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Regular Article

Social gaze behavior and hyperarousal in young females with fragile X syndrome: A within-person approach

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Abstract

Children with fragile X syndrome (FXS) often avoid eye contact, a behavior that is potentially related to hyperarousal. Prior studies, however, have focused on between-person associations rather than coupling of within-person changes in gaze behaviors and arousal. In addition, there is debate about whether prompts to maintain eye contact are beneficial for individuals with FXS. In a study of young females (ages 6–16), we used eye tracking to assess gaze behavior and pupil dilation during social interactions in a group with FXS ($n = 32$) and a developmentally similar comparison group ($n = 23$). Participants engaged in semi-structured conversations with a female examiner during blocks with and without verbal prompts to maintain eye contact. We identified a social–behavioral and psychophysiological profile that is specific to females with FXS; this group exhibited lower mean levels of eye contact, significantly increased mean pupil dilation during conversations that included prompts to maintain eye contact, and showed stronger positive coupling between eye contact and pupil dilation. Our findings strengthen support for the perspective that gaze aversion in FXS reflects negative reinforcement of social avoidance behavior. We also found that behavioral skills training may improve eye contact, but maintaining eye contact appears to be physiologically taxing for females with FXS.

Keywords: eye gaze avoidance; females; fragile X syndrome; pupil dilation

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Introduction

Fragile X syndrome (FXS) is a genetic condition characterized by mutation within the fragile x messenger ribonucleoprotein 1 gene (*FMR1*) of the X chromosome, leading to hypermethylation of the promoter region of *fMR1* and reduced production of fragile X messenger ribonucleoprotein 1 (FMRP). Decreased FMRP production leads to aberrant dendritic pruning and synaptic plasticity, likely contributing to a range of neural, cognitive, behavioral, and social phenotypes observed in individuals with FXS (Cordeiro et al., 2011; Lee et al., 2021; Schneider et al., 2009). FXS is more common and is typically characterized by a more severe clinical phenotype in males compared to females (Garber et al., 2008; Hagerman et al., 2017; Hunter et al., 2014). Many males with FXS meet criteria for intellectual disability (ranging from mild to severe) and frequently present co-occurring conditions of attention problems, hyperactivity, anxiety, and autism (Bailey et al., 2008). Nevertheless, females with FXS are at increased risk for experiencing significant internalizing and social difficulties (Bartholomay et al., 2019; Freund et al., 1993; Hagerman et al., 2017; Miller et al., 2021), but are underrepresented in research relative to males with FXS (Bartholomay et al., 2019).

One of the most striking behavioral features of social anxiety in both males and females with FXS is severe eye contact aversion (Bruno et al., 2014; Cohen et al., 1991; Hall et al., 2006), especially when interacting with unfamiliar people (Cohen et al., 1988). For example, prior eye tracking research found that compared to an age- and developmental functioning-matched control group, males and females with FXS spent less time looking at the face of an unfamiliar partner during conversation (Hall et al., 2015). Social avoidance behaviors in FXS, or those that support social withdrawal (e.g., gaze avoidance, physically turning away, social shyness) (Roberts et al., 2007), may be driven by increased susceptibility to experiencing hyperarousal states (Klusek et al., 2015; Roberts et al., 2001), particularly during social interaction (Hall et al., 2009). For example, individuals with FXS (sample of primarily males) demonstrate heightened pupil dilation in response to faces (Farzin et al., 2011), potentially indicating increased arousal driven by sympathetic nervous system activation (Bradley et al., 2008). The sympathetic branch of the autonomic nervous system plays an important role in preparing for defensive responses to stress, whereas the parasympathetic nervous system downregulates arousal and contributes to calm, soothed states that are potentially conducive to social engagement (Porges, 2007). It is important to note that the majority of research documenting proneness to hyperarousal states and physiological

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dysregulation in FXS have found evidence for increased heart rate, which reflects both sympathetic and parasympathetic nervous system activity, or decreased high-frequency heart rate variability, which reflects decreased parasympathetic activity (Klusek et al., 2015). Fewer studies of FXS have assessed physiological arousal states driven by sympathetic activation. In addition, despite findings suggesting that FXS is characterized by hyperarousal, the research linking physiological measures to eye gaze avoidance in FXS has produced mixed findings. In research with males and females with FXS, Hall et al. (2009) did not find associations between measures of cardiovascular activity (i.e., heart rate and heart rate variability) during social interaction and eye gaze avoidance. In contrast, Hessl et al. (2006) found that among male and female children with FXS, lower cortisol reactivity was associated with less eye contact. One interpretation of these findings is that in children with FXS, gaze aversion is a behavioral strategy for regulating and decreasing physiological arousal during social interaction. To the extent that physiological arousal during eye contact is experienced as distressing, avoiding eye contact may temporarily remove aversive stimulation, thus serving as negative reinforcement of social avoidance behavior. In other words, gaze aversion may lead to removal of aversive stimulation, which strengthens or encourages the avoidant behavior.

It is important to note, however, that the majority of prior studies have focused on between-person associations of average physiological arousal and average eye gaze behavior. In other words, prior analyses have tested whether individuals with higher or lower levels of physiological arousal tend to engage in more or less gaze aversion relative to others. One limitation of this approach is that it does not speak to within-person processes, which require a consideration of intra-individual differences in repeated measures (Curran & Bauer, 2011). Modeling within-person processes is important for more directly testing the hypothesis that for individuals with FXS, physiological arousal increases while engaging in eye contact and decreases while engaging in eye gaze avoidance. Thus, one of the primary aims of the current study was to assess within-person effects to test the hypothesis that aberrant eye gaze aversion behaviors in FXS regulate physiological arousal during social interaction.

