Seeing Eye to Eye with Threat: Atypical Threat Bias in Children with 22q11.2 Deletion Syndrome

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Running Title: Threat Bias in Children with 22q11DS

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Abstract:

Individuals with 22q11.2 deletion syndrome (22q11DS) show high rates of anxiety associated with their increased risk of developing schizophrenia. Biased attention is associated with anxiety and is important to investigate in those with 22q11DS given this association. We analyzed attention bias to emotional faces in 7 to 17-year olds with 22q11DS and typically developing controls (TD) using a dot probe threat bias paradigm. We measured response time, eye tracking, and pupilometry. Those with 22q11DS showed no significant changes in early versus late trials, while those who were TD showed differing patterns in both gaze and pupilometry over time. The patterns in those who are TD may indicate adaptation that is lacking or slower in individuals with 22q11DS.

KEYWORDS: 22q11.2 Deletion Syndrome; Anxiety; Eye Tracking; Attentional Bias

MANUSCRIPT TEXT:

BACKGROUND

Chromosome 22q11.2 deletion syndrome (22q11DS) results from the most common human chromosomal microdeletion, affecting as many as 1 in every 2000 live births (Grati et al., 2015; Kobrynski & Sullivan, 2007). Physical manifestations include craniofacial and palatal abnormalities, cardiac defects, and endocrine problems (Shprintzen, 2008). Intellectual and behavioral impairments and psychiatric disorders are also prevalent. Cognitive features include borderline IQ and impaired attentional, executive, and numerical abilities, delayed speech, social impairments, and comprehension impairments (Gur et al., 2014; Woodin et al., 2001).

Behavioral and psychiatric manifestations include anxiety and mood disorders, attention disorders, and a 25-30-fold increased risk for schizophrenia compared to the general population (Bassett & Chow, 2008; Green et al., 2009; Schneider et al., 2014). In fact, although childhood anxiety disorder does not guarantee later psychosis, it presents a significant risk factor for conversion in 22q11DS (Gothelf et al., 2013; Tang et al., 2013). Further, anxiety disorders are common in 22q11DS, with recent reports suggesting around 50% of children and adults with 22q11DS experience anxiety disorders (Schneider et al., 2014; Tang et al., 2013). Despite this, many with 22q11DS and an anxiety disorder are not receiving treatment for their anxiety (Tang et al., 2013). Because anxiety and stress have negative health consequences, both physically and mentally (McEwen, 2013; McEwen, Eiland, Hunter, & Miller, 2012) , learning more about anxiety in 22q11DS may build a literature that will help close the gap between anxiety diagnosis and treatment in those with 22q11DS.

We particularly note, that while IQ predicts adaptive functioning scores in typically developing (TD) children (Sparrow, Balla, & Cicchetti, 1984) and among individuals with most neurodevelopmental disorders (Loveland & Kelley, 1991), in a previous study it was found that anxiety, not IQ, predicts adaptive functioning in children with 22q11DS (Angkustsiri et al., 2013). In the Angkustsiri study, the authors examined 78 children with 22q11DS and 36 TD children between the ages of 7 and 14 years. The authors measured adaptive function using the Adaptive Behavior and Assessment System, 2nd Edition (ABAS-II) (Harrison & Oakland, 2003) and the Behavior Assessment System for Children, 2nd Edition (BASC-2) (Reynolds & Kamphaus, 2008). They measured anxiety using the Spence Children's Anxiety Scale (SCAS)

(Spence, 1998). In the group of children with 22q11DS, higher anxiety scores on the SCAS were related to lower adaptive function ($r=-0.27$, $p=0.015$), while there was no relationship between full scale IQ and adaptive function. The TD children, unlike those with 22q11DS, showed a significant relationship between full scale IQ and adaptive function ($r=0.6$, $p=0.002$) (Angkustsiri et al., 2013). Given the relevance of anxiety to adaptive function in this population we aimed to examine how anxiety might impact a particular cognitive function, attentional deployment, in this population.

In the present study, we examine rates of anxiety in children with 22q11DS using self and parent reports, which provide a good estimate of the anxiety symptoms and severity that participants experience. We predict this analysis will confirm higher rates of anxiety in our sample of children with 22q11DS. Because anxiety impacts attentional deployment to emotional stimuli in typical adults (MacLeod & Mathews, 1988), it is likely that attentional control in children with 22q11DS will also be affected when responding to emotional stimuli.

We measure attentional deployment to emotional stimuli. In this study, the emotional stimuli are only mildly threatening. As participants evaluate mildly threatening stimuli, they may adapt to the emotional content. They may also realize, even within a single trial, that the perceived threat is not real. This adaptation and evaluation may allow the participants to change their attentional deployment, even within a single trial. This is consistent with the observation that typically developing individuals often show high levels of emotion in response to salient stimuli when they are first presented (Hare, Tottenham, Davidson, Glover, & Casey, 2005; Thomas et al., 2001). However, this initial response will quickly habituate with repeated exposures (Hare,

Tottenham, Davidson, Glover, & Casey, 2005; Thomas et al., 2001). Individuals with high anxiety, however, may show a potentiated initial response, coupled with reduced habituation to threat. This pattern is consistent with poor extinction shown in anxious adolescent humans and animals (Lee et al., 2014; Pattwell et al., 2012).

The Dot Probe Threat Bias (DPTB) experimental paradigm is appropriate for investigating attention to emotional stimuli in children with 22q11DS. In the DPTB, participants are asked to respond to a target that appears in one of two screen locations. Targets are preceded by emotional or neutral stimuli, which can capture attention to facilitate target identification. Facilitated response time for targets that subsequently appear in the same location as angry, but not neutral, faces, is presumed to indicate a threat bias (Mogg & Bradley, 1998; Pérez-Edgar et al., 2011), which is a measure of attention to emotional stimuli. Many studies (Bar-Haim, Lamy, & Glickman, 2005; B. Bradley, Mogg, & Millar, 2000; Salum et al., 2013; a M. Waters, Bradley, & Mogg, 2014) have shown that children and adults with, or at risk for, anxiety show systematic attention biases to threatening or emotional stimuli (Roy et al., 2008). Further, since the task requires multiple trials of each emotional valence, habituation to multiple exposures can be measured, capturing the dynamic time course of attention to threat.

Early DPTB experiments exclusively used the facilitated RT measure to examine threat bias to stimuli including affective faces or words (MacLeod & Mathews, 1988; Wilson & MacLeod, 2003). This early work presented findings including the supposition that individuals experiencing stress or anxiety may attend to, and have difficulty disengaging from threatening stimuli (MacLeod & Mathews, 1988). Later work, indicated that the facilitation of RT was dependent on

experimental as well as individual factors, such as the length of time stimuli were presented (B. P. Bradley, Mogg, Falla, & Hamilton, 1998; Koster, Crombez, Verschuere, & De Houwer, 2004), possibly indicating that RT facilitation subsumes multiple cognitive processes. Work in children further showed that younger individuals may show threat bias (Dawel, Palermo, O'Kearney, Irons, & Mckone, 2014), however the results may be specific to particular subtypes of anxiety (Salum et al., 2013; Waters & Valvoi, 2009). However, threat bias measured by response time (RT) alone has also yielded inconsistent results (Koster, Crombez, Verschuere, & De Houwer, 2004). Further, using the DPTB along with other methodologies has produced a more nuanced understanding of attentional deployment to threat. For example, the analysis of event-related potentials from electroencephalography showed that while non-anxious individual all show initial capture by threat, they do not show longer term perseveration on threatening stimuli (Kappenman, MacNamara, & Proudfit, 2014). Finally, results from eye tracking studies indicate that analysis or eye movements, while correlated with RT scores, may produce a more robust measure of attention bias (B. P. Bradley, Mogg, & Millar, 2000).

