYP groups across all valence and social type conditions. The FXS group also had significantly faster heart rates than the TYP group for both social conditions, as well as the neutral and unpleasant conditions, and trended towards significantly faster on the pleasant condition (Z = −1.66, p = 0.10). The FXS group was somewhat faster than the ASD group across all conditions, but only met statistical significance for the social condition (Z = −1.97, p = 0.05).

## Discussion

The goal of this study was to determine whether unique profiles of psychophysiological responses to social and emotional stimuli could be identified in individuals diagnosed with autism, with fragile X syndrome, with comorbid autism and fragile X syndrome, and typically developing males. While the results suggest that while those with comorbid ASD +FXS have similarities with both the ASD and FXS groups, each group also has distinguishable patterns of autonomic function. The similarity between the ASD+FXS and FXS groups was evident when considering sympathetic arousal. Both the ASD+FXS and FXS groups had hyper-responsive sympathetic activity, while the ASD group did not, and thus supports the stated hypotheses and is consistent with prior studies demonstrating hyperarousal of the sympathetic nervous system in FXS (Miller et al., 1999; Farzin et al., 2009; Cohen, 1995; Roberts et al., 2001). However the ASD+FXS and ASD groups both showed parasympathetic dysregulation, and autonomic imbalance, while the FXS group did not. The hypothesis of low vagal tone in the ASD+FXS group was supported, and low vagal tone was also observed in the ASD group, while the FXS groups performed better. This appears consistent with the Polyvagal theory, and with prior reports of low vagal tone in ASD (Ming et al 2005; Bal et al 2009) in so far as vagal withdrawal was associated with the two study groups characterized by clinically significant social reciprocity deficits.

In addition, there was evidence of patterns in startle potentiation (a probe of amygdala function), and arousal modulation unique to those with ASD+FXS. Although prior theories about gaze avoidance in FXS implicated amygdala hyper-activation mediating arousal and anxiety (Hessl, Rivera, & Reiss, 2004), fMRI studies examining brain activation while individuals with FXS viewed human faces do not clearly support this hypothesis. In one study (Dalton et al., 2008), social eye gaze was most strongly associated with fusiform gyrus activation, although amygdala activation was positively correlated with autism symptoms. However, our recent fMRI study (Kim et al., 2012) revealed *attenuated* amygdala activation to fearful faces in individuals with FXS compared to controls, with decreased amygdala activation correlated with higher anxiety and social deficits. These inconsistent findings may be related to differences in emotional valence of stimuli used for given studies (as suggested by our findings). Given that the *fmr1* knockout mouse model studies implicate an imbalance of inhibitory and excitatory transmission in the amygdala (Suvrathan & Chattarji, 2011), further investigation in this area should be fruitful and may lead to more consistent results and clinical implications.

With regards to sympathetic arousal, both those with ASD+FXS and those with FXS show similarly high sympathetic tone. This was evident by similarly long EDR duration, relatively fast average heart rate, and higher magnitude of EDRs (the latter occurring only during the valence conditions). The ASD+FXS group demonstrated even stronger EDR responses than all three comparison groups during the general and baseline conditions. Cardiac measurements provided additional evidence of similar sympathetic hyper-arousal in ASD +FXS and FXS groups. These results corroborate those of Roberts and colleagues (2001) who also found that boys with either FXS alone, or with comorbid ASD+FXS, had faster heart rates than the TYP group.

Results of this study suggest that parasympathetic dysregulation contributes to autonomic dysfunction in both ASD+FXS and ASD groups. This was evidenced by lower vagal tone for the ASD+FXS group compared with the TYP and FXS groups during the general and baseline conditions. That there was no measurable difference in baseline vagal tone between the ASD+FXS and the ASD groups suggests similar parasympathetic processing deficiency in maintaining homeostasis. Evidence of low vagal tone in the ASD+FXS group supports the findings of Roberts and colleagues (2001). In that study, the boys with ASD+FXS also had lower vagal tone than those with FXS, during both passive and active tasks. Low parasympathetic activity in the ASD group is also consistent with similar observations in prior studies (Bal et al., 2010; Ming et al., 2005; Vaughan Van Hecke et al., 2009), suggesting that vagal tone may play a role in regulating at least some aspects of social reciprocity behaviors as suggested by Porges' theory (Porges, 2007).