Considering within-person associations between measures of physiological arousal and gaze behavior, as well as how these processes unfold over the course of social interaction, has significant clinical implications. There is debate as to whether interventions for individuals with FXS should focus on increasing eye contact. Some researchers have suggested that encouraging eye contact promotes hyperarousal, leading to anxiety and behavioral problems that undermine intervention efforts (Dykens et al., 2000; Morris et al., 2014; Scharfenaker et al., 2002). On the other hand, eye contact is thought to play an important role in social and communicative functions, such as language development, emotion recognition, and joint attention-based learning (Emery, 2000; Itier & Batty, 2009; Mirenda et al., 1983; Senju & Johnson, 2009). Thus, some researchers have argued that improving social gaze behavior is a crucial target for interventions in individuals with FXS (Gannon et al., 2018; Hall et al., 2009). In studies of male and female youth with FXS, these researchers have found evidence that gaze avoidance and physiological arousal decrease over the course of conversations that include prompts to maintain eye contact and suggest that eye contact training may facilitate social skills (Gannon et al., 2018; Hall et al., 2009). Thus, individuals with FXS may "warm up" to behavioral skills training rather than experience escalating avoidance and arousal. Given that females with FXS typically present with a less severe clinical phenotype than their male counterparts, it is possible that this population might be particularly amenable to potential improvements in social gaze behavior and reduction of physiological arousal through behavioral skills training. More research focused on young females with FXS, however, is clearly needed.

The current study compared young females with FXS to a developmentally-similar and sex-matched comparison group. We assessed eye gaze behaviors and physiological arousal during naturalistic conversations which included blocks with and blocks without verbal prompts to maintain eye contact. We tested whether young females differed from the comparison group in (1) mean durations of social gaze behavior and mean levels of physiological arousal during conversations, (2) within-person coupling of social gaze behavior and physiological arousal during conversations, and (3) individual trajectories of social gaze behavior and physiological arousal over the course of the conversation.

Methods

Participants and procedures

Participants included 32 females with FXS ($M = 11.22$ years, $SD = 3.03$, range = 6.25–16.25; 6% Asian, 3% Black or African American, 3% Multiracial, 88% White; 9% Hispanic or Latino) and a comparison group of 23 females without FXS ($M = 10.87$ years, $SD = 2.34$, range = 6.67–14.75; 13% Asian, 4% Black or African American, 39% Multiracial, 4% unknown or not reported, 39% White; 43% Hispanic or Latino). Participants in the comparison group exhibited a range of idiopathic developmental delays and learning and intellectual disabilities. This group was considered developmentally matched or similar to the FXS group based on age and verbal IQ as assessed by Differential Ability Scales, Second Edition (DAS-II) (both t's(53) < 1.56, p's > .127). Table 1 presents the descriptive statistics for age, verbal IQ, and adaptive behaviors and functional skills as assessed by the Vineland-3 Adaptive Behaviors Scales composite score (Sparrow et al., 2016), for the FXS and comparison groups. The Supplement presents a group comparison on autism symptoms.

The diagnosis of full-mutation FXS (>200 CGG repeats in the FMR1 gene) was confirmed by molecular genetic testing. Participants were excluded if they had evidence of current or past major neurological or psychiatric conditions such as bipolar disorder, epilepsy and schizophrenia, sensory deficits which prevented them from completing the study tasks, preterm birth (<34 weeks gestation), or a history of concussion.

Participants were part of a multiyear longitudinal study, and current analyses focused on data that were collected at the baseline assessment (Time 1). Participants with FXS were recruited using the current Stanford FXS family registry, contacts with the National Fragile X Foundation, regional fragile X organizations, the Fragile X Clinical and Research Consortium and FORWARD registry, and electronic media. Participants in the comparison group were recruited through various parent organizations, regional centers, school districts, social media, and flyer services throughout California. In the larger study, 101 participants had the opportunity to complete the conversations task at the baseline assessment (56 in the FXS group and 45 in the comparison group). We excluded participants who experienced technical difficulties (e.g., could not calibrate eye tracker) or were noncompliant (e.g., excessively touched or refused to wear the eye tracking goggles). The Stanford University Institutional Review Board approved this research. All research was performed in accordance with the latest version of the Declaration of Helsinki. Participants and their parents/

Table 1. Descriptive statistics for age, verbal IQ, adaptive behaviors, and autism behaviors

	FXS Group			Comparison Group			Group-Matching Statistics			
	Mean	SD	Range	Mean	SD	Range	*t*	*p*	Cohen's d	Variance Ratio
Age	11.22	3.03	6.25–16.25	10.87	2.34	6.67–14.75	0.47	.643	.13	1.68
DAS Verbal IQ	84.03	15.49	34–114	91.65	21.12	31–133	1.55	.128	−.42	0.54
Vineland-3 ABC score	82.59	11.86	64–114	79.17	12.09	63–105	1.05	.300	.29	0.96

Note. ABC = Adaptive Behavior Composite score based on the Communication, Daily Living Skills, and Socialization domains of Vineland-3; DAS = Differential Abilities Scale; FXS = Fragile X Syndrome. The *t*-statistics, *p*-values, and Cohen's d statistics are specific to comparisons of the means in the FXS and in the comparison groups. The variance ratio is the ratio of the variance in the FXS group to the variance in the comparison group.

guardians were fully informed about the purpose of the research, and written consent was obtained from the parent or guardian for each participant. Study data were collected and managed using REDCap electronic data capture tools (Harris et al., 2019).

Conversation task

Participants engaged in a conversation task with a female examiner who was novel to the participant (Kover et al., 2012; Li et al., 2022). The task consisted of two conditions that were presented in a fixed order across participants. In the first condition (no prompt), participants were instructed to engage in a natural conversation with the examiner. Following the conclusion of the first condition, participants were told that they were going to continue their conversation but that they should look at the examiner's eyes (prompt condition). In the prompt condition, the examiner requested participants to look at their eyes (i.e., "remember to look in my eyes" or "don't forget to look in my eyes"), or positively reinforced the participant's eye contact if already present (i.e., "good looking at my eyes" or "thank you for looking at my eyes"), approximately every 15 s. Specifically, the examiner held a MotivAider (Behavioral Dynamics, Thief River Falls, MN, USA) in their lap which was set to vibrate every 15 s. After receiving the vibration, the interviewer began their next opportunity to speak with an eye contact prompt or affirmation. Each condition consisted of eight conversation blocks that were approximately 1 min in duration. Each block was structured around a particular topic such as participant interests, school, pets, and family. We used topics of conversation that have previously been shown to effectively elicit language in individuals with FXS (Berry-Kravis et al., 2013). These topics are listed in Table S1 in the Supplement. Participants were not apprised of conversation topics ahead of time. Conversation blocks were allowed to be longer than 1 min if the participant was still talking (i.e., examiners did not cut off participants while they were speaking). The number of conversation blocks and their duration were meant to limit participant burden and to present participants with a range of topics that would elicit varying levels of interest and engagement. Each block was separated by a 10 s interval in which the participant was presented with a fixation cross on a white 8.5 × 11 inch card that covered the examiner's face. The task began and ended with presentation of the fixation cross in the same manner for 15 s.