Based on this literature review, we used concurrent eye tracking to measure gaze bias as a complementary, and possibly more reliable, measure of threat bias than RT alone. The eye tracking apparatus was only available for a subset of participants, but was added because RT subsumes motor, cognitive, and perceptual processes into a single value. By contrast, eye-tracking directly measures quantitative and qualitative aspects of gaze (overt attention), thereby providing more sensitive indices of the cognitive processing participants engage in between stimulus presentation and motor response (B. Bradley et al., 2000). Further, many eye-tracking instruments, such as the one used here, generate a rudimentary measure of pupil diameter. To

our knowledge, pupil dilation has not been measured in previous DPTB paradigms. However, previous work has used pupilometry as a measure of autonomic activation (M. Bradley, Miccoli, Escrig, & Lang, 2008). The Bradley paper related pupil diameter to other known indicates of autonomic activity (heart rate and skin conductance during affective picture viewing) during affective picture viewing. They found that pupil diameter increased when viewing affective pictures, and that this increase was correlated with heart rate and skin conductance, indicating that it may be a good measure of sympathetic nervous system activity. Additional work has shown that changes in pupil diameter are also correlated with processing load (Beatty, 1982; Laeng, Ørbo, Holmlund, & Miozzo, 2011). In the Laeng paper, the authors showed that pupil dilations were correlated with the greater cognitive load of inhibiting a prepotent response during a stroop paradigm. Taken together, the previous research indicates that larger pupil dilation during the DPTB may indicate the combination of emotional reactivity to affective pictures and the greater processing load of controlling one's attentional deployment to these pictures. Disentangling the effects of emotional reactivity and attentional control will require further study, possibly using additional indicators of emotional reactivity (such as skin conductance) and cognitive load (such as event-related potential from electroencephalography).

The present study was performed in children aged 7 to 17 years. As stated, the DPTB has been used in younger children, who have shown RT facilitation and eye movements consistent with threat bias (B. P. Bradley et al., 2000; Pérez-Edgar et al., 2010). Other studies using affective stimuli indicate that adolescents often perform worse than adults and children in response to emotional stimuli (Casey & Caudle, 2013). This may be because during adolescence the "bottom-up" limbic system of the brain is more developed than the "top-down" frontal system of

the brain (Lago, Davis, Grillon, & Ernst, 2017). Additionally, adolescence is a period where social approval is particularly important, which may lead to different responses to social stimuli used in studies (including this one) (Blakemore & Mills, 2014). Particularly, emotional regulation to social stimuli is worse in adolescence (Silvers et al., 2012). These findings in typically developing individuals emphasize the importance of studying attentional regulation to emotional stimuli in children with 2211DS in this age group.

Notably, children with 2211DS show a lower full-scale IQ than age-matched typically developing children. While the DPTB has not been leveraged in children with 22q11DS before, a study was performed in children with Fragile X (Burris et al., 2017). The study was able to collect meaningful data from a similarly cognitively impaired population. Additionally, the Burris study further used a group of mental age-matched control, who comprised children with low IQ who did not have fragile X. Clean data was also collected from this low IQ group. Finally, the study found a different pattern between the group of children with fragile X and the mental age-matched controls, indicated the DPTB is likely sensitive to more subtle effects than those from IQ alone.

Hypotheses

Based on previous findings (Angkustsiri et al., 2013) we predicted higher anxiety and lower adaptive functioning in children with 22q11DS than TD controls.

We predicted that the 22q11DS group would show bias to negative stimuli (angry faces) in both RT and gaze. We did not expect the TD group to show any threat bias. Indeed, data showing the effect of positive stimuli on youth cognition suggest that the TD group will show a bias to happy faces instead (Casey & Caudle, 2013), again evident in both RT and gaze. Because gaze is a more direct index of threat processing we also predicted that eye-tracking and pupilometry would produce a stronger and more reliable response pattern than RT data.

Analyzing responses to early versus late trials within our experiment allows us to examine both initial threat response and adaptation. Based on evidence from extinction paradigms (Pattwell et al., 2012), we expected that the TD children would show gaze adaptation to the emotional stimuli (Ochsner & Gross, 2005), evident in how their gaze and pupilometry change over multiple stimulus exposures. We predicted less gaze adaptation in the children with 22q11DS.

An additional benefit of looking at gaze rather than RT alone, is that we can observe whether participants are viewing the faces on the screen at all, or if they are looking at non-face areas of the screen. We predict that children who are TD would initially show gaze to happy and non-face areas of the screen, perhaps showing more gaze to angry faces with additional trials as they learn they are not a threat. We predicted those with 22q11DS would not show a change in non-face looking over time, consistent with our prediction of less gaze adaptation.

Since pupilometry can be used as an indicator of emotional reactivity, we predicted that the TD children would show pupil responses consistent with reducing autonomic activation and cognitive effort over time, particularly, more pupil dilation to emotional stimuli (Beatty, 1982;

M. Bradley et al., 2008; Kahneman & Beatty, 1966; Laeng, Ørbo, Holmlund, & Miozzo, 2011). We expected to see a more dysregulated pattern in the children with 22q11DS, particularly, less change in pupil diameter to emotional stimuli.

Additionally, to examine the effect of age on our findings, we included age as a regressor in our models, however removed it if the effect was not significant. We predicted that all measures would show an effect of age, with the more anxious adolescents showing more RT facilitation, more gaze to emotional stimuli, and more pupil dilation to emotional stimuli, consistent with greater anxiety in adolescence.

METHODS

Participants

This study was submitted to and approved by the university's Institutional Review Board (#268067) in compliance with the Helsinki Declaration. Children in both groups were recruited through flyers at the UC Davis MIND institute, the MIND Institute Subject Tracking System, web postings and social media, and who had previous participated in Dr. Simon's studies. Children in both groups were excluded if they had a history of head trauma or were part of a multiple birth. Children who were TD were additionally excluded if they had any known axis 1 disorders. Parents provided written informed consent approved by the IRB for participation and children verbally assented before participation. The total sample comprised 79 children (41 males) aged between 7 and 17 years (mean ± SD = 10.92 years ± 2.33 years), there was no significant difference of age between groups ($p=0.64$). Of these, 32 children were TD and 47

children had a 22q11DS diagnosis, confirmed via fluorescence in situ hybridization or similar genetic test.

Materials

IQ Measures

We measured IQ using either the Wechsler Intelligence Scale for Children – Fourth Edition (WISC-IV) (Wechsler, 2004) or the Wechsler Abbreviated Scale of Intelligence (WASI) (Wechsler, 1999). Trained research personnel or clinicians at the research institute performed assessments. IQ scores for 7 participants (3 22q11DS) were unavailable (Table 1). IQ scores for 40 children with 22q11DS came from the WISC-IV and IQ scores for 25 TD children were generated from the WASI.

Anxiety Measures

Anxiety was measured using the Spence Children's Anxiety Scale (SCAS) (Spence, 1998). This scale assesses six domains of anxiety: generalized anxiety, panic/agoraphobia, social phobia, separation anxiety, obsessive-compulsive disorder and physical injury fears. The SCAS is typically used in a research setting to examine the structure of anxiety symptoms in a study population.

We collected both parent and self reports on the SCAS. Research personnel received in-person training by a developmental neuropsychologist on administering the SCAS to children. Parents or guardians completed the SCAS prior to the study visit. Because young, developmentally

delayed children are limited in self-reporting abilities, our primary measure was the SCAS total parent score.

Adaptive Functioning Measures

We measured adaptive functioning, or daily living skills, with the parent report Adaptive Behavior Assessment System-Second Edition (ABAS-II) (Harrison & Oakland, 2003). This scale assesses nine subdomains (Home Living, Health and Safety, Leisure, Self-Care, Self-Direction, Functional Academics, Community Use, Communication, and Social); three composites (Social, Practical, and Conceptual); and one overall measure of adaptive behavior (general adaptive composite - GAC). Parents or guardians completed the ABAS-II form prior to the study visit.