Cardiac activity is influenced by the relative balance of sympathetic and parasympathetic systems, and can be measured via the LF/HF ratio. Examination of this ratio reveals additional support of autonomic dysregulation in ASD+FXS. This group had a high LF/HF ratio compared to the FXS and TYP groups in the general and baseline conditions. These results suggest that those with ASD+FXS have relatively more sympathetic than parasympathetic activity compared with the FXS and TYP groups. Roberts and colleagues came to the same conclusion in their 2001 study. Lack of difference with the ASD group suggests that those with idiopathic autism may also have autonomic imbalance (see Anderson & Colombo, 2007). This is likely driven by relatively low parasympathetic tone, since sympathetic tone is notably lower than for ASD+FXS, and no different from TYP. Although this result is notably different from Watson and colleagues (2011) who did not observe lowered parasympathetic responses to child-directed speech in boys with autism, our findings of elevated heart rate (average R-R interval during exposure to all images) and increased electrodermal activity in the ASD+FXS group adds further evidence that this group can be distinguished from idiopathic autism on a physiological basis.

Patterns of electrodermal activity can also indicate autonomic modulation difficulties. The ASD+FXS group had an increased number of EDR responses/second compared with all three groups during both the general and valence type conditions. The large number of EDRs indicates either a prolonged sympathetic response, a lack of response inhibition due to low parasympathetic tone, or most likely both. It is clear that multiple aspects of autonomic regulation are impaired in ASD+FXS.

The study identified areas that distinguished ASD+FXS from both the FXS and ASD groups. For example, The ASD+FXS group had significantly more as well as stronger EDRs than both the FXS and ASD groups. It was also only the ASD+FXS group that had such strong magnitude of startle response to the pleasant visual stimuli. Because the potentiated startle response is linked to amygdala function, these results suggest that this brain region functions atypically in this subgroup. Because amygdala activity is typically associated with fear responses, the result may suggest that the pleasant stimuli were perceived as threatening. This finding may also be consistent with the high rates of anxiety disorders seen in patients with FXS (Cordeiro et al, 2011), and the well known avoidant, shy, or fearful responses these individuals demonstrate, even to positive, though arousing stimuli. From a

psychophysiological perspective, ASD+FXS shares characteristics with both ASD and FXS alone, as well as unique responses not shared with either FXS or ASD. This is consistent with several researchers including Kaufmann and colleagues (2004) and Hazlett and colleagues (2009) who have asserted that autism in FXS is quite different from idiopathic ASD, at least at the neurophysiological level.

## Limitations

While the results of this study provide a unique perspective on physiological profiles in the groups of interest, there were a number of limitations to consider. First, the sample sizes of our groups were small, increasing the likelihood of a Type II error. The current study was designed to allow for physiological functioning while being less invasive than other techniques such as functional neuroimaging. To this end, our protocol is moderately successful. Tactile defensiveness, or extreme sensitivity to touch, is common in both FXS and autism. That is, there were several children in each group that would not allow all of the electrodes to be placed, particularly for those around the eye region. Because of this, there is likely to be a bias towards inclusion of higher functioning children, or those with better coping mechanisms that are more capable of tolerating the present protocol. In the future, alternative measures of startle response should be considered to increase participant success in completing the protocol. In addition to the unease with the electrodes, several children refused to wear the headphones through which the acoustic probe was delivered. In the future, it may be advantageous to deliver the acoustic probe via speakers placed near to the child.

Another limitation of this study was that eye-tracking technology was not employed. Advances in eye tracking technology have made it possible to know specifically where an individual is focusing gaze. Further, recent eye tracking investigations have demonstrated that both those with FXS (Farzin et al., 2011) and autism (Pelphrey et al, 2002) look less often at the eye region of human faces.

