Heightened facial muscle reactivity in preadolescent girls with pathological anxiety

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Abstract

Anticipatory anxiety and heightened responses to uncertainty are central features of anxiety disorders (ADs). We examined facial emotional responding in a sample of preadolescent girls with a range of anxiety symptoms: no/low anxiety (controls) to subthreshold anxiety (subthreshold-AD) to DSM-5 diagnoses of separation, social, and/or generalized ADs. Using a threat anticipation paradigm, we assessed how variations in image valence (negative vs. neutral) and image anticipation (uncertain vs. certain timing) impacted activity of the corrugator supercilii, a forehead muscle implicated in the 'frown' response that modulates to emotional stimuli (negative>neutral). Average corrugator magnitude and corrugator time-course were compared between groups. Findings demonstrate greater corrugator activity during anticipation and viewing of negative stimuli, with overall increased corrugator reactivity in subthreshold-AD and AD girls. Time-course analyses revealed anxiety-related sustained corrugator activity during uncertain anticipation of negative images. Results extend the physiological characterization of childhood pathological anxiety, highlighting the impact of subthreshold-AD symptoms.

Highlights

- Emotion-related facial muscle activity elevated in girls with pathological anxiety
- Highlights differences between girls with subthreshold anxiety and healthy controls
- Corrugator muscle activity modulates during both image anticipation and viewing

Introduction

Anxiety disorders (ADs) commonly emerge during childhood, and it is estimated that up to 20% of youth suffer from ADs (Costello et al., 2005). In general, ADs are characterized by persistent and excessive worries, leading to physiological and cognitive symptoms that result in functional impairments in social, academic, and professional domains (Connolly et al., 2007). Childhood anxiety symptoms have also been linked with increased risk for the later development of psychopathology, including ADs, depression, and comorbid substance abuse (Kendall et al., 2010; Patel et al., 2007). Young girls are at particular risk because with the transition to adolescence there is an increase in the prevalence of anxiety and depressive disorders which is two-fold greater in girls compared to boys (Angold et al., 1998; Wade et al., 2002; Wesselhoeft et al., 2015). While a growing number of studies have focused on characterizing the pathophysiological features of children and adolescents with ADs, fewer studies have examined the numerous children with subthreshold anxiety symptoms that are associated with negative impacts to their daily living but do not reach the level of a DSM-5 diagnosis. Due to the dimensional nature of anxiety, there has been a growing need to examine the wide range of anxiety symptoms as this approach may provide insights into factors that contribute to the spectrum of distress, disability, and risk for later life psychopathology experienced by children with pathological anxiety.

Clinical data demonstrate that adults and children with ADs are particularly sensitive to conditions of uncertainty, exhibiting distress and difficulty functioning in ambiguous situations (Comer et al., 2009; McEvoy & Mahoney, 2012) Functional brain imaging (Grupe & Nitschke, 2013; Sarinopoulos et al., 2010; Williams et al., 2014) and physiological (Grillon, 2008; Grillon et al., 2004; Nelson & Shankman, 2011) data confirm increased reactivity to anticipation and uncertainty in relation to a potentially negative event in AD individuals. Despite the early onset and high prevalence of ADs during childhood, relatively few studies have investigated pathophysiological responses to uncertainty and prolonged anticipation in children with ADs. Therefore, we sought to understand behavioral and physiological responses to uncertain anticipation in young girls (age 9-11) with ADs, as well as girls with persistent subthreshold-AD symptoms. We extended the scope of our study to include girls with persistent anxiety symptoms that did not meet DSM-5 AD criteria to better characterize potential physiological alterations in this group of youth that are symptomatic and at increased risk for the development of later psychopathology (Clauss & Blackford, 2012).

Studying the physiological correlates of emotion processing is highly relevant for studies of anxiety as ADs are commonly associated with physiological symptoms (e.g., increased muscular tension, head, or muscle aches), and laboratory studies have demonstrated increased and/or sustained physiological responding in AD individuals measured by skin conductance, heart rate, and eyeblink startle response (Campbell et al., 2013; Eckman & Shean, 1997; Lader & Wing, 1964). In the current study we used facial electromyography (EMG) to quantify responses of the corrugator supercilii muscle during the anticipation period preceding the presentation of negative pictures, as well as the response to negative images themselves, in girls with a range of pathological anxiety. We also examined corrugator responses over time to the repeated presentations of these stimuli to characterize prolonged exposure to the anticipation and viewing of negative images.