Some anxiety scores and adaptive scores were unavailable (see Table 1) due to skipped questions by the parent or participant.

Procedure

The DPTB task parameters were the same as in Perez-Edgar et al.'s 2011 study (Pérez-Edgar et al., 2011) except for the addition of eye tracking. The task was presented on a Dell T5500 computer running Windows XP Service Pack 3 Professional using E-Prime 2.0 software on a 1280 x 1024 pixel resolution monitor. The DPTB task consisted of 8 practice trials and 80 experimental trials, presented in two 40-trial blocks. Each trial began with the presentation of a central fixation cross (children were required to fixate for 250 ms before stimuli were presented), followed by the presentation of a pair of faces depicting emotions (500 ms, 4.8 degrees to the left

and right of center, subtending 5.3 x 4 degrees of visual angle). Immediately after the face pair disappeared, an asterisk probe appeared (2500 ms) in the location previously occupied by one of the faces.

Children were seated 60cm from a 17-inch Tobii™ T120 Eye Tracker Monitor using a chin rest to limit head movement. Before beginning the task, each child completed a 5-point gaze calibration sequence using Tobii Studio Enterprise Edition version 2.3.2.0. After successful calibration, children proceeded to the task. The software required the child to maintain fixation within 4.1° of the fixation cross for 250ms to initiate each trial, but they were explicitly permitted to look anywhere during stimulus presentation. Children used the index and middle fingers of their dominant hand on a Psychology Software Tools Serial Response Box to indicate as quickly and accurately as possible on which side of the screen the probe appeared.

There were three types of face pairs presented: angry/neutral (32 trials), happy/neutral (32 trials), and neutral/neutral (16 trials). 30 different actors (50% female) from the NimStim face stimulus set portrayed facial expressions (Tottenham, Borscheid, Ellersten, Marcus, & Nelson, 2002). Congruent trials were those where the probe appeared in the same location as the emotional face (angry or happy). In incongruent trials the probe appeared at the same location as the neutral face. Laterality of the emotional face, trial congruency, sex of the face, and probe location were counterbalanced across trials. RTs and response accuracy were recorded for each trial.

ANALYSIS PLAN

PARTICIPANT CHARACTERISTICS

Two-tailed independent t-tests were used to compare groups on IQ, SCAS Parent Total anxiety score, and ABAS GAC. We also examined anxiety medications from a health questionnaire parents completed, though there was insufficient data for formal statistical testing.

RELIABILITY OF MEASURES

In order to confirm the reliability of the different measures we used throughout the study, we split each of the participant's trials (in the case of RT) and epochs (in the case of gaze and pupilometry) into even- and odd-numbered trials. We then tested to see if measures correlated between the even and odd trials using Pearson's r.

ANALYSIS 1: RT AND GAZE

We removed outliers using previous criteria (Pérez-Edgar et al., 2010, 2011). Trials with errors and trials with responses that were anticipatory (<200ms) or unusually slow (>2.5 SD from mean RT of the individual) were removed from analyses. In addition, we removed individual participants with accuracy below 75% on any condition. This resulted in removing 6 children with 22q11DS. Four TD children were also removed due to technical difficulties with the recording software, leaving a final sample of 42 children with 22q11DS and 28 TD children with RT data. There were no significant effects of age, gender, IQ, anxiety level, or adaptive function on who was removed from the data (all $p > 0.05$). The resulting participants had an average of 8.5% of trials removed.

Attention bias was defined as the mean RT on congruent trials subtracted from the mean RT on

incongruent trials for each emotion condition ("angry bias" and "happy bias," respectively) for correct trials only. Between-group Student's two-tailed t-tests were performed to assess the difference in angry bias or happy bias between TD and 22q11DS groups. Analyses were performed in R version 3.1.3.

While we acknowledge the poor reliability of RT measures in previous DPTB work, we feel is important to report these analyses given that most previous DPTB papers have used RT measures. Including these results will increase researchers' ability to compare our findings with the broader literature.

Eleven children completed the study before eye-tracking technology was obtained and thus could not be included in gaze analyses. Two children did not have their gaze recorded due to technical errors. Before eye tracking analyses were performed, time points during which tracking was lost on one or both eyes were marked "invalid" based on Tobii's validity column. Tracking can be lost due to technical problems, the participant blinking, or the participant looking away from the screen. All other time points were considered valid and we used only those for analyses. We excluded participants if ≥70% of their time points were invalid on any condition resulting in exclusion of 10 children (6 22q11DS), resulting in eye tracking data for 46 participants (27 22q11DS). See figure 1, for information on participant removal. The resulting participants had an average of 17.3% invalid eye-tracking data. Tests showed that the sample with eye-tracking data did not differ from the full sample on diagnostic distribution, age, gender distribution, SCAS parent total, ABAS GAC, or IQ.

Tobii Studio software calculated eye position as the average position tracked from both eyes. For each trial, percent of total time looking at the emotional and neutral face was calculated (time on emotional face or on neutral face divided by total presentation time respectively). We use the terms “angry gaze bias” and “happy gaze bias” to refer to percent total time spent looking at each of the respective emotional faces.

Analyses were performed as mixed effects linear models on angry or happy trials (separately) including a random effect of participant and effects of interest for percent time looking at emotional/neutral faces and diagnosis. Since an interaction of emotion and diagnosis did not reach significance, separate models were used for 22q11DS and TD. We did not combine the two groups because we wanted to look for differences between these two genetically distinct samples. Age, gender, and IQ were included as factors in the model only if they contributed significantly. The percent of time spent on overall emotional face, overall calm face gaze, and overall non-face gaze would by definition sum to 100%, therefore to avoid issues of non-independence, gaze to non-face areas was not included in the overall models.

ANALYSIS 2: CHANGE IN GAZE OVER TIME

To investigate whether children with 22q11DS or TD children adapted differently to emotional stimuli, we compared gaze patterns on early versus late trials on the DPTB. Early trials were designated as the first 25% of trials for each stimulus type, while late trials were designated as the last 25% of trials for each stimulus type. Gaze as a function of diagnosis (22q11DS versus TD) and trial (early versus late) was entered into a 2 x 2 mixed effects model. In this case since we are comparing gaze to each respective area on early trials to gaze to that same area on late

there is no reason to remove non-face areas from analysis. For example, it is not the case that non-face gaze early and non-face gaze late necessarily sums to a given value.

Exclusion criteria were the same as above, except before analyses were conducted we decided to remove children who made saccades on fewer than 10% of trials. While in analysis 1, if a child stared at the same area of the screen (the center, for example) this data could meaningfully indicate attention to that area of the screen, when comparing early to late this child's scores would subtract out to zero, because they never moved their gaze. This would preclude the calculation of meaningful eye movement scores (B. Bradley et al., 2000). This resulted in the removal of one additional child (with 22q11DS) (figure 1). Because we ultimately found no significant difference in eye movements between early and late trials in children with 22q11DS, this child's non-zero change scores do not change our results if included.

ANALYSIS 3: PUPIL DIAMETER

To measure dilation and contraction, we compared the pupil size recorded by the Tobii eye tracker on the emotional face trials (happy versus neutral and angry versus neutral) to our neutral/neutral face control trials (emotional trial size minus neutral trial size) for early versus late trials. Pupil size was calculated as an average of pupil diameter during the first/last 25% of angry/happy trials minus pupil diameter during the first/last 25% of neutral trails. Comparing pupil diameter in response to neutral faces also allowed us to control for the effects of viewing faces in general, such as luminance and contrast. This allows us to use pupil diameter as a preliminary measure of autonomic reactivity to emotional stimuli.

For each family of tests where multiple comparisons correction were reported they were corrected for using the false discovery rate correction (Benjamini & Hochberg, 1995).

RESULTS

PARTICIPANT CHARACTERISTICS

Mean IQ for 22q11DS was 73.7 (mean IQ ± SD =73.7 ± 14.4), and for TD was 116.5 (mean IQ ± SD =116.5 ± 14.9) (Table 1).