Social gaze behaviors and pupil dilation

Eye tracking glasses (SensoMotoric Instruments) were worn by participants to track their eye movements during both the prompt and no prompt conversation blocks. Eye tracking data were processed with Behavioral and Gaze analysis software (BeGaze V3.7, SMI, Germany). Eye movements were classified into saccades, fixations, and blinks. A saccade is identified when the instantaneous acceleration or instantaneous velocity of the eye gaze is greater than a specific threshold, while a fixation is bordered by two saccades with gaze present. Conversely, a blink is defined by absent gaze. Fixations were utilized as the eye gaze variable of interest in the current analyses. We determined the percentages of fixations within four regions of interest (ROIs) to assess the eye gaze behaviors of the participants. The interviewer's face was divided into two regions: above the bottom of the nose (eye region) and below the bottom of the nose (lower face). Fixations that were on a part of the researcher's body other than the face were coded as being on-body, whereas fixations that were not on the researcher's face or body region were defined as off-body.

Four researchers received training on in-house gaze coding software to manually code the ROI of each fixation. One researcher coded every dataset while the other three researchers coded every tenth dataset for reliability purposes. Cohen's kappa values were calculated for each pair of raters for each reliability set. The mean kappa value was 0.924 with a range from 0.827 to 0.977, suggesting excellent interrater reliability. The raw pupil diameter data were preprocessed and used to calculate mean pupil diameter based on guidelines provided by Kret and Sjak-Shie (2019). Regarding lighting as a potential extraneous influence on pupil dilation, we note that all testing was performed in the same room under the same lighting conditions. The walls of the room were lightly colored, so examiners were instructed to wear a darker colored shirt to contrast with the walls.

Statistical analyses

Measurements of eye gaze behaviors (proportion of fixations per ROI) and pupil dilation were collected simultaneously in conversation blocks nested within each individual. We focused on three levels of analysis for examining associations among FXS group status, physiological arousal, and eye gaze behavior: (1) mean levels of pupil dilation and eye gaze behaviors (i.e., proportion of fixations per ROI) for each conversation task condition, (2) within-person coupling between fluctuations of pupil dilation and eye gaze behaviors, and (3) trajectories of pupil dilation and eye gaze behaviors over the course of the conversation task conditions.

We conducted a series of multilevel models to explore within-person coupling between pupil dilation and eye gaze behavior (e.g., proportion of time looking above the nose, below the nose, on-body, and off-body). Further, we used this approach to test potential group differences in this coupling. These models considered nested conversation blocks (Level 1)

Table 2. Descriptive statistics and correlations

Variable	1	2	3	4	5	6	7	8	9	10	11
1. Group status (FXS = 1)	1										
2. NP- Pupil Dilation	.05	1									
3. P- Pupil Dilation	.11	.97***	1								
4. NP- Looking Above Nose	−.38**	−.18	.17	1							
5. P- Looking Above Nose	−.33*	−.26+	−.19	.80***	1						
6. NP- Looking Below Nose	.22	.09	.08	−.31*	−.37**	1					
7. P- Looking Below Nose	.37**	.13	.14	−.45***	−.51***	.78***	1				
8. NP- Looking On-body	.16	.26+	.25+	−.61***	−.57***	.05	.23+	1			
9. P- Looking On-body	.09	.17	.11	−.54***	−.69***	.05	.02	.72***	1		
10. NP- Looking Off-body	.17	.03	.02	−.60***	−.32*	−.39**	−.18	.13	.15	1	
11. P- Looking Off-body	.08	.16	.06	−.49***	−.63***	−.24+	−.20	−.19	.38**	.63***	1
Mean	58%	3.39	3.43	.32	.42	.17	.21	.16	.15	.33	.20
SD		0.59	0.60	.24	.28	.14	.17	.11	.12	.17	.16
Min		2.39	2.46	.01	.00	.00	.00	.01	.01	.07	.03
Max		4.99	4.92	.82	.94	.60	.67	.44	.48	.89	.83

Note: ***$p < .001$, **$p < .01$, *$p < .05$, +$p < .10$. NP = No Prompt Condition; P = Prompt Condition. Pupil dilation and eye gaze behaviors are average values from across conversation blocks (within task condition).

within individuals (Level 2). All eye gaze variables were person-centered, such that values represented deviations from individual participants' mean eye gaze behavior. These analyses proceeded in three steps. First, we tested a random effects model to examine whether proportion of time looking above the nose was associated with concurrent pupil dilation, regardless of task condition and FXS group status (i.e., across the entire conversation task and entire cohort). Second, we examined whether the strength of the coupling between eye gaze behavior and pupil dilation was different for participants with and without FXS (i.e., moderated by FXS group status). Third, we repeated these analysis steps for each eye gaze ROI regardless of condition, and then again separately for the no prompt and prompt conditions.

We used a latent basis modeling approach to estimate trajectories of pupil dilation and eye gaze behaviors over the course of the conversation task (Grimm et al., 2011). This approach estimates a latent intercept factor for the outcome variable in the initial conversation block (e.g., pupil dilation) and a second latent slope factor to describe change in the outcome over the course of the task (i.e., each conversation block). In the latent basis model, some of the latent slope factor loadings are estimated from the data. This modeling approach allows for the estimation of nonlinear shaped trajectories of change, and has been used in prior research to model dynamic changes in psychophysiological processes (Miller et al., 2016; Ugarte et al., 2021). Outcome variable error variance in each conversation block was constrained to be equal. We conducted a series of structural equation models to test whether group status predicted variability in latent intercepts and slopes of eye gaze behaviors and pupil dilation in the no prompt and prompt conditions.

For models with significant effects, we found evidence for homoscedasticity (e.g., for multilevel model of covariation between looking above nose and pupil dilation, Levene's test of equality of residuals across individuals $p = .453$). Visual inspection of QQ plots suggested normality of residuals.

Results

Mean levels of eye gaze behavior and pupil dilation

Table 2 presents the descriptive statistics and correlations among group status, pupil dilation, and eye gaze behavior, by condition. These correlations show that, compared to females without FXS, females with FXS spent significantly less time looking above the nose during the no prompt ($r = -.38$, SE = .13, $p = .004$) and prompt conditions ($r = -.33$, SE = .13, $p = .013$), and more time looking below the nose during the prompt condition only ($r = .37$, SE = .13, $p = .005$).