Facial EMG responses related to emotion processing in ADs are an interesting target because subtle variations in facial expressions are a crucial component of emotion expression and emotion recognition. Indeed, historical studies of facial expressions were a rudimentary examination of facial muscle activity, with Charles Darwin being one of the first to highlight how facial muscle activity evolved as an adaptive process supporting the nonverbal expression and communication of underlying emotional states (Darwin, 1872). Understanding corrugator supercilii activity in relation to anxiety is particularly relevant as it is fundamentally involved in the frown response and has been shown to exhibit increased activity during the viewing of negative images (Cacioppo et al., 1986; Heller et al., 2011, 2014; Lang et al., 1993; Larsen et al., 2003).

By examining the corrugator response during the anticipation and viewing of negative images, as well as sustained responses to the repeated exposure to these stimuli under conditions of uncertainty, we sought to characterize anxiety-related differences in facial muscular responses. Our specific interest was to not only characterize responses in girls with separation, social and generalized ADs, but to also characterize physiological reactivity across the range of pathological anxiety, including girls with significant anxiety symptoms that did not meet DSM-5 AD criteria (subthreshold-AD). We hypothesized that girls with

pathological anxiety would have increased corrugator activity evident in their responses to negatively valenced stimuli, and further enhanced by uncertain conditions. More specifically, we expected that subthreshold-AD girls would display responses that were intermediate between AD girls and control girls. Because anxiety is characterized by prolonged worry and physiological activation, we also predicted that these effects would be apparent in girls with pathological anxiety when examining sustained responses over time.

Materials And Methods

Participants

Data were collected as part of a longitudinal study examining risk factors for AD development in preadolescent girls that includes repeated assessments of clinical, multimodal brain imaging, behavioral, and physiological measures over a 3-year period. Study data was managed using REDCap (Research Electronic Data Capture) tools hosted at the University of Wisconsin-Madison, School of Medicine and Public Health, a secure, web-based application designed to support data capture for research studies (Harris et al., 2009, 2019). Here we present EMG data collected during the first year of study participation. Preadolescent girls, ages 9 to 11 years old, were recruited from the Madison community. Study procedures were approved by the University of Wisconsin–Madison Institutional Review Board (IRB), and informed consent from parent and assent from children were acquired. Prior to enrollment, potential participants and their parent or guardian completed an online screening form that included the parent and child Screen for Child Anxiety Related Disorders (SCARED) scale (B Birmaher et al., 1999) and MRI eligibility questions. Girls who met eligibility criteria for an MRI scan with either 1) a parent or child SCARED >15 (potential pathological anxiety participant) or 2) both parent and child SCARED $\leq$ 10 (potential control participant) were eligible for an initial study visit which included the Kiddie Schedule for Affective Disorders and Schizophrenia (KSADS) semi-structured interview (Boris Birmaher et al., 2009). KSADS were administered by a trained study team member and all cases were reviewed and approved by a senior psychiatrist (Dr. Ned Kalin or collaborator Dr. Daniel Pine, Chief of the Section on Development and Affective Neuroscience, National Institute of Mental Health). Based on the KSADS and the Clinical Global Impression (CGI) scale (Busner & Targum, 2007), girls were categorized into one of three diagnostic groups using the following criteria: Control – no past or current KSADS diagnoses, CGI =1; subthreshold-AD – no past or current KSADS diagnoses other than specific phobia (n=4), CGI = 2 or 3; and AD - current KSADS diagnosis of either separation, social, and/or generalized ADs, CGI 4+. Children with ADHD were included in the AD group if anxiety was the primary source of dysfunction/distress. Participants with symptoms or diagnoses of depression, post-traumatic stress disorder (PTSD), obsessive compulsive disorder (OCD), psychotic disorders or autism spectrum disorders were excluded. All subjects were treatment naïve and not treatment seeking. Corrugator EMG data was collected from 47 Control girls, 83 subthreshold-AD girls, and 48 AD girls. Data from 3 AD, 10 subthreshold-AD, and 6 control subjects were not included in the final analysis due to technical issues associated with lack of adequate EMG signal quality resulting from sensor application issues, incomplete session data, and/or excessive

movement artifacts. The final analysis sample included 41 Control girls, 73 subthreshold-AD girls, and 45 AD girls. Groups did not significantly differ on age, scores on clinical measures followed the expected pattern of AD > subthreshold-AD > Control (Table 1).