As predicted, initial two-tailed t-tests indicated participants with 22q11DS had higher SCAS Parent Total $t(70.9) = 7.06$, $p<0.0001$ (indicating higher anxiety in those with 22q11DS) and lower ABAS GAC $t(70.6) = -11.37$, $p<0.0001$ (indicating lower adaptive function in those with 22q11DS), scores than TD children (Table 1).

Unfortunately, we did not directly inquire about if children were receiving psychotherapy or counseling for anxiety. However, parents of 5 children (all 22q11DS) indicated “anxiety” as a medical problem their child had on a medical history questionnaire. Of these 5 children, 3 were receiving some type of anxiety medication (2 zyprexa, 1 prozac). No other children had anxiety medications listed. Additionally, we know that while anxiety is prevalent in those with 22q11DS, it is also undertreated (Tang et al., 2013).

In summary, we see the expected pattern of lower IQ, lower adaptive function, and higher anxiety in children with 22q11DS than TD children.

RELIABILITY OF MEASURES

The RT measures showed low reliability. Specifically, although the data in the TD group showed significant correlations between even and odd angry face trials ($r = 0.45$, $p < 0.05$) they did not show any correlation between even and odd happy face trials ($r = -0.17$, $p = 0.40$). The data from the 22q11DS group did not show any correlation in the angry face trials ($r = 0.17$, $p = 0.25$) and in fact showed a negative correlation between even and odd trials for the happy face trials ($r = -0.57$, $p < 0.05$).

By contrast, gaze and pupilometry each showed very high reliability (between odd and even trials). Specifically, both groups showed strong correlations between odd and even trials across all conditions (all $r > 0.98$, all $p < 0.0001$).

In summary, we see the expected pattern of low reliability on RT facilitation and high reliability on gaze and pupilometry during the DPTB experiment.

ANALYSIS 1: RT AND GAZE

We initially built a model including diagnostic group, emotion of faces, age, IQ, and gender to assess RT facilitation. However, age, gender and IQ were not significant predictors of RT bias if included as regressors, and thus were not included in final models. Therefore, between-group Student's two-tailed t-tests were performed to assess the difference in angry bias or happy bias between TD and 22q11DS groups. As shown in Figure 2A, there were no significant between

groups differences for angry or happy RT bias (angry: p=0.32, TD mean ± SD = 4.8msec ± 56.1, 22q11DS mean ± SD = -8.1msec ± 66.4; happy: p=0.24, TD mean ± SD = -19.6msec ± 34.9, 22q11DS mean ± SD = -2.6msec ± 65.3). Direct comparisons of happy bias to angry bias between groups were not significant. In the TD group, RT bias was significantly different from zero for happy trials ($t(27) = -2.75$, $p < 0.05$) but not angry trials ($p>0.05$). Bias was not significantly different from zero in either condition for children with 22q11DS (both $p > 0.05$).

Analyses were performed as mixed effects linear models on angry or happy trials (separately) including a random effect of participant and effects of interest for percent time looking at emotional/neutral faces and diagnosis for each group (22q11DS and TD). Age, gender, and IQ were initially included as factors in the model, but for RT bias gender and IQ were not significant so were dropped from the model. Both groups showed gaze bias toward angry faces on angry vs. neutral face trials (TD $t(18) = -4.15$, $p<0.0001$, angry: mean ± SD = 33.73% ± 12.27; neutral mean ± SD =24.71% ± 8.42; 22q11DS $t(26) = -5.97$, $p<0.0001$, angry: mean ± SD = 34.87% ± 9.54; neutral: mean ± SD = 24.90% ± 7.53) (Figure 2B). Both groups also exhibited a significant bias towards happy faces on happy vs. neutral trials (TD $t(18) = -2.22$ $p=0.01$ happy: mean ± SD = 32.28% ± 12.14 neutral: mean ± SD = 25.83% ± 8.03; 22q11DS $t(26) = -3.07$, $p<0.01$ happy: mean ± SD = 32.92% ± 11.09; neutral: mean ± SD = 24.94% ± 9.39). These results indicate bias toward emotional faces in all children, regardless of group membership (for summary of significant findings, see Table 2).

IQ and gender were not a significant predictors of gaze bias and were not included in final models. Age was a significant predictor in both groups (Figure 3), with older children showing

less bias toward emotional faces than younger children (angry trials: $t(43) = -3.79$ $p < 0.01$; happy trials: $t(43) = -2.81$ $p < 0.01$).

In summary, there were few significant findings from RT facilitation, the only finding was RT facilitation to happy faces in the TD group. When examining overall gaze both groups showed a bias toward emotional faces. Finally, older children showed less bias toward emotional faces than younger children.

ANALYSIS 2: CHANGE IN GAZE OVER TIME

To investigate whether children with 22q11DS or TD children adapted differently to emotional stimuli, we compared gaze patterns on early versus late trials on the DPTB. Models initially included parameters for age, gender, and IQ. No effects were seen regarding age or gender. There was an interaction of diagnosis and IQ, with participants with 22q11DS showing more gaze to happy faces over time with higher IQ, however this effect did not survive correction for multiple comparisons. Because the effect was not robust with multiple comparisons corrections and there was no consistent pattern across tests showing an influence of IQ, IQ was not included in the final model.

An omnibus 2 (early vs. late trials) by 2 (22q11DS vs. TD) test did not find a significant interaction $t(43) = 0.24$, $p > 0.05$ for angry trials (Figure 4). Given the novel nature of this analysis in this population, we performed exploratory pairwise follow-up tests. On angry trials, the TD children showed a significantly higher percentage of gaze to angry faces in the late versus early

trials $t(18) = 3.83$, $p < 0.05$, mean ± SD = +10.2% ± 11.7%). Additionally, though TD children did not increase gaze to neutral faces over time ($p > 0.05$) they looked less at non-face areas and towards faces over time $t(18) = -3.45$, $p<0.05$, mean ± SD = -10.5% ± 19.0%. The children with 22q11DS showed no significant changes in gaze to neutral faces, angry faces, or non-face areas (all $p > 0.05$) over time. There was a trend towards a significant group difference for the non-face change between children with 22q11DS and TD analysis $t(43) = 1.73$, $p = 0.09$, mean ± SD for TD = -10.5% ± 19.0% mean ± SD for 22q11DS = -1.5% ± 2.9%. There were no significant group differences in gaze change to neutral or angry faces (both $p > 0.05$). (For summary of significant findings, see Table 3)

An omnibus 2 (early vs. late trials) by 2 (22q11DS vs. TD) test for happy trials found a significant interaction $t(43) = 2.21$, $p<0.05$, supporting follow-up tests (Figure 4B). On happy trials, TD children increased gaze to neutral faces over time $t(18) = 3.46$ $p<0.05$, mean ± SD = +6.3% ± 7.7%), increased gaze to happy faces over time $t(18) = 2.19$ $p<0.05$ mean ± SD = 7.4% ± 7.8%, and again showed less non-face gaze as time went on $t(18) = -3.45$ $p<0.05$, mean ± SD = -13.7% ± 15.9%) (summary of significant findings in Table 3). Again, children with 22q11DS showed no significant change in gaze to neutral, happy, or gaze to non-face regions with time (all $p < 0.05$). The difference between groups for change in gaze to happy faces over time reached significance $t(43) = 2.21$ $p < 0.05$, mean ± SD for TD = 7.4% ± 7.8% mean ± SD for 22q11DS = -1.6% ± 2.6%. The difference between groups for change in gaze to non-face areas over time also reached significance, $t(43) = -3.22$, $p <0.05$ mean ± SD for TD = -13.7% ± 15.9%, mean ± SD for 22q11DS = 1.2% ± 3.0%. There was no apparent difference between groups for change in neutral gaze ($p > 0.05$).