In the entire cohort, relative to the no prompt condition, the prompt condition was characterized by higher mean levels of pupil dilation ($t(54) = 2.05$, $p = .045$) and proportion of time looking above the nose ($t(54) = 4.67$, $p < .001$) and below the nose ($t(54) = 2.90$, $p = .006$). Conversely, the proportion of time looking off-body was higher during the no prompt condition than during the prompt condition ($t(54) = 6.70$, $p < .001$). Females with FXS demonstrated significant increases in mean pupil dilation in the prompt condition relative to the no prompt condition ($t(31) = 2.60$, $p = .014$) while mean pupil dilation did not differ between conditions for females in the comparison group ($t(22) = 0.05$, $p = .964$)

Coupling between eye gaze behavior and pupil dilation

Table 3 presents the results of multilevel models testing coupling between eye gaze behavior and pupil dilation.

In a multilevel model analysis considering the entire task (i.e., all 16 conversation blocks from both conditions) and the whole sample (females with and without FXS), we found significant within-person coupling such that eye contact was positively associated with pupil dilation ($b = 0.26$, SE = 0.06, $p < .001$). Analyses of conditions separately showed that eye contact significantly covaried with pupil dilation in the prompt condition ($b = 0.22$,

Table 3. Multilevel models testing coupling between eye gaze behavior and pupil dilation and group differences

	Entire Task			No-Prompt			Prompt		
	Est	*SE*	*p*	*Est*	*SE*	*p*	*Est*	*SE*	*p*
Testing Coupling Between Looking Above Nose and Pupil Dilation									
Intercept	3.41***	0.08	<.001	3.39***	0.08	<.001	3.43***	0.08	<.001
Looking Above the Nose	0.26***	0.06	<.001	0.10	0.09	.257	0.22*	0.08	.013
Testing Group Differences in Coupling									
Intercept	3.36***	0.09	0.03	3.36***	0.12	<.001	3.35***	0.12	<.001
Looking Above the Nose	0.12	0.09	.183	0.04	0.13	.756	0.15	0.12	.229
Group Status	0.10	0.16	.533	0.06	0.16	.694	0.14	0.16	.400
Looking Above the Nose × Group Status	0.24^{+}	0.12	.051	0.12	0.17	.509	0.12	0.17	.472
Testing Coupling Between Looking Below Nose and Pupil Dilation									
Intercept	3.41***	0.08	<.001	3.39***	0.08	<.001	3.43***	0.08	<.001
Looking Below the Nose	0.21*	0.09	.039	0.14	0.10	.153	0.14	0.07	.077
Testing Group Differences in Coupling									
Intercept	3.36***	0.12	<.001	3.35***	0.12	<.001	3.35***	0.12	<.001
Looking Below the Nose	0.16	0.16	.299	0.16	0.15	.299	0.06	0.11	.598
Group Status	0.06	0.16	.694	0.06	0.16	.694	0.14	0.16	.400
Looking Below the Nose × Group Status	−0.03	0.19	.866	−0.03	0.19	.866	0.14	0.14	.308
Testing Coupling Between Looking On-Body and Pupil Dilation									
Intercept	3.41***	0.08	<.001	3.39***	0.08	<.001	3.43***	0.08	<.001
Looking On-Body	−0.23*	0.10	.035	−0.00	0.12	.974	−0.16	0.15	.281
Testing Group Differences in Coupling									
Intercept	3.36***	0.12	<.001	3.36***	0.12	<.001	3.36***	0.12	<.001
Looking On-Body	−0.45**	0.16	.009	−0.18	0.19	.335	−0.47*	0.22	.036
Group Status	0.10	0.16	.532	0.06	0.16	.694	0.14	0.16	.399
Looking On-Body × Group Status	0.36	0.21	.090	0.29	0.24	.228	0.54	0.28	.065
Testing Coupling Between Looking Off-Body and Pupil Dilation									
Intercept	3.41***	0.08	<.001	3.39***	0.08	<.001	3.43***	0.08	<.001
Looking Off-Body	−0.22**	0.08	.007	−0.13	0.08	.120	−0.13	0.13	.327
Testing Group Differences in Coupling									
Intercept	3.36***	0.12	<.001	3.36***	0.12	<.001	3.35***	0.12	<.001
Looking Off-Body	−0.06	0.12	.626	−0.13	0.13	.334	0.21	0.20	.300
Group Status	0.10	0.16	.532	0.06	0.16	.694	0.14	0.16	.399
Looking Off-Body × Group Status	−0.26	0.15	.096	−0.00	0.17	.978	−0.57*	0.26	.034

Note: $^{***}p < .001$, $^{**}p < .01$, $^{*}p < .05$, $^{+}p = .051$. Est = unstandardized estimates; SE = standard error. Models for the entire task (i.e., both conditions) included 880 observations nested within 55 dyads, whereas models for the no-prompt and prompt conditions included 440 observations nested within 55 dyads.

SE = 0.08, $p = .013$), but not in the no prompt condition ($b = 0.10$, SE = 0.09, $p = .257$). We next investigated whether females with and without FXS differed in the strength of positive coupling between eye contact and pupil dilation across the entire task. Group status moderated the covariation between eye contact and pupil dilation ($b = 0.24$, SE = 0.12, $p = .051$). Specifically, covariation between eye contact and pupil dilation across conversations was weak and not statistically significant in females without FXS ($b = 0.12$, SE = 0.09, $p = .183$) but was magnified in females with FXS ($b = 0.36$, SE = 0.10, $p < .001$). Figure 1 presents the average within-person covariation of looking above the nose and pupil dilation across conversations in females with and without FXS.

In the entire cohort, we also found significant positive coupling between the proportion of time looking below the nose and pupil dilation ($b = 0.19$, SE = .09, $p = .039$), and time looking on-body and off-body were both negatively associated with pupil dilation ($b = -0.23$, SE = 0.10, $p = .035$ and $b = -0.22$, SE = 0.08, $p = .007$, respectively). Unlike looking above the nose, however, the strength of these associations did not differ between females with and without FXS (all $ps > .090$).