Task Structure

To elicit prolonged states of uncertainty and threat preparedness, we utilized an emotional picture anticipation and reactivity paradigm. The task was structured using a mixed block-event-related task design to simultaneously measure states of acute threat and prolonged anticipatory threat (Somerville et al., 2013). Participants completed the task twice, once during an MRI scan and once outside the scanner during which psychophysiological data were collected. Corrugator EMG data from the physiology session are presented here.

As outlined in Figure 1, each task block presented fifteen 3-second images from the International Affective Picture System (IAPS) (Lang et al., 1998) that were either negative or neutral in valence. Picture valence was held constant within a 2-minute block period. Different sets of pictures were used between the MRI and psychophysiology sessions to ensure stimulus novelty between each task presentation. At the start of each block, subjects viewed a 10-second fixation cross, which was used as a baseline period for EMG analyses. A text screen indicated which condition would be presented during the subsequent two-minute block. Prior to each picture, an anticipation clock period, pre-randomized to be between 2 to 7 seconds long, was presented to vary the time before picture onset. Clock images were presented either sequentially in a “countdown” order or in a “random” order to vary the predictability of picture onset. The countdown order provides certainty in relation to the timing of the onset of each picture, and the random order induces uncertainty as to the timing of the picture presentation. Task blocks varied by picture valence and picture predictability to produce 4 different block conditions (Uncertain-Negative, Certain-Negative, Uncertain-Neutral, Certain-Neutral), each of which were presented twice over the course of the data collection session (8 blocks total). To ensure task compliance and attention throughout the duration of the task, subjects were also asked to respond via keyboard presses as to whether the pictures showed an object or scene that was either “indoors” or “outdoors.” The order of presentation of task blocks was counterbalanced across subjects. Participant ratings on picture likeability and nervousness during each block type were also collected at the end of the session to measure how each subject perceived the intensity of the emotional stimuli and examine task-evoked anxiety across conditions (see supplement for more details).

Electromyography (EMG) Data Collection, Processing, and Analysis

Electromyography (EMG) data from the corrugator supercilii was recorded using two 4mm electrodes placed over the left or right brow region, counterbalanced across participants, in accordance with guidelines provided by Fridlund and colleagues (Fridlund & Cacioppo, 1986). Raw EMG signals were filtered and cleaned for noise artifacts and processed via a Fast Fourier transform (FFT) to derive

estimates of spectral power density ($\mu V^2/Hz$) every 500 milliseconds. These data values were log-transformed and baseline corrected within each 2-minute block by subtracting the mean activity during the 10-second pre-block fixation epoch. To separately examine the magnitude of corrugator response to pictures and anticipation clocks, data from each of the 3-second picture exposures (45 seconds per block, 90 seconds per condition) and the anticipation clock periods (75 seconds per block, 150 seconds per condition) were separated and then averaged within condition. Analysis of the corrugator EMG response over time analyzed the averaged time-series of the artifact-free log-transformed power values across each condition presentation. Full details outlining equipment, signal processing, data reduction steps, and data exclusion criteria can be found in the supplement.

Statistical Analyses

Separate linear mixed effects models were used to test the effects of certainty, valence, group, and their interactions for: corrugator magnitude during picture exposure periods, corrugator magnitude during the anticipation clock periods, baseline periods, and task-evoked anxiety self-report rating measures. For the picture and anticipation clock magnitude analyses, data from the two presentations of each experimental condition were averaged. Models included 3 fixed effects: anticipation clock certainty (uncertain timing vs. certain timing), picture valence (neutral vs. negative), and group (AD vs. subthreshold-AD vs. Control). The model included a by-subject random intercept and random slopes for the certainty and valence parameters to account for repeated measurements across the four conditions.