In summary, while TD children showed more gaze to all types of faces on late trials than early trials, children with 22q11DS showed no significant changes in gaze over time.

ANALYSIS 3: PUPIL DIAMETER

We initially built a model including diagnostic group, time in experiment (early versus late), age, gender and IQ. Age, IQ, and gender were not significant predictors of pupil contraction or dilation and so were removed from the model. The 2 (early versus late) by 2 (22q11DS versus TD) omnibus tests for angry faces and happy faces both failed to reach significance (both $p > 0.05$) (Figure 5). Given the novel nature of this analysis in this population, we performed exploratory pairwise follow-up tests. The only significant finding was greater pupil contraction to happy trials compared to neutral trials in the TD population, $t(18) = -2.14$, $p < 0.05$, for later trials only. There was also a trend for more contraction in late trials than early trials for happy stimuli in the TD children, $t(18) = 1.93$, $p = 0.062$. (All other $p > 0.05$).

DISCUSSION

This study explored attention to threat in a DPTB paradigm performed by children with 22q11DS. We examined the effect of positive and negative emotional stimuli compared to neutral stimuli on attentional control as a function of genetic diagnosis.

Scores on the SCAS and ABAS confirmed that the group of children with 22q11DS was more anxious, and had lower adaptive functioning than their TD peers, as predicted.

The RT facilitation for threat stimuli reported in numerous other studies was not observed in our sample. We concluded that this was likely attributable to the fragility of the RT facilitation measure and the more variable response patterns of our young sample. In fact, previous research has criticized the use of RT bias on the DPTB as an unreliable measure (Schmukle, 2005). This fragility was confirmed in our analyses showing very low consistency in either group's RT bias responses to odd and even trials, consistent with recent work showing the reliability of eye-tracking in this paradigm (Burris, Barry-Anwar, & Rivera, 2017).

In contrast to the RT bias responses, both our gaze and pupilometry measures were consistent across trials within the same experiment. This novel analysis of the consistency and reliability of the different measures deployed in the study strongly increases our confidence in the following interpretations of each group's behavior in response to differently valenced affective stimuli.

Gaze measures showed a bias towards both positive and negative emotional faces in both the TD and 22q11DS groups. This indicates that within our sample, regardless of valence or diagnostic group, participants oriented toward the emotional faces. This may be due to the greater social relevance of emotional, as opposed to neutral, faces. Alternately, emotional faces may be more interesting than neutral faces.

Assessing response to perceived threat averaged over multiple exposures ignores differences in adaptation that might produce divergent patterns (Hare et al., 2005). As such, we also compared responses to early versus late trials. This analysis produced different patterns in our two groups. While TD children had markedly different gaze patterns on early versus late trials, children with

22q11DS showed no significant changes in gaze between initial and later exposures to either positive or negative affective stimuli. We found that TD children gradually attended more to all types of faces over time while children with 22q11DS made no significant changes to gaze with repeated exposures. The gaze pattern in TD children indicates controlled gaze, primarily staying on the center of the screen, early in the experiment. Then with repeated exposures the children who were TD evaluated the faces more regardless of valence. The children with 22q11DS, however, maintained gaze to non-face areas throughout. It could be that children with 22q11DS do not shift in response over time as they do not habituate to the faces enough to want to explore their content, or that they would have needed many more trials to reach the same pattern of exploration as the TD children. Alternately, the lack of shift in attention may be due to lower flexibility in those with a 22q11DS, an effect of the lower executive function in this population (Shapiro et al., 2014).

In addition, we made exploratory use of pupilometry data to provide an autonomic index of arousal. We chose to examine pupil dilation as it is an objective measure of arousal and processing load providing continuous values for analysis. In addition, pupil dilation, unlike the other available measures, is not under conscious control. Here, TD children showed increased pupil contraction to happy faces with repeated exposure, perhaps indicating that the happy faces produced less arousal, or required less effortful processing, as these children became accustomed to the stimuli. In contrast, children with 22q11DS showed no real changes over time, possibly suggesting that their arousal levels did not change or that they exerted the same amount of cognitive effort in scrutinizing and interpreting the facial emotions that were presented. This pattern parallels their static gaze patterns.

Taken together, our findings suggest that TD children increased viewing of positive, negative, and neutral emotion faces over the course of the DPTB experiment. Pupilometry data suggest, in turn, that the increase in processing time was linked to less arousal and cognitive load. One possible interpretation of this pattern is that effective emotional regulation mechanisms enable typically developing children to continually view and evaluate the emotional content of the faces, without accompanying affective reactivity. This may be in contrast to previous work that has found decreasing RT bias to threat with repeated exposures (Staugaard, 2009). Those studies, were different from ours in that the participants were young adults rather than children. Thus, it may be that developmental differences result in different habituation processes (i.e., increasing emotion regulation abilities in older children). In fact, research has shown that while children without anxiety diagnoses can be trained to attend more to threat faces, it is difficult to train them to attend more toward neutral ones (Eldar, Ricon, & Bar-Haim, 2008). On the other hand, our differing results may be due to our use of gaze and pupilometry rather than RT bias. It may be that with repeated exposures and decreasing salience though participants explore the faces more they are less “stuck” on this area of the screen. They are able to disengage when the target asterisk appears and show less RT bias in spite of the increased exploration, and therefore processing, of the emotional faces.

By contrast, the children with 22q11DS, who were as a whole highly anxious, showed no significant changes in viewing patterns or in pupil responses across the task. A possible interpretation of this pattern is that their reduced emotion regulation abilities did not allow them to modulate their response to the faces.

It was somewhat unexpected that we did not see major differences dependent on the valence of the trial type (happy or angry), a finding that has also been shown using event related potentials from EEG (Thai, Taber-Thomas, & Pérez-Edgar, 2016). Recent research has suggested different response patterns for "fearful" versus "distress" anxiety subtypes in pediatric populations (Salum et al., 2013; a M. Waters et al., 2014) it is possible that these subgroups were present within our sample. Fearful children (mainly those with phobias and separation anxiety) display avoidance of mildly threatening stimuli, while distressed children (mainly those with generalized anxiety disorder (GAD), major depressive disorder, and post-traumatic stress disorder) display vigilance toward mildly threatening stimuli. However, the size and variability of our sample of children with 22q11DS, did not produce sufficiently large groups for the different anxiety subtypes to allow us to examine our data in this way. With a much larger sample, we might have observed differences between anxiety-type groups as well as between stimulus type.

These findings contribute to the attention bias literature in several ways. We supported the consensus that RT measures may be too variable to accurately index the noisy patterns of threat bias in certain populations, especially phenotypically variable children with neurodevelopmental disorders like those in our study. We also found that the relationship of attention bias to anxiety is not as simple as those with anxiety show more threat bias. While many studies have shown threat bias in anxious populations and not in non-anxious populations, our results differed because we observed a gaze bias to emotional faces, regardless of valence, in both of our groups. It is possible that different subtypes of anxiety produce different response patterns, or that response patterns change during childhood and that our age group showed a propensity toward a

broad emotional bias. The correlation between age and reduced gaze to emotional faces in the older participants in our sample, supports this assertion. We also supported the importance of examining responses to threat stimuli over the course of an experiment rather than collapsing all trials together. Finally, our exclusion of inaccurate trials and requirement that participants fixate before stimuli are presented allowed us to look at the effect of emotional stimuli beyond just inattention to the task.

Our findings also have important implications for clinical practice with children with 22q11DS and others suffering from anxiety. Interventions addressing attention bias in anxiety have tried to reduce threat bias or increase positivity bias (Hakamata et al., 2010; A. M. Waters, Pittaway, Mogg, Bradley, & Pine, 2013) by training participants to pay increasing attention to positively valenced stimuli. Attention bias modification has shown positive results in anxious children and teenagers without 22q11DS (Sylvester, Petersen, Luby, & Barch, 2016; Waters, Pittaway, Mogg, Bradley, & Pine, 2013). Our findings indicate those with 22q11DS are already avoiding the negative stimuli, and so training them to continue avoiding negative stimuli may not be effective. Indeed, this could have the unintended effect of increasing, rather than reducing, their anxiety. Additionally, we encourage clinicians to discuss and address anxiety concerns patients with 22q11DS are experiencing, and incorporate anxiety treatment into the patient's treatment plan.