In models constrained to the prompt condition, group status moderated the covariation between looking off-body and pupil dilation ($b = -0.57$, SE = 0.26, $p = .034$). For females with FXS, looking off-body covaried negatively with pupil dilation across

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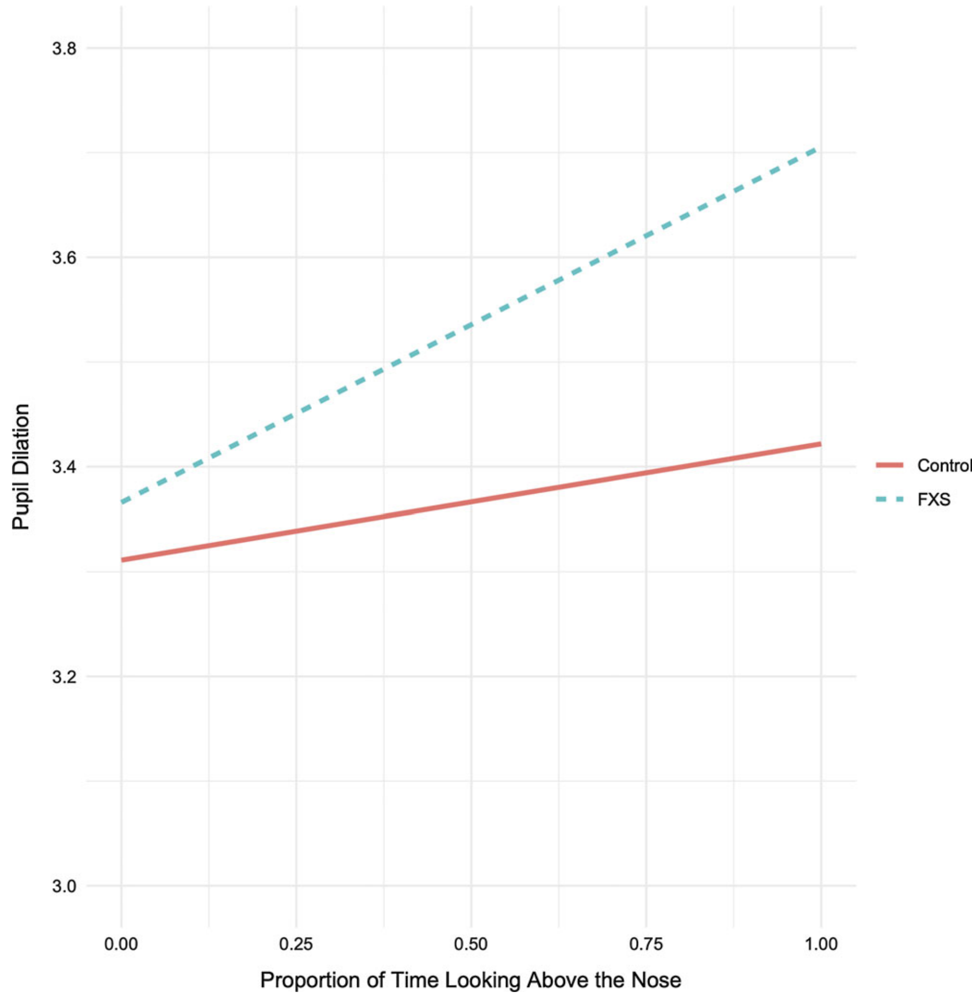

Figure 1. Covariation between proportion of time looking above nose and pupil dilation across all conversations for females with and without FXS.

conversations in the prompt condition ($b = -0.36$, SE = 0.16, $p = .034$). In contrast, looking off-body did not significantly covary with pupil dilation in females without FXS ($b = 0.21$, SE = 0.20, $p = .300$). Figure 2 presents the average within-person covariation of looking off-body and pupil dilation across prompt condition conversations in females with and without FXS. Group status did not moderate coupling of looking on-body or looking below the nose with pupil dilation in the prompt condition (both $ps >$ .064). Further, group status did not moderate coupling between gaze behaviors and pupil dilation in the no prompt condition (all $ps > .508$).

Trajectories of eye gaze behavior and pupil dilation over the course of the task

We tested a series of latent basis growth models treating FXS group status as a predictor of eye gaze behavior and pupil dilation trajectories. We conducted separate models for the no prompt and prompt conditions. Table 4 presents the results of the models. Figure 3 presents the estimated trajectories for females with and without FXS based on the results of the latent basis growth models. The path diagram used to estimate trajectories as a function of group status is presented in the Supplement (Figure S1).

In models focused on group differences in looking above the nose, FXS status was associated with lower latent intercept values in the no prompt ($b = -.14$, SE = .066, $p = .033$) and prompt conditions ($b = -.22$, SE = .078, $p = .006$). Group status was not significantly associated with latent slope factors in either condition (both $ps < .404$).

In models focused on group differences in pupil dilation, group status was not associated with latent intercept or slope values in the no prompt condition (both $ps > .686$). In the prompt condition, FXS group status was negatively associated with latent slope values, albeit not at a statistically significant level ($b = -.10$, SE = .055, $p = .066$). Females without FXS showed slight increases in pupil dilation over the course of the prompt condition (mean slope = .07, SE = .042, $p = .095$). Conversely, females with FXS showed more stable levels of pupil dilation over the course of the prompt condition (mean slope = −.03, SE = .035, $p = .392$).

In models focused on group differences in looking below the nose, group status was not associated with latent intercept or slope values in the no prompt condition or slope in the prompt condition (all $ps > .374$). In contrast, females with FXS had higher latent intercept values of looking below the nose in the prompt condition than females without FXS ($b = .12$, SE = .047, $p = .012$). Models focused on looking on-body and off-body either did not show group differences in trajectories in the no prompt or prompt conditions (all $ps > .135$) or failed to converge (i.e., models focused on looking on-body in prompt condition and looking off-body in the no prompt condition).