Finally, it is difficult to be sure what role IQ plays in the processing of valence and social content. In the presented study, controlling for IQ did not contribute to the clarity of the substantive inferences. As presented before, there are good reasons not to control for IQ; including, but not limited to the possibility that IQ deficits may be linked to the same underlying CNS abnormalities that causes the social and valence processing deficits. Therefore, one can argue that IQ is not causing the social and valence processing deficits, but rather has a shared cause rooted in the underlying neurodevelopmental disorder (Dennis et al., 2009). Due to the IQ, communication, and insight deficits in the clinical groups studied, it was not possible to collect behavioral data, such as valence or affect ratings, which might have otherwise contributed to result interpretation. Future studies would benefit from including mental age-matched typically developing children and/or a group of mental-age matched children with intellectual disabilities (such as Down syndrome).

## Conclusions and Study Implications

This study provides evidence that autonomic arousal and regulation profiles could be useful to distinguish subgroups of autism and shed light on the variability underlying emotional

responsivity. Although it is not possible to determine from this study whether any of the physiological factors actually cause or contribute to autism symptom expression (e.g., clinical correlates were not examined), the results clearly point to future directions of research. For example, given the high rates of hyperarousal and anxiety in FXS, it would be clinically useful to examine links between sympathetic or amygdala responsiveness to particular environmental stimuli and the expression of social deficits or repetitive, stereotyped behaviors. If these causal links exist (either in FXS or within a subgroup of children with idiopathic autism with similar profiles), treatments might be developed focused on reducing these responses, pharmacologically or behaviorally. Also, we do yet have a complete understanding of the neurobiological mechanisms and processes underlying the development of these symptoms and physiological abnormalities. Recent work using the fragile X animal models has provided important clues, however. For example, numerous behavioraland neurobiological abnormalities of the *fmr1* knockout mice and fruitfly are rescued by agents that regulate glutamate and GABA systems (Berry-Kravis, Knox, & Hervey, 2011). Several clinical trials of medications that target these systems have been completed or are underway in children and adults with FXS, with some promising results (Jacquemont et al., 2011; Berry Kravis et al., 2012). In the near future, it will be critical to examine whether children with ASD or other neurodevelopmental disorders having similar neurobiological and psychophysiological markers are equally responsive to these treatments. Consistent with the RDoC project, treatments such as these may increasingly target the underlying genetic and neurobiological abnormalities of disease, leading to better outcomes for children with ASD.

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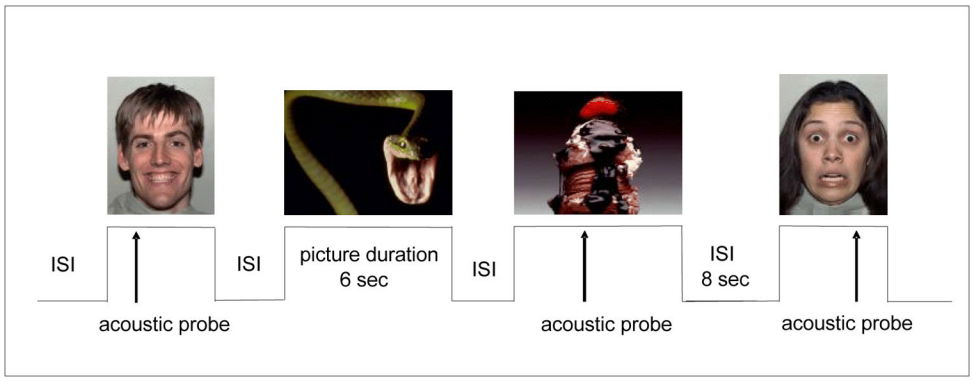


**Figure 1.**

Examples of emotionally evocative visual stimuli used in the study protocol. Images are displayed for 6 sec, with an interstimulus interval (ISI) of 8 sec. Two-thirds of the 36 positive, negative, or neutral images were accompanied by a 50 ms 95 db burst of white noise (acoustic probe) in order to examine emotion potentiation of the eyeblink startle reflex.