To examine the time course of corrugator response across task blocks, a locally estimated scatterplot smoothing (LOESS) function was applied to each subject's 2-minute block responses using their log-transformed, baseline-corrected, power values (Figure 2A). The alpha parameter for smoothing was set at a default span value of 0.7 to set a scale of simplification desired for visual inspection of time course differences. Smoothed data from each subject was averaged by condition within subject (Figure 2B), and then averaged within group and condition (Figure 2C). Because of our interest in the timecourse of the threat response in pathological anxiety, a linear mixed effects model that incorporated a linear spline framework was used to test the effects of group, certainty, and time on corrugator activity using separate models for the neutral and negative conditions. To explore potential habituation of the corrugator response throughout the block period, the time parameter of our linear spline analysis included a single knot point placed at the midway point (60-second) of the 2-minute response window. This approach simultaneously provides separate but connected linear slope parameters for the first and second minute of the response. The model also included fixed effects for anticipation clock certainty (certain vs. uncertain) and group (AD vs. subthreshold-AD vs. Control). And finally, the model included a by-subject random intercept and random slopes for the certainty to account for repeated parameter measurements between the certain and uncertain conditions.

Analyses were run in R (Team, 2020) using the lme4 (Bates et al., 2015), lmSupport (Curtin, 2018), lspline (Bojanowski, 2017), and HLMdiag (Loy & Hofmann, 2014) packages to run the mixed models, apply the linear spline transformations, and test for potential influential data. To test for influential data points,

Cook's distance was calculated using measures of internal scaling (three times the interquartile range) as cutoff criterion and models were run with and without influential subjects to evaluate sensitivity of our findings to individual subjects (Cook, 1977; Loy & Hofmann, 2014). Full model results are reported here; results of analyses that exclude influential data points are largely similar and are detailed in the supplement (Tables S1-S12). Differences in task-evoked anxiety, picture likeability, corrugator magnitude, and corrugator response trajectories were estimated as a function of fixed effects with t-tests used to measure significance. P-values less than 0.05 were considered significant. Certainty, valence, and time parameters were numerically coded, scaled from 0 to 1, and were centered to interpret the main effects across the sample. Between group effects were calculated through dummy coding and reference group (AD vs. Control, AD vs. subthreshold-AD, subthreshold-AD vs. Control) model interpretations to provide contrast estimates for each group effect and their interactions.

Results

Self-Report Ratings of Nervousness and Likeability of Task Stimuli

Analyses of self-reported ratings of nervousness during each task condition revealed a main effect of valence across the sample, such that subjects rated feeling more nervous during blocks that contained negative pictures relative to blocks that contained neutral pictures (Est.=1.4, SE= 0.14, t=10.6, p<0.01). There also was an overall main effect of certainty such that all subjects rated themselves as more nervous during uncertain relative to certain blocks (Est.=0.6, SE=0.10, t=5.9, p<0.01). Across all conditions, AD girls reported higher levels of nervousness relative to control girls (AD vs. control: Est.=0.42, SE=0.19, t=2.1, p<0.05). Additionally, there was a significant interaction between the valence by AD vs. control contrast such that AD girls rated negative, relative to neutral, conditions as more anxiety-provoking compared to control girls (Est.=1.0, SE=0.37, t=2.7, p<0.01). Ratings for subthreshold-AD girls did not significantly differ from either AD or control girls (subthreshold-AD vs. AD: Est.=-0.04, SE=0.03, t=-1.3, p=0.20; subthreshold-AD vs. control: Est.=0.06, SE=0.04, t=1.6, p=0.11). As expected, overall participants rated negative pictures as less likeable than neutral pictures (Est.=-1.3, SE=0.07, t=-17.6, p<0.01). No differences in picture likeability ratings between groups were found.

Average Corrugator Magnitude During Anticipation Clock Periods

Corrugator responses during baseline fixation periods did not vary by task condition or anxiety group (see supplementary Table S5). During the anticipation clock periods prior to the picture presentation, we found a main effect of valence such that corrugator activity during the anticipation of negative pictures was significantly greater than activity during the anticipation of neutral pictures (Est.=0.06, SE=0.01, t=4.5, p<0.01) (Figure 3). We also found that girls with pathological anxiety exhibited significantly higher corrugator magnitude across all conditions relative to controls (AD vs. control: Est.=0.10, SE=0.04, t=2.6,

p<0.05; subthreshold-AD vs. control: Est=0.07, SE=0.03, t=2.0, p<0.05) while overall corrugator magnitude between AD and subthreshold-AD girls did not significantly differ (Est.=-0.03, SE=0.03, t=-0.9, p=0.36) (Figure 1C).