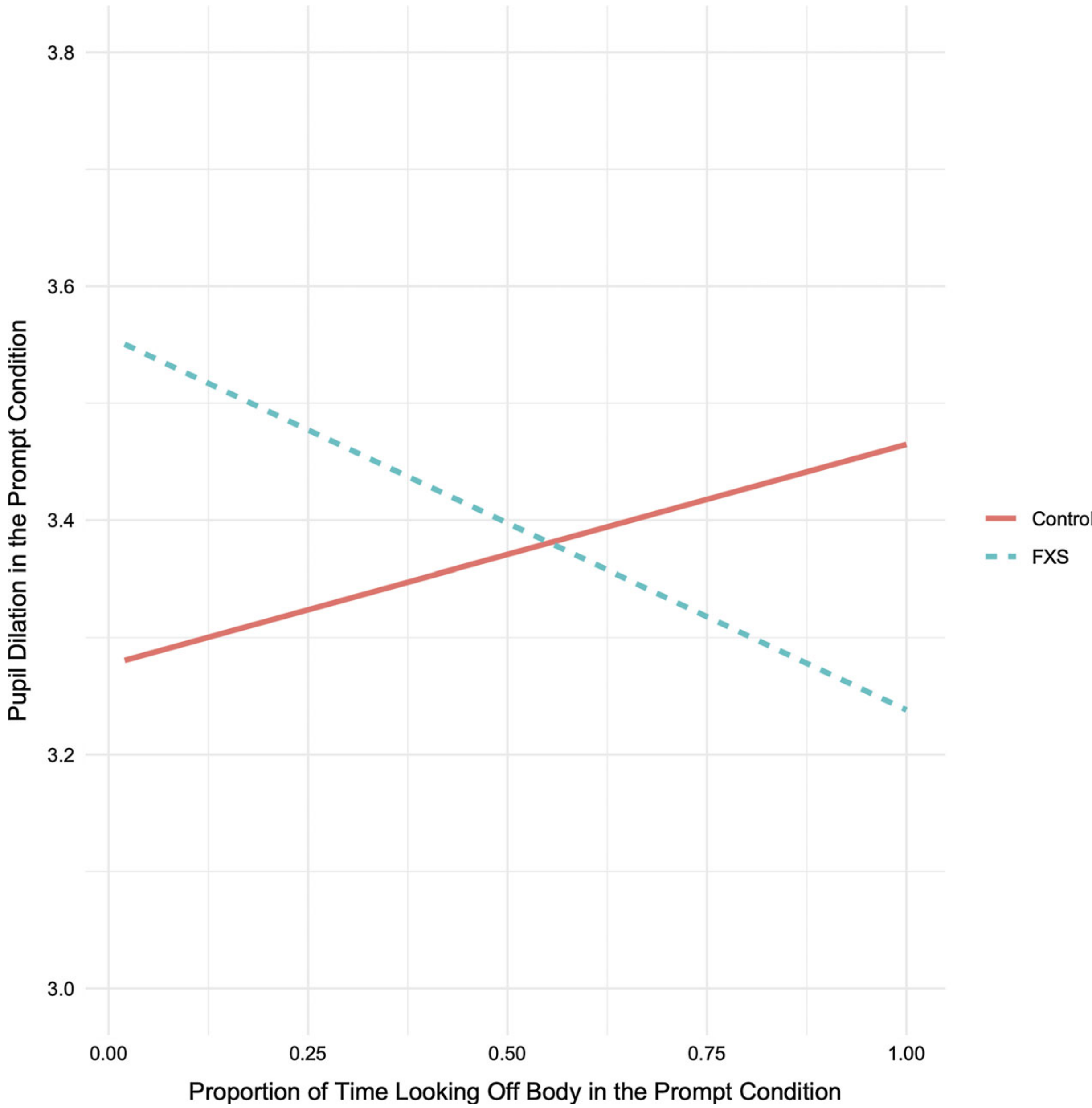

Figure 2. Covariation between proportion of time looking off-body and pupil dilation across conversations in the prompt condition for females with and without FXS.

Discussion

The current study identified a profile of social–behavioral and psychophysiological responses in young females with FXS. This profile describes FXS-specific gaze behavior and arousal at three levels: mean durations, trajectories, and within-person coupling. Consistent with prior work suggesting that gaze aversion is a feature of FXS (Cohen et al., 1991), we found that females with FXS demonstrated lower mean levels of eye contact than a comparison group of females without FXS. The females with FXS also demonstrated higher mean levels of pupil dilation when prompted to make eye contact. In analyses of trajectories, we did not find that females with FXS "warmed-up" during conversations; rather, they demonstrated relatively stable levels of eye gaze behavior and pupil dilation over the course of each task condition. In analyses of within-person coupling of gaze behavior and pupil dilation, we found positive coupling between eye contact and pupil dilation that was stronger in the FXS group than in the comparison group. Further, in the entire cohort, we found that spending more time looking away from the examiner's body was negatively coupled with pupil dilation. In the prompt condition, this negative coupling was stronger in the FXS group than in the comparison group. In other words, when females with FXS spent more time looking at the eyes of the examiner, they also showed physiological signs of hyperarousal (i.e., increased pupil dilation mediated by increased sympathetic nervous system activation). When females with FXS spent more time looking away from the examiner during the prompt condition, they showed physiological signs of decreased arousal, potentially mediated by increased parasympathetic activation (i.e., pupil constriction). These findings suggest that for females with FXS, engaging in eye contact is physiologically evocative, and gaze aversion is an effective behavioral method for regulating arousal during social interaction. By focusing on