Work in fragile X syndrome showed that these children showed RT facilitation to angry, but not happy faces (Burris et al., 2017). It is difficult to directly compare these results to our own because our RT results were noisy and unreliable. However, the results we see from gaze show more gaze to both angry and happy faces in those with 22q11DS, perhaps indicating that while

the angry face bias is common across these two developmentally delayed groups, the bias toward happy is unique to 22q11DS. Future research may wish to directly compare different genetically distinct groups on the same paradigm to produce a more reliable comparison. Exploring patterns in multiple groups with different genetic backgrounds could additionally connect our results to genetics, particularly if the genetic profile of participants were known. It may be interesting, for example, for future studies to correlate these type of results with COMT genotype, which is implicated in 22q11DS and known to affect executive function tasks (Shapiro, Takarae, Harvey, Cabaral, & Simon, 2012; Shapiro, Tassone, Choudhary, & Simon, 2014).

Limitations

First, the lack of an interaction affect in our original models is concerning. Performing separate models for each group means we are not statistically comparing the two groups for group differences, but rather discussing the results of two separate models. However, we note that the sample size needed to detect an interaction effect may be 7-9 times as large as that needed to detect a simple effect (Wahlsten, 1991). As such, in a rare population such as 22q11DS we argue that examining main effects in two models between groups is necessary to performing preliminary research. We hope that similar studies will be conducted in larger samples to confirm our findings.

Second, while we initially attempted to perform correlations between task responses and continuous anxiety scores, we did not find significant results. Upon performing a power analysis, we found to reach an 80% chance of identifying such an effect in a sample of our size, the correlation would have to be at least $r = 0.41$. Since this is a very large effect size for this type of

data, we did not further pursue this route, since we did not feel we had sufficient power to address this question.

Finally, 22q11DS is a complicated disorder, and while we find that the anxiety and adaptive functioning explains a substantial amount of the variance in our outcomes, it is likely that other aspects of this disorder are also important. The common spatiotemporal attention impairments in 22q11DS (Simon, Bearden, Mc-Ginn, & Zackai, 2005) may contribute to performance in the DPTB. Second, social stimuli may have induced children of both groups to attend to the more socially relevant and salient emotional rather than neutral faces. Third, it is possible that angry faces, while mildly threatening, were not threatening enough to elicit a threat bias behavior. It is also possible that we did not have enough trials in our experiment to induce differential patterns of adaptation. Our study is also limited by the relatively small sample size, an unfortunate consequence of working with a special population. These results nevertheless provide valuable information about attention bias as measured by gaze in this population.

CONCLUSIONS

Gaze and pupilometry were more reliable measures in a DPTB paradigm than RT alone. Additionally, when examining performance overtime children with 22q11DS showed less adaptation than those who were TD. This may indicate fewer emotion regulation skills in this population, making emotion regulation a potential treatment target.

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Table 1: participant demographics

	22q11DS	**TD**	**p**
Count	47	32	--
Age (years)	10.83 ± 2.08 (n=47)	11.08 ± 2.4 (n=32)	0.6368
Gender	F=25; M=22	F=13; M=19	0.3854
IQ	73.7±14.4 (n=44)	116.5±14.9(n=28)	**<0.001**
Spence Parent Total	65.4±11.8 (n=43)	47.8±9.6 (n=31)	**<0.001**
Spence Child Total	60.4±16.4 (n=40)	51.5±11.7 (n=28)	**0.011**
ABAS GAC	69.2±16.1 (n=47)	105.4±11.4 (n=28)	**<0.001**

Table 2: summary of significant findings, overall gaze

	Diagnosis	**Mean ± SD**	***t***	***p***
gaze bias to angry faces on angry vs. neutral face trials	TD	angry: 33.73% ± 12.27 neutral: 24.71% ± 8.42	-4.15	<0.0001
gaze bias to angry faces on angry vs. neutral face trials	22q11DS	angry: 34.87% ± 9.54 neutral: 24.90% ± 7.53	-5.97	<0.0001
gaze bias to happy faces on happy vs neutral face trials	TD	happy: 32.28% ± 12.14 neutral: 25.83% ± 8.03	-2.22	<0.01
gaze bias to happy faces on happy vs neutral face trials	22q11DS	happy: 32.92% ± 11.09 neutral: 24.94% ± 9.39	-3.07	<0.01

Table 3: summary of significant findings, change in gaze over time

	Diagnosis	**Mean ± SD**	***t***	***p***
change in angry face gaze, early vs late trials	TD	+10.2% ± 11.7	**3.83**	**<0.05**
change in non-face gaze, early vs late trials with angry faces	TD	-10.5% ± 19.0	**-3.45**	**<0.05**
change in neutral gaze, early vs late trials with happy faces	TD	+6.3% ± 7.7	**3.46**	**<0.05**
change in happy face gaze, early vs late trials with happy faces	TD	7.4% ± 7.8	**2.19**	**<0.05**
change in non-face gaze, early vs late trials with happy faces	TD	-13.7% ± 15.9%	**-3.45**	**<0.05**

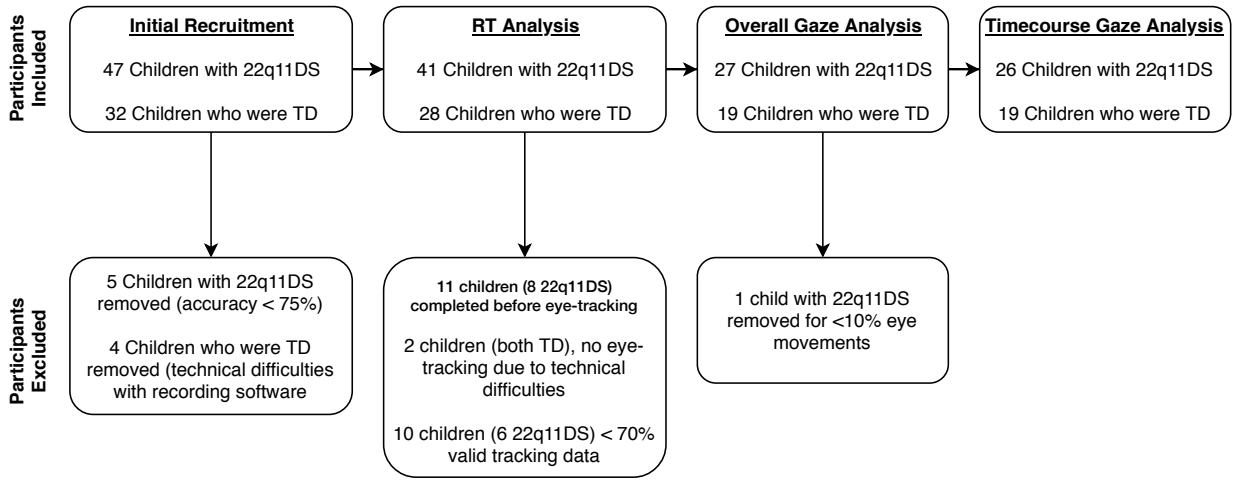

Figure 1: Participants recruited and included or excluded for each stage of analysis.