Average Corrugator Magnitude During Picture Exposure Periods

During the picture exposure periods there was a main effect of valence, such that participants demonstrated greater corrugator magnitude in response to negative relative to neutral pictures (Est.=0.06, SE=0.01, t=4.5, p<0.01) (Figure 3A). Across all pictures, corrugator magnitude in AD girls was significantly greater than control girls (AD vs. control: Est.=0.10, SE=0.04, t=2.6, p<0.05) but did not differ from subthreshold-AD girls (subthreshold-AD vs. AD: Est.=-0.04, SE=0.03, t=-1.3, p=0.20) (Figure 3B). Additionally, corrugator activity in subthreshold-AD girls did not significantly differ from control girls (subthreshold-AD vs. control: Est.=0.06, SE=0.04, t=1.6, p=0.11).

Corrugator Responses Over Time During Negative Conditions

The previous analyses examined the average corrugator activity during the picture and clock anticipation periods. A unique characteristic of this paradigm was the opportunity to further examine the timecourse and pattern of corrugator responses as they evolved over time. Because of the relevance of threat anticipation and processing to pathological anxiety, we focused the timecourse analyses on the negative conditions, and tested whether the trajectory of corrugator responses across the entire 2-minute block period differed between AD, subthreshold-AD, and control girls. To explore potential habituation effects of the corrugator response throughout the block period, we employed a spline model which provided separate slope estimates for the first and second minutes of each negative task block, as well as the corrugator magnitude across the entire block.

Response trajectories in the first minute of the block showed corrugator activity to be increasing in both the certain and uncertain negative conditions (Est.=0.16, SE=0.01, t=26.7, p<0.001) (Figure 4). The rate of increase of the corrugator response trajectory across uncertain-negative and certain-negative conditions was greater for AD compared to control and subthreshold-AD girls (AD vs. control: Est.=0.13, SE=0.02, t=7.6, p<0.001; AD vs. subthreshold-AD: Est.=0.19, SE=0.04, t=13.2, p<0.001). Further details on linear spline model outputs for the first minute trajectory results can be found in supplementary Table S11. Results from the second half of the block demonstrated anxiety-related differences in corrugator response to uncertain negative blocks relative to certain negative blocks. Specifically, girls with ADs showed more sustained corrugator responses during the uncertain, relative to certain, condition compared to subthreshold-AD and control girls (AD vs. control: Est.=0.17, SE=0.03, t=5.1, p<0.001; AD vs. Subthreshold-AD: Est.=0.1, SE=0.03, t=3.0, p<0.05) (Figure 4). Subthreshold-AD girls also showed more sustained responses in the uncertain vs. certain conditions compared to control girls (Est.=0.08, SE=0.03, t=2.8, p<0.05).

Discussion

In the current study we sought to understand potential alterations in physiological reactivity in girls across the spectrum of pathological anxiety to characterize their short- and long-term responses to negative stimuli and uncertainty. When examining corrugator activity during the anticipation clock and picture periods we found a significant main effect of valence, with greater corrugator activity during negative, relative to neutral, conditions. These findings are consistent with significant main effects of valence found in both the post-task condition nervousness and picture likeability ratings. Although subjects rated feeling more nervous during uncertain relative to certain conditions, contrary to our expectations, we did not find a main effect of uncertainty on corrugator activity. While we hypothesized that girls with pathological anxiety would show specific heightened reactivity to negative and uncertain conditions compared to control girls, results demonstrated that girls with pathological anxiety had increased corrugator activity across all task conditions, not just during negative and uncertain conditions. With respect to subjective experience during the task, AD girls rated feeling more nervous overall compared to control girls and had a larger valence effect (negative>neutral) relative to controls. While nervousness ratings in subthreshold-AD girls did not differ between AD and control girls, our physiological findings demonstrate that even subthreshold symptoms are associated with significant increases in physiological responding, highlighting a dimensional feature of anxiety-related physiology separate from subjective experience. Importantly, corrugator activity during baseline periods did not significantly differ between groups. To our knowledge, no studies have reported differences in corrugator responses in adults or children with ADs. However, one study in healthy adults has linked higher levels of state anxiety to increased negative image induced corrugator activity (Smith et al., 2005). Previous studies using other physiological measures (heart rate, skin conductance, eye-blink response) that have primarily examined responses to negatively valanced stimuli have demonstrated increased physiological reactivity in individuals with ADs (McTeague et al., 2011; McTeague & Lang, 2012). The current finding of overall anxiety-related increases in corrugator responses regardless of valence is consistent with recent studies of anxiety and fear conditioning, which have demonstrated that individuals with ADs exhibit increased physiological responses to neutral cues as well as those associated with threat (Abend et al., 2019, 2021).