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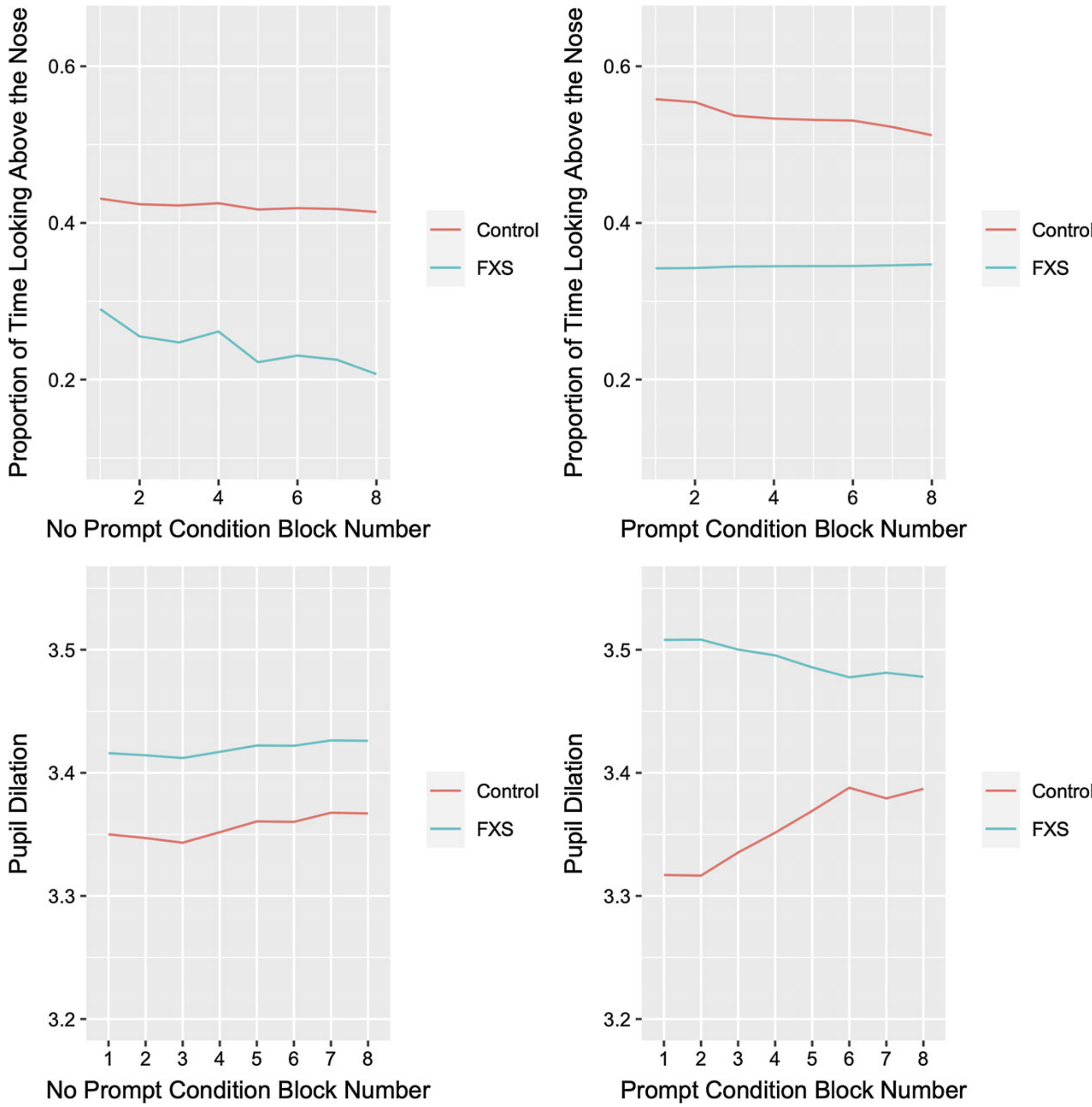

Figure 3. Trajectories of proportions of time looking above the eyes and pupil dilation levels in the no prompt and prompt conditions. *Note:* Numbers on the *x*-axis indicate the conversation block number within either the No Prompt or Prompt Conditions (i.e, 1 = first conversation block, 2 = second conversation block, etc.).

within-person processes, our findings strengthen support for the perspective that gaze aversion in FXS reflects negative reinforcement of social avoidance behavior. Collectively, the observed profile of social–behavioral and psychophysiological responses in females with FXS may contribute to or reflect social anxiety in this population.

Gaze aversion in FXS has been viewed as a behavioral sign of hyperarousal (Reiss & Dant, 2003). In accordance with this perspective, FXS has been associated with altered neurobiological functioning that may indicate hyperarousal, such as reduced neural habituation to faces (Bruno et al., 2014). Further, children with FXS who engage in less eye contact demonstrate less reactivity in stress biology systems, including the hypothalamic pituitary adrenal axis (Hessl et al., 2006). Prior studies, however, have not directly tested whether eye gaze behaviors covary with physiological activity that is indicative of arousal within individuals with FXS. Our findings suggest that fluctuations in eye gaze behavior and physiological arousal are more tightly coupled in females with FXS than in developmentally-similar females without FXS. One potential explanation for this specificity in FXS is that coupling between gaze behaviors and physiological arousal is related to reduced FMRP, which leads to impaired synaptic plasticity and abnormal dendritic spines that may contribute to social anxiety (Spencer et al., 2005).

It is important to note that our study used pupil dilation as a measure of physiological arousal primarily driven by sympathetic activation, whereas many prior psychophysiological studies of FXS have considered cardiovascular measures reflecting parasympathetic activation (Klusek et al., 2015). The frequent focus on parasympathetic activation may be guided, in part, by the theoretical perspectives of neurovisceral integration (Thayer & Lane, 2009)

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Table 4. Parameter estimates and fit indices for growth curve models

	No Prompt: Looking Above Nose		Prompt: Looking Above Nose		No Prompt: Pupil Dilation		Prompt: Pupil Dilation	
	Est	SE	Est	SE	Est	SE	Est	SE
Latent Slope Factor Loadings								
Conversation 1	0		0		0		0	
Conversation 2	.42**	.13	.08	.11	−.18	.25	−.01	.13
Conversation 3	.51***	.12	.46***	.10	−.40	.28	.26*	.13
Conversation 4	.35**	.13	.54***	.11	.10	.22	.42**	.13
Conversation 5	.82***	.15	.58***	.10	.62**	.21	.74***	.14
Conversation 6	.72***	.13	.60***	.10	.60**	.19	1.01***	.14
Conversation 7	.78***	.14	.77***	.09	1.04***	.23	.89***	.13
Conversation 8	1		1		1		1	
Means								
Intercept	.43***	.05	.56***	.06	3.35***	.13	3.32	.13
Slope	−.02	.04	−.05	.05	.02	.03	.07	.04
Variances								
Intercept	.05***	.01	.08***	.02	.36***	.07	.37***	.07
Slope	.02**	.01	.04***	.01.	.02*	.009	.03**	.01
Covariance								
Intercept and Slope	−.01	.007	−.02	.010	−.02	.02	−.03	.02
Regressions								
FXS -> Intercept	−.14*	.07	−.22**	.08	.07	.16	.19	.17
FXS -> Slope	−.07	.05	.05	.06	−.01	.05	−.10	.06
Fit Indices								
χ^2(df = 38)	63.31**		56.23*		50.26		93.59***	
CFI	.96		.98		.99		.95	
TLI	.96		.98		.99		.96	

Note: ****p* < .001, ***p*<.01, **p*<.05. Est = unstandardized estimates; SE = standard error; df = degrees of freedom; CFI = comparative fit index; TLI = Tucker Lewis index; FXS = fragile X syndrome. In all models, latent intercept factor loadings were set at 1 for each conversation. For FXS, 0 = comparison group and 1= fragile X syndrome group.

and polyvagal theory (Porges, 2007). These perspectives suggest that decreased parasympathetic regulation of cardiac activity allows for activation of sympathetic states that drive stress and negative affect in challenging or threatening contexts. Polyvagal theory posits that increased parasympathetic activation via the myelinated vagus nerve supports social engagement behaviors in safe contexts (Hastings & Miller, 2014). From this perspective, our findings may indicate that females with FXS evaluate eye contact as a threat-related stimulus. Future research considering within-person coupling of gaze behavior and parasympathetic and sympathetic activity may provide further support for this perspective.