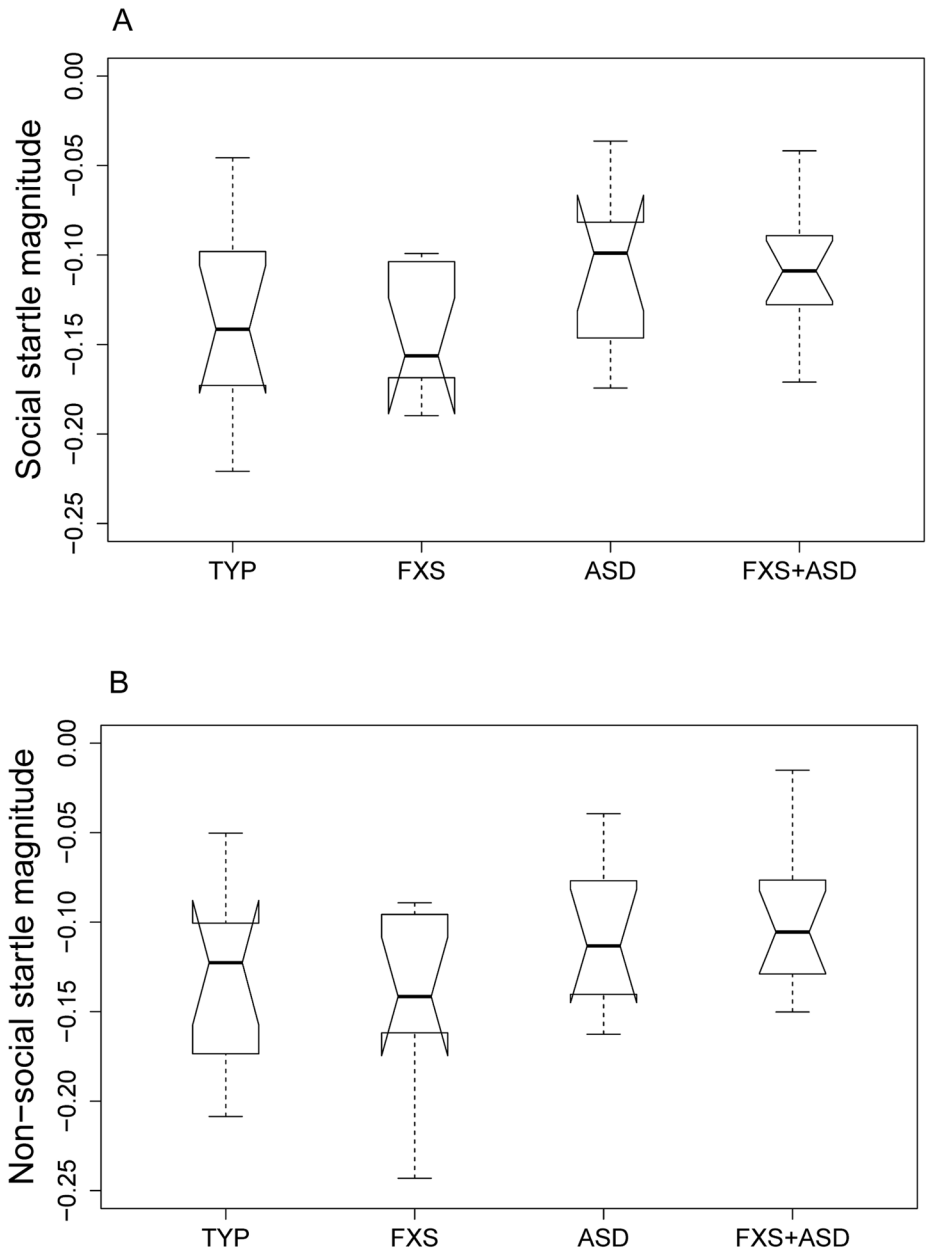


**Figure 2.**

Notched side-by-side box plots of standardized residual eyeblink startle responses to (A) social (i.e., faces) and (B) non-social (i.e., objects) for each of the four groups, adjusted for baseline startle magnitude. The length of the "whiskers" is 1.5 IQR; the notch on the box represents the 95% CI for the median. (Note: Each of the three clinical groups had one outlier with a value beyond the corresponding top whisker outside the plot area.)