When examining the time course of the corrugator response across block conditions that contained repeated exposures to negative pictures, we found that girls with ADs had sustained elevated corrugator activity during the uncertain-negative, relative to certain-negative, condition compared to controls. Subthreshold-AD girls demonstrated intermediate effects that were significantly different from both AD and control girls (AD > subthreshold-AD > control). This physiological finding demonstrating differences between subthreshold-AD and control girls further supports extending the concept of pathological anxiety to subthreshold-AD girls. Previous work in adults and children with anxiety have reported heightened intolerance of uncertainty (Grillon et al., 2004, 2008, 2017; Grupe & Nitschke, 2013; Schmitz & Grillon, 2012). The findings from our timecourse analysis demonstrating uncertainty-related modulation of corrugator activity in girls with pathological anxiety provide a potential physiological correlate of this cognitive predisposition.

While the study of facial muscle activity has an extensive history in the study of emotional expression, it holds promising relevance for more contemporary and therapeutically oriented approaches to understanding pathophysiology. For example, similar to our findings in the discrete corrugator magnitude analysis, a study on adults with depression found that when participants were asked to imagine sad and negative situations, corrugator activity was greater in depressed relative to non-depressed individuals (Schwartz et al., 1976). Based on the idea that facial muscle activity may influence the somatic perception of emotional states (Darwin, 1872; James, 1890), facial muscle activity has been an interesting clinical intervention target for the treatment of depression in recent years (Finzi & Rosenthal, 2016; Hennenlotter et al., 2009). Specifically, clinical trials that have used botulinum toxin A injections to immobilize facial muscles associated with sad and melancholic mood-like facial expressions, such as the corrugator supercilii, frontalis, and procerus muscles, have been shown to reduce depression symptoms in individuals with major depression (Finzi & Rosenthal, 2014; Magid et al., 2014; Wollmer et al., 2012). While the clinical significance and efficacy of this depression treatment intervention is still in its early stages, these promising advancements may provide a framework in which the current findings could be relevant for not only identifying a more clinically robust marker for pathological anxiety but also for the potential consideration of the corrugator supercilii as a similar intervention target for pathological anxiety.

In conclusion, our study highlights several new significant contributions for understanding the physiological correlates of threat and uncertainty processing in pathological anxiety. From a methodological standpoint, we demonstrate that noninvasive facial EMG recordings of the corrugator supercilii muscle are well-suited to measure valence-modulated changes in both the anticipation of and reactivity to negative images, as well as uncertainty-modulated changes of the timecourse of sustained response patterns. With respect to anxiety, we show that emotion-related facial muscle activity is increased in girls with subthreshold-AD symptoms and in girls with ADs. These findings suggest the consideration of evaluating girls with subthreshold anxiety symptoms for potential treatment. Additionally, we note that previous studies may have considered girls with subthreshold anxiety as controls, which could influence the interpretation of results. Future studies focused on the early onset and pathophysiology of childhood ADs would benefit from understanding the emergence of heightened and sustained physiological responding. In summary, the current findings extend the physiological characterization of childhood pathological anxiety, bring into focus the potential importance of the corrugator supercilii muscle, and highlight the utility of complementary analytic approaches assessing discrete physiological responses as well as their temporal dynamics.