The current study also focused on the effects of prompting eye contact on gaze behavior and physiological arousal. Consistent with prior findings suggesting that behavioral prompts improve eye contact in individuals with FXS (Gannon et al., 2018), we found that females with FXS (and without FXS), on average, demonstrated increased eye contact during conversations that included prompts. The comparison group, however, engaged in more eye contact than the FXS group in both task conditions. In addition, females with FXS had higher mean levels of physiological arousal during the conversations with prompts than during the conversations without prompts. Our trajectory analyses suggested that gaze avoidance and pupil dilation were stable over the course of both task conditions (in both groups). Collectively, these findings suggest that prompting eye contact elicits heightened and sustained physiological arousal in young females with FXS.

There is a debate in the literature regarding whether behavioral interventions for FXS should focus on encouraging eye contact (Dykens et al., 2000; Gannon et al., 2018; Hall et al., 2009). Some researchers have argued that encouraging eye contact can lead to hyperarousal states that lead to anxiety and behavioral problems (Dykens et al., 2000; Morris, Kondratenko, & Griffiths, 2014; Scharfenaker et al., 2002). Conversely, other researchers (Gannon et al., 2018; Hall et al., 2009) have adopted the perspective that appropriate social gaze behavior is crucial for social and communicative functions, including language development, emotion recognition, and joint attention-based learning (Emery, 2000; Itier & Batty, 2009; Mirenda et al., 1983; Senju & Johnson, 2009); thus, improving social gaze behavior such as eye contact may be an important target for behavioral intervention for children with FXS. Our findings suggesting that behavioral prompts improve eye contact and increase psychophysiological

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demands for females with FXS could be interpreted as providing support for both sides of this debate. Although we did not observe that females with FXS “warmed up” over the course of the prompt condition, it is possible that this pattern could eventually be observed with training over a longer period of time. For example, Hall et al. (2009) observed that children with FXS slightly decreased their eye gaze avoidance over the course of a 25-min conversation task. On the other hand, for females with FXS, it is possible that heightened physiological arousal during conversations with prompts may contribute to states of anxiety. Prior studies suggest that heightened physiological arousal may be an indicator of anxiety (Friedman, 2007). From this perspective, our findings may provide support for the notion that encouraging eye contact elicits hyperarousal that has negative affective and behavioral implications in individuals with FXS (Dykens et al., 2000). It is important to note, however, that we did not collect data on subjective states of anxiety, and that is unclear from our findings whether increased physiological demands necessarily undermine other aspects of social behavior. Psychophysiological research that considers subjective and behavioral measures of anxiety, in addition to eye gaze behavior, would further advance our understanding of the impact of encouraging eye contact in females with FXS.

It is important to note that this study focused on 6–16 year old females, which is a wide age range. Prior analyses of the current sample focused on age-based differences (i.e., cross-sectional analyses) suggest that social avoidance seems to worsen as females with FXS enter adolescence (Lightbody et al., 2022). Given these findings, it is possible that the effects of FXS on social gaze behavior and psychophysiological response, as well as the impact of the prompting condition, may differ depending on age. The current study is underpowered to test these possibilities, which would require interaction effects involving age as a moderator variable (e.g., three-way cross-level interaction of age, group status, and eye gaze behavior in multilevel model predicting pupil dilation). However, longitudinal data are currently being collected, which will lead to analyses focused on the developmental implications of our findings.

We note five limitations of our study. First, we relied on pupil dilation as a single measure of autonomic activity. Pupil dilation and constriction are mediated by sympathetic and parasympathetic activation, respectively (Szabadi, 2018), but autonomic activity can also be characterized by patterns of coactivation and coinhibition of sympathetic and parasympathetic activity (Berntson et al., 2008). Capturing these kinds of autonomic states requires separate measures of sympathetic and parasympathetic activity. This kind of research would advance our understanding of the specific within-person autonomic processes that characterize gaze avoidance in FXS. Second, each task condition was 8 min in duration, and this may not have been enough time to observe “warm-up” effects in the FXS group. For example, Hall et al. (2009) found that eye gaze avoidance and heart rate slightly decreased over the course of a 25 min conversation task with behavioral prompts. Third, we did not include a typically developing comparison group. Thus, the degree of gaze aversion, pupil dilation, and coupling between these measures in females with FXS relative to females without developmental difficulties (i.e., without idiopathic developmental delays or learning disabilities) is not clear from our findings. On the other hand, the fact that our comparison group included age-matched females without FXS, but with developmental difficulties and similar levels of verbal IQ, increases our confidence that our findings are specific to FXS. Fourth, we did not correct for multiple comparisons in our analyses. Future studies, potentially with larger samples, are necessary to replicate our findings using more conservative statistical thresholds. Lastly, our region of interest for defining eye contact may have included participants looking at other areas of the face above the nose (e.g., forehead).

In conclusion, our study replicates and builds upon prior studies of gaze behavior and psychophysiology in FXS. To our knowledge, our study is the first to adopt a within-person approach to considering coupling between gaze behavior and physiology that is specific to FXS. Our findings point to a behavioral and psychophysiological profile in females with FXS that is characterized by heightened gaze aversion and enhanced coupling between gaze behavior and physiological arousal. To the extent that heightened physiological arousal during social interaction is distressing, our findings provide support for the perspective that gaze aversion is a form of negative reinforcement of social avoidance in females with FXS. Our findings also provide support for the perspective that it is possible to improve eye contact in females with FXS using basic behavioral skills training, but not necessarily to the levels observed in developmentally-similar females without FXS. In addition, maintaining eye contact appears to be particularly physiologically taxing for females with FXS.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S095457942300038X.

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Conflicts of interest. None.

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