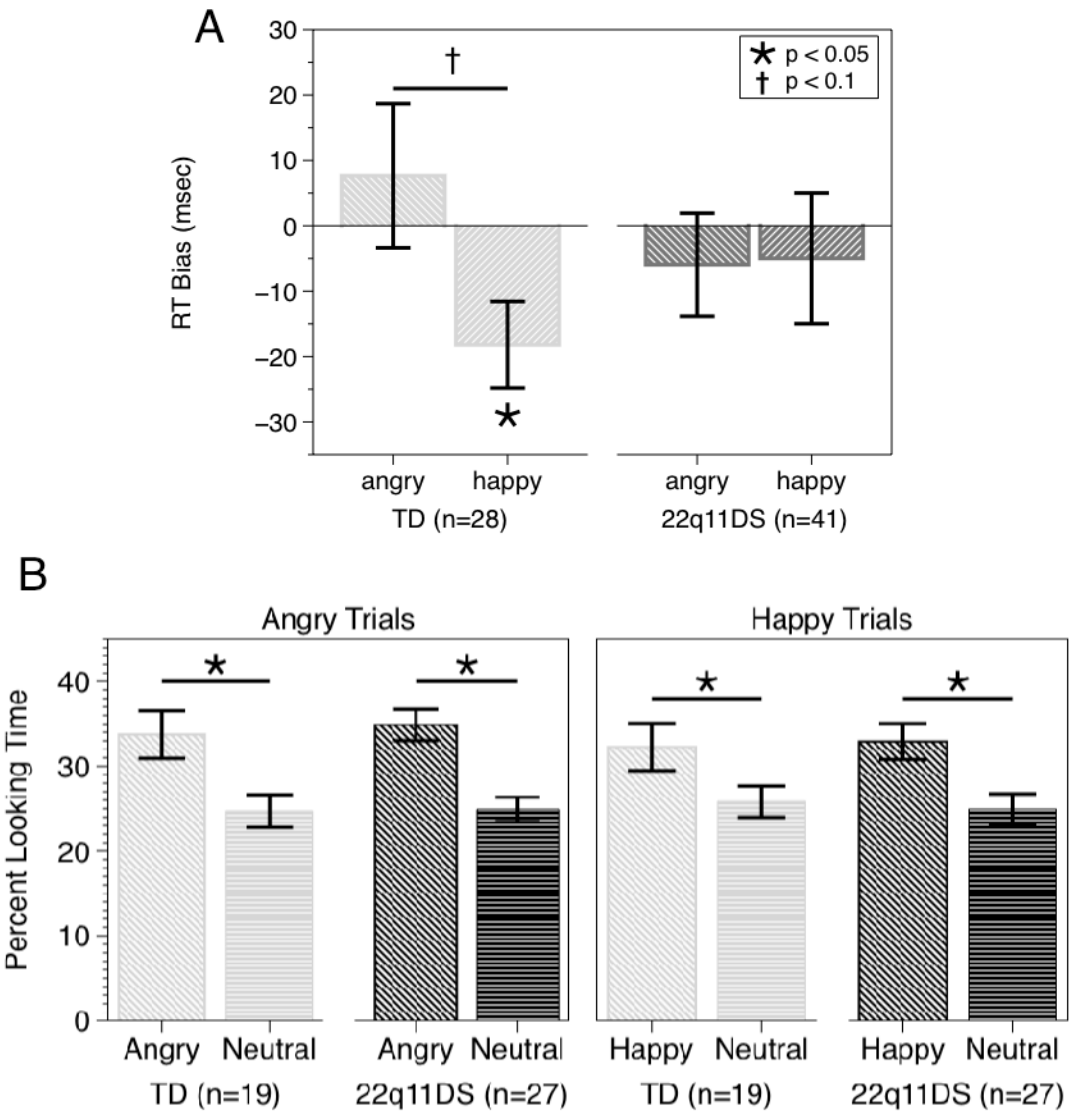

Figure 2: Panel A shows Mean RT bias (incongruent RT – congruent RT) scores by emotion condition and genetic diagnosis. Error bars indicate SEM. Panel B shows percent looking time for each face type by emotion condition and diagnosis. Error bars indicate SEM. * indicates $p<0.05$; † indicates $p=0.05$.

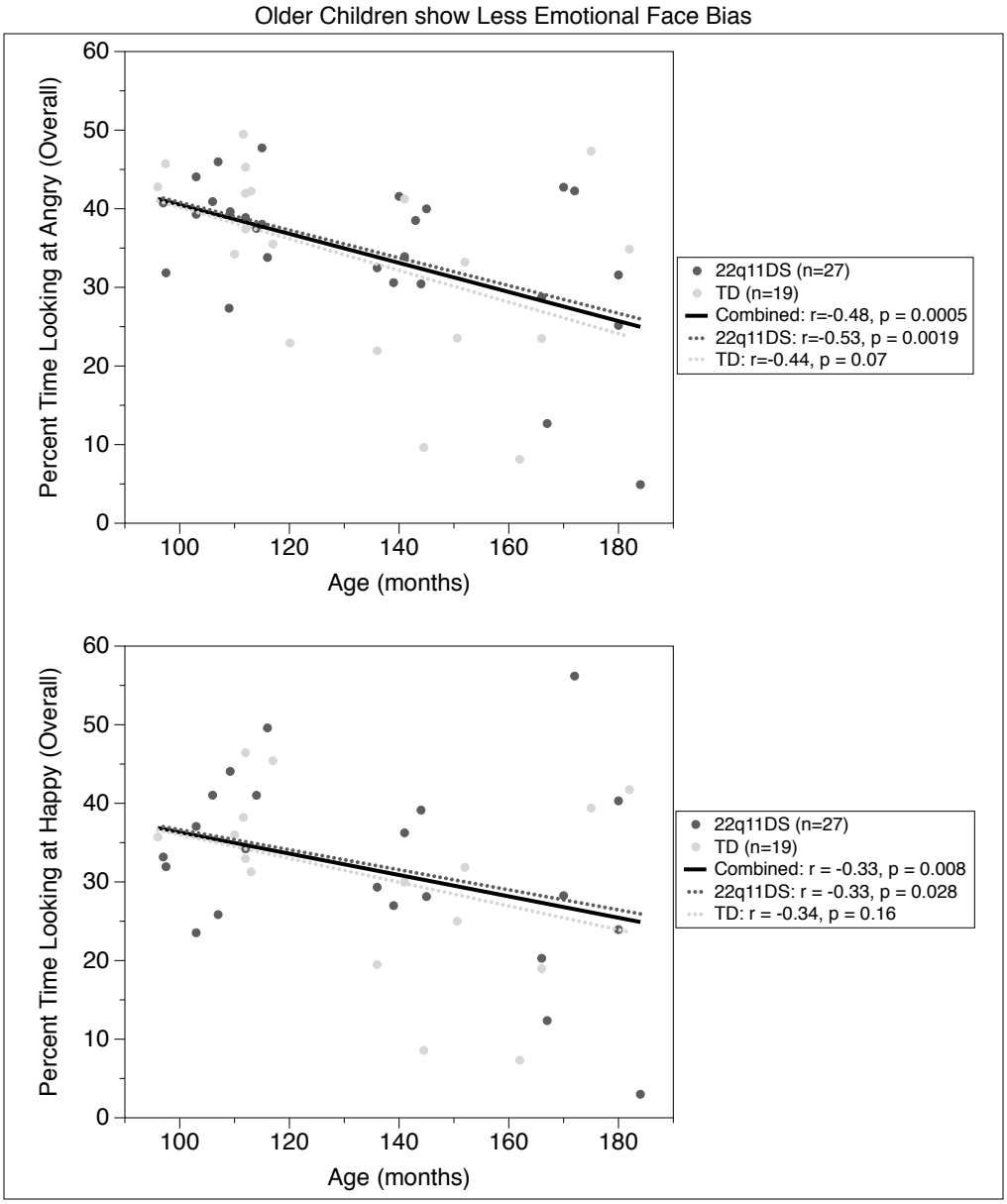

Figure 3: Effect of age in months on overall gaze bias to Angry and Happy.