Declarations

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Table 1

Table 1
Sample Characteristics

	Anxiety Disorder	Subthreshold Anxiety Disorder	Control	Statistic
Count	(n=45)	(n=73)	(n=41)	-
Age, years, mean ± SD	10.55 (0.77)	10.43 (0.84)	10.4 (0.85)	t=0.86 p=0.393
Race, n (%)				
White	37 (82.22)	61 (83.56)	35 (85.37)	-
Black or African American	1 (2.22)	2 (2.74)	1 (2.44)	-
Asian	-	-	1 (2.44)	-
Native American or Alaskan Native	-	2 (2.74)	-	-
Native Hawaiian or Other Pacific Island	-	-	-	-
More than one	7 (15.56)	7 (9.59)	3 (7.32)	-
Unknown	-	1 (1.37)	-	-
Ethnicity, n (%)				
Hispanic or Latino	1 (2.22)	4 (5.48)	3 (7.32)	-
CGI-Severity, mean ± SD	4.18 (0.49)	2.44 (0.5)	1 (0)	t=34.07 p<0.001
SCARED, mean ± SD				
Parent Report	31.18 (10.86)	19.48 (9.32)	2.9 (2.76)	t=14.90 p<0.001
Child Report	33.91 (13.64)	23.96 (9.94)	6.97 (6.04)	t=11.59 p<0.001
Diagnosis, n (%)				
Generalized anxiety disorder	18 (40)	-	-	-
Separation anxiety disorder	23 (51.11)	-	-	-
Social anxiety disorder	21 (46.67)	-	-	-

Abbreviations:
SCARED = Screen for Child Anxiety Related Disorders
CGI-Severity = Clinical Global Impressions Scale Severity

Figures

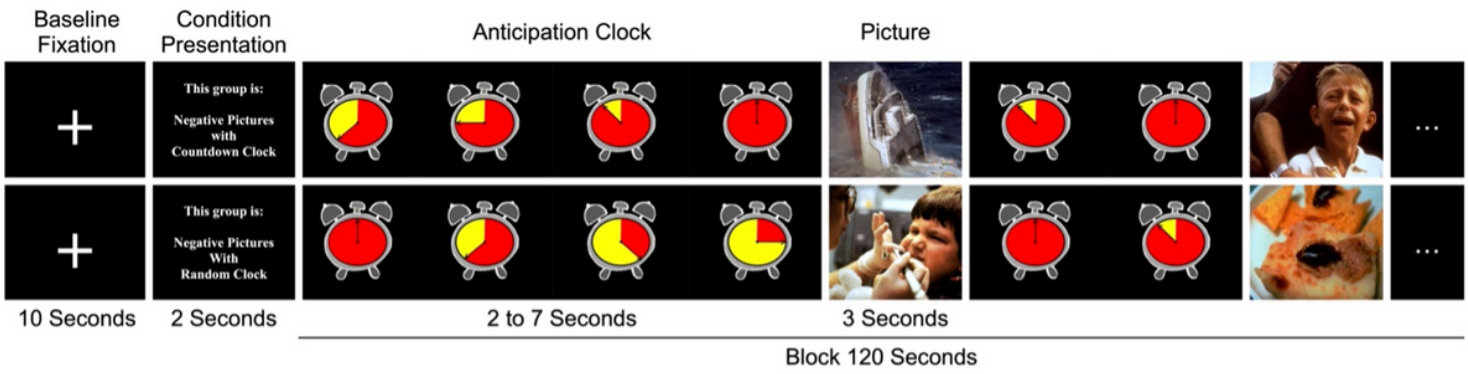

Figure 1

Experimental paradigm. A series of negative or neutral pictures were presented in a mixed-event block-related design where each 3 second picture presentation is preceded by a series of clocks images (2-7) that either “countdown” to picture presentation (certain timing, top row) or are presented in a random order (uncertain timing, bottom row). A 2 (negative, neutral) x 2 (certain timing, uncertain timing) task design yields 4 distinct block types