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**Table 1**

Summary Table for Analysis of Variance on Ranks[a] of Picture Type[b] Startle Amplitude

| | Mean Amplitude Ranks[c] | | | | | |
|---|---|---|---|---|---|---|
| **Group** | **P-S** | **P-NS** | **N-S** | **N-NS** | **U-S** | **U-NS** |
| TYP | 16.45 | 19.55 | 22.09 | 19.27 | 19.73 | 17.64 |
| FXS | 17.40 | 15.90 | 17.60 | 19.30 | 19.80 | 21.20 |
| ASD | 23.00 | 27.80 | 29.50 | 25.40 | 25.90 | 24.10 |
| ASD+FXS | 33.07 | 28.60 | 24.47 | 28.13 | 27.13 | 28.93 |
| $\chi^2$, $df$ = 3 | 12.73 | 7.35 | 4.13 | 4.06 | 3.05 | 4.87 |
| $p$ | .005* | .06 | .25 | .26 | .38 | .18 |
| $\eta^2$ | 0.28 | 0.16 | 0.09 | 0.09 | 0.07 | 0.11 |

[a] Kruskal-Wallis one-way analysis of variance by ranks

[b] P-S/NS: pleasant-social/nonsocial; N-S/NS: neutral-social/nonsocial; U-S/NS: unpleasant-social/nonsocial

[c] Adjusted for baseline

* denotes significance at adjusted level of $\alpha = .026$

**Table 2**

Summary Table for Analysis of Variance on Ranks[a] of EDR Characteristics by Picture Type[b]

| | **Mean Frequency Ranks** | | | | | |
|---|---|---|---|---|---|---|
| **Group** | **P-S** | **P-NS** | **N-S** | **N-NS** | **U-S** | **U-NS** |
| TYP | 26.00 | 24.64 | 27.45 | 27.91 | 25.27 | 22.64 |
| FXS | 24.20 | 21.15 | 21.60 | 25.80 | 25.50 | 24.80 |
| ASD | 15.73 | 16.23 | 18.27 | 14.45 | 13.50 | 16.82 |
| ASD+FXS | 29.69 | 31.13 | 28.56 | 28.25 | 29.77 | 29.73 |
| $\chi^2$, $df$ = 3 | 6.65 | 8.06 | 4.44 | 7.56 | 9.33 | 5.79 |
| $p$ | .08 | .05 | .22 | .06 | .025* | .12 |
| $\eta^2$ | 0.14 | 0.17 | 0.09 | 0.16 | 0.20 | 0.12 |
| | Mean Magnitude Ranks | | | | | |
| Group | P-S | P-NS | N-S | N-NS | U-S | U-NS |
| TYP | 22.64 | 19.55 | 24.09 | 24.45 | 27.36 | 23.64 |
| FXS | 32.50 | 30.25 | 29.50 | 33.20 | 25.30 | 29.10 |
| ASD | 17.64 | 18.77 | 14.82 | 15.55 | 16.64 | 21.73 |
| ASD+FXS | 25.50 | 26.93 | 28.31 | 25.25 | 26.07 | 22.53 |
| $\chi^2$, $df$ = 3 | 6.19 | 5.53 | 7.73 | 8.42 | 4.27 | 1.87 |
| $p$ | .10 | .14 | .05 | .04 | .23 | .60 |
| $\eta^2$ | 0.13 | 0.12 | 0.16 | 0.18 | 0.09 | 0.04 |
| | Mean Duration Ranks | | | | | |
| Group | P-S | P-NS | N-S | N-NS | U-S | U-NS |
| TYP | 20.55 | 22.63 | 19.36 | 21.36 | 24.27 | 19.64 |
| FXS | 29.90 | 26.15 | 31.00 | 29.30 | 26.70 | 28.70 |
| ASD | 13.18 | 15.86 | 19.36 | 15.09 | 13.18 | 17.45 |
| ASD+FXS | 31.63 | 29.73 | 27.50 | 30.13 | 29.93 | 28.87 |
| $\chi^2$, $df$ = 3 | 13.70 | 6.90 | 5.85 | 9.29 | 10.06 | 6.69 |

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| | Mean Frequency Ranks | | | | | |
|---|---|---|---|---|---|---|
| **Group** | **P-S** | **P-NS** | **N-S** | **N-NS** | **U-S** | **U-NS** |
| *p* | .003*** | .08 | .12 | .025*** | .018*** | .08 |
| $\eta^2$ | 0.29 | 0.15 | 0.12 | 0.18 | 0.21 | 0.14 |

[a] Kruskal-Wallis one-way analysis of variance by ranks

[b] P-S/NS: pleasant-social/nonsocial; N-S/NS: neutral-social/nonsocial; U-S/NS: unpleasant-social/nonsocial