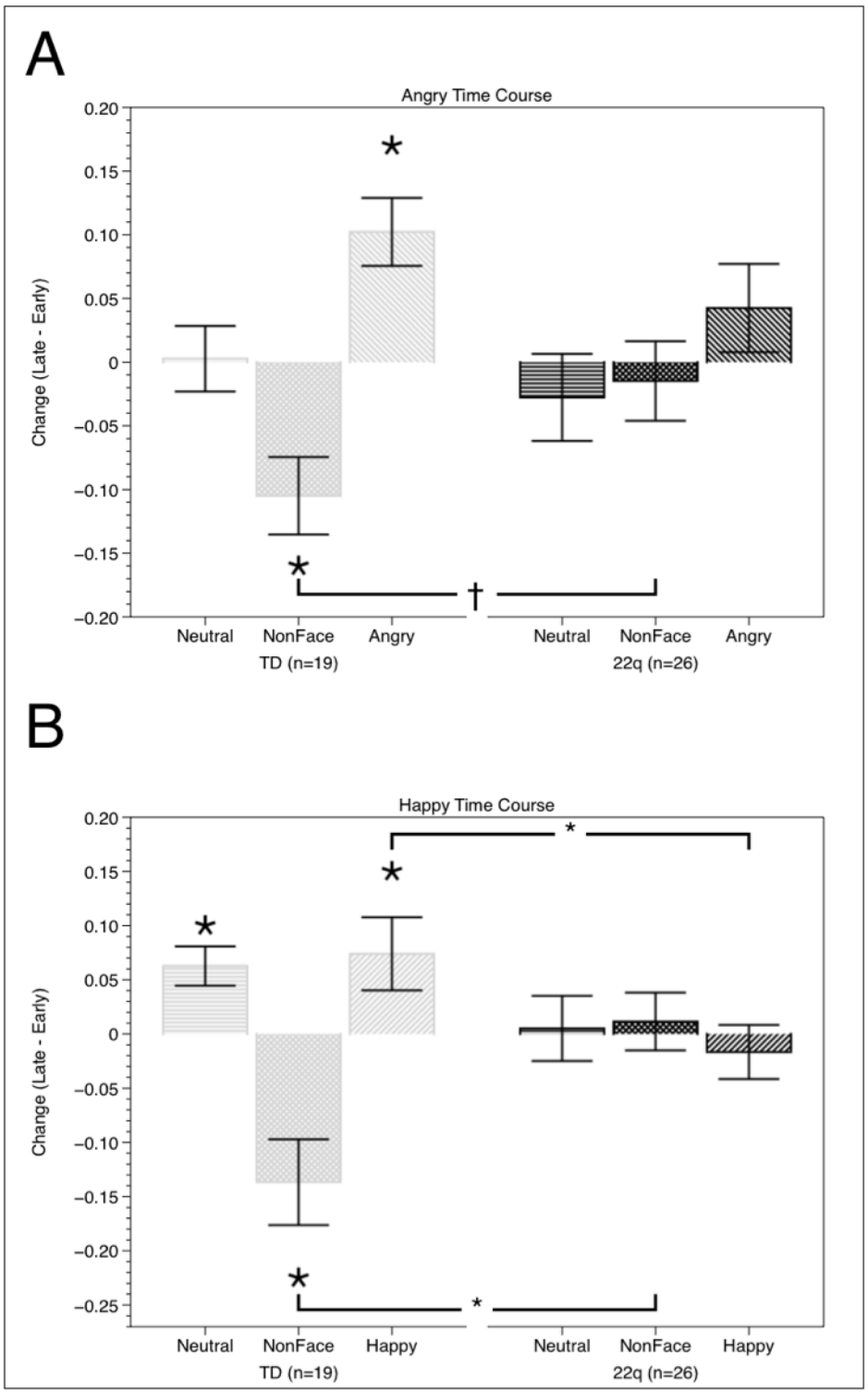

Figure 4: Panel A shows mean change in gaze with standard error bars for angry (* $p < 0.05$, † $p < 0.1$). Panel B shows mean change in gaze with standard error bars for happy (* $p < 0.05$, † $p < 0.1$). Note, bracketed comparisons are between group comparisons of change scores while un-bracketed symbols indicate significant early versus late gaze.

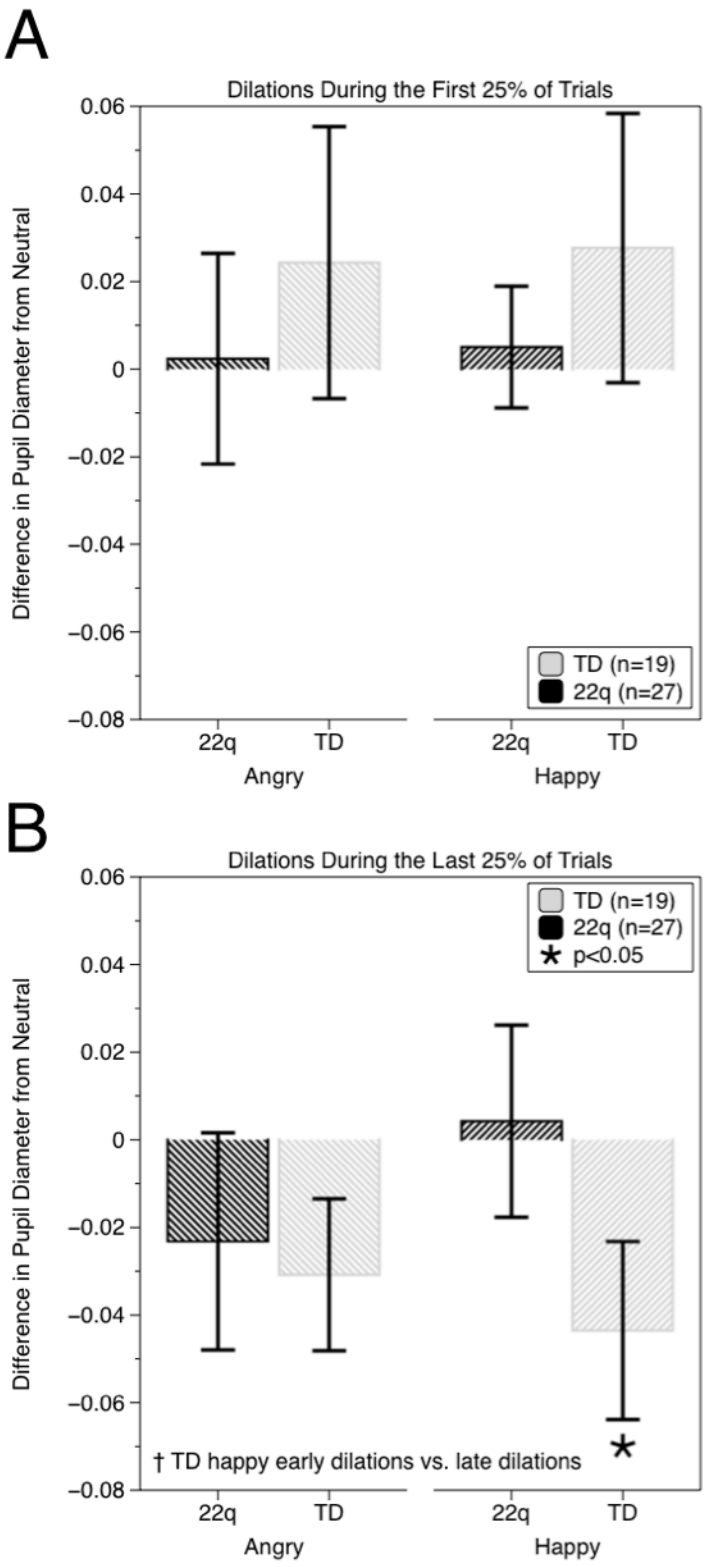

Figure 5: Mean pupil dilation/contraction with standard error bars for early (panel A) and late (panel B) trials by group. (* $p<0.05$, † $p < 0.1$)