* denotes significance at adjusted level of α = .029,

** α = .024,

*** α = .025

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**Table 3**

Summary Table for Analysis of Variance on Ranks[a] of Relative Change in R-R Due to Onset of Specific Picture Stimuli[b]

| | Mean Relative Change in R-R Ranks | | | | | |
|---|---|---|---|---|---|---|
| **Group** | **P-S** | **P-NS** | **N-S** | **N-NS** | **U-S** | **U-NS** |
| TYP | 29.55 | 22.40 | 14.20 | 22.80 | 23.00 | 19.70 |
| FXS | 22.40 | 21.30 | 26.60 | 21.90 | 22.60 | 20.30 |
| ASD | 25.18 | 25.27 | 24.64 | 21.45 | 22.64 | 31.09 |
| ASD+FXS | 22.32 | 27.12 | 29.24 | 29.00 | 27.71 | 22.60 |
| $\chi^2$, $df = 3$ | 1.96 | 1.38 | 7.58 | 2.77 | 1.39 | 4.96 |
| $p$ | .58 | .71 | .06 | .43 | .71 | .18 |
| $\eta^2$ | 0.04 | 0.03 | 0.16 | 0.06 | 0.03 | 0.11 |

[a] Kruskal-Wallis one-way analysis of variance by ranks

[b] P-S/NS: pleasant-social/nonsocial; N-S/NS: neutral-social/nonsocial; U-S/NS: unpleasant-social/nonsocial

* denotes significance at adjusted level of $\alpha = .005$

**Table 4**

Mean Rank Scores of Analysis of Variance on Ranks[a] for Sustained Attention Adjusted Mean R-R by Picture Type[b]

| | Mean Sustained Attention Adjusted Mean R-R Ranks | | | | | |
|---|---|---|---|---|---|---|
| **Group** | **P-S** | **P-NS** | **N-S** | **N-NS** | **U-S** | **U-NS** |
| TYP | 33.90 | 32.00 | 32.10 | 35.00 | 32.40 | 30.00 |
| FXS | 20.10 | 17.78 | 19.50 | 17.70 | 18.00 | 18.89 |
| ASD | 27.00 | 32.30 | 31.64 | 27.36 | 30.18 | 26.30 |
| ASD+FXS | 19.94 | 16.35 | 18.35 | 20.47 | 20.00 | 17.13 |
| $\chi^2$, $df$ = 3 | 7.65 | 14.76 | 10.36 | 9.85 | 8.91 | 7.61 |
| $p$ | .05 | .002* | .016* | .020* | .031 | .05 |
| $\eta^2$ | 0.16 | 0.31 | 0.22 | 0.21 | 0.19 | 0.16 |

[a] Kruskal-Wallis one-way analysis of variance by ranks

[b] P-S/NS: pleasant-social/nonsocial; N-S/NS: neutral-social/nonsocial; U-S/NS: unpleasant-social/nonsocial

* denotes significance at adjusted level of $\alpha = .03$

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**Table 5**

Mean Rank Scores of Analysis of Variance on Ranks[a] for Sustained Attention for Adjusted Mean R-R by Valence and Social Types

| | Mean Sustained Attention Adjusted Mean R-R Ranks | | | | |
|---|---|---|---|---|---|
| **Group** | **Pleasant** | **Neutral** | **Unpleasant** | **Social** | **Non-social** |
| TYP | 33.80 | 35.10 | 33.50 | 34.00 | 35.00 |
| FXS | 19.90 | 18.60 | 19.20 | 18.40 | 19.80 |
| ASD | 30.00 | 29.64 | 28.73 | 29.82 | 28.91 |
| ASD+FXS | 18.18 | 18.41 | 19.59 | 19.06 | 18.24 |
| $\chi^2$, $df$ = 3 | 10.66 | 12.20 | 8.66 | 10.66 | 11.25 |
| $p$ | .01* | .01* | .03* | .01** | .01** |
| $\eta^2$ | 0.23 | 0.26 | 0.18 | 0.23 | 0.24 |

[a] Kruskal-Wallis one-way analysis of variance by ranks

* denotes significance at adjusted level of $\alpha$ = .04,

** $\alpha$ = .05