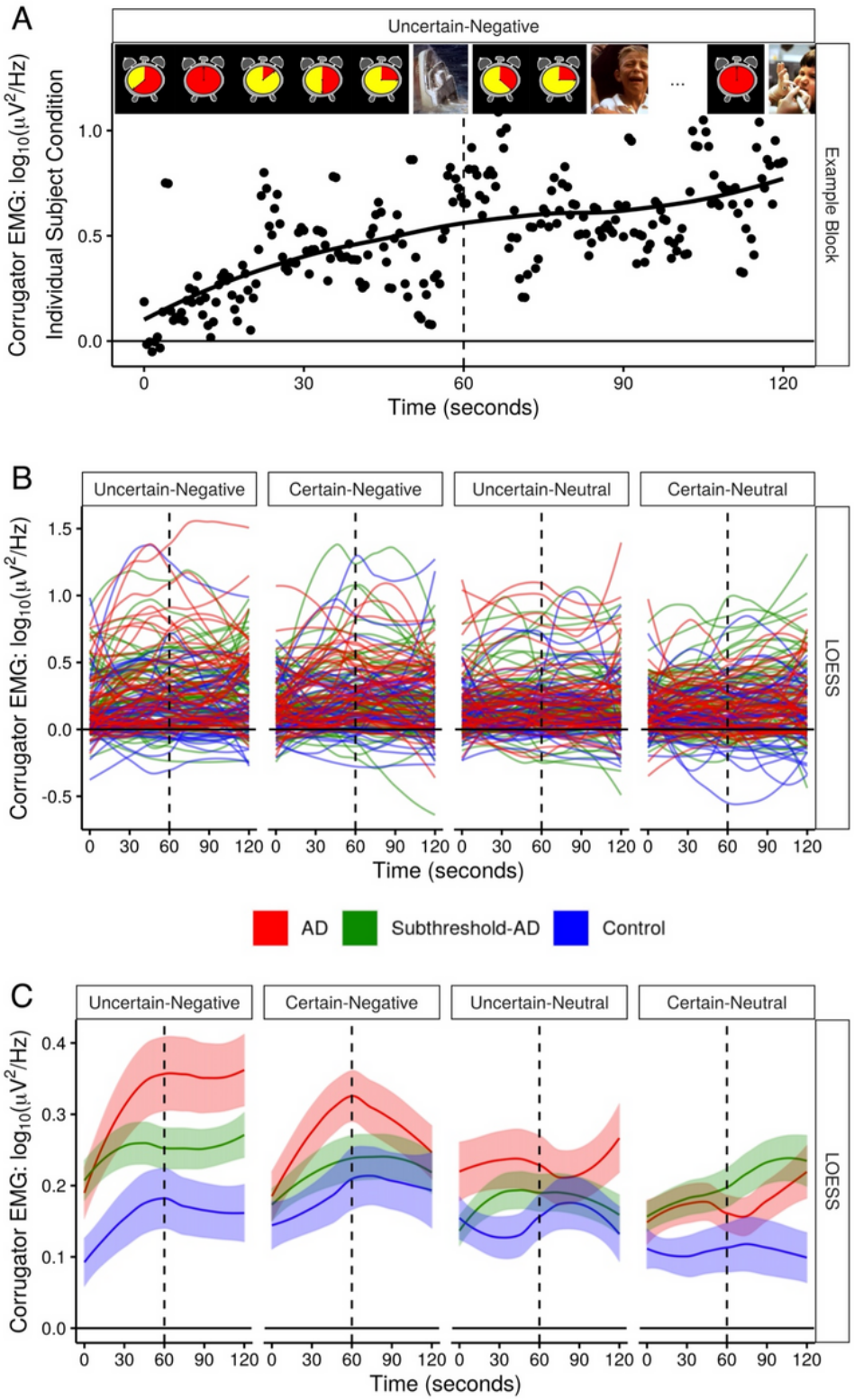

Figure 2

Time course of corrugator response across the 2-minute block by task condition and diagnosis. A) Example of an individual subject's processed electromyography (EMG) trace over the time course of a 2-minute block response window. Points represent log-transformed and baseline-corrected EMG magnitude values with an overlay of a smoothing trace calculated by a locally estimated scatterplot smoothing (LOESS) function with a span value of 0.7 for visualization purposes. B) Individual subjects' smoothed

(LOESS) corrugator response time course, colored by group and sorted by condition. C) Within-group mean smoothed (LOESS) corrugator response time course by condition. Colors red, green, and blue represent Anxiety Disorder, Subthreshold-AD, and Control groups respectively. Shaded regions represent mean +/- standard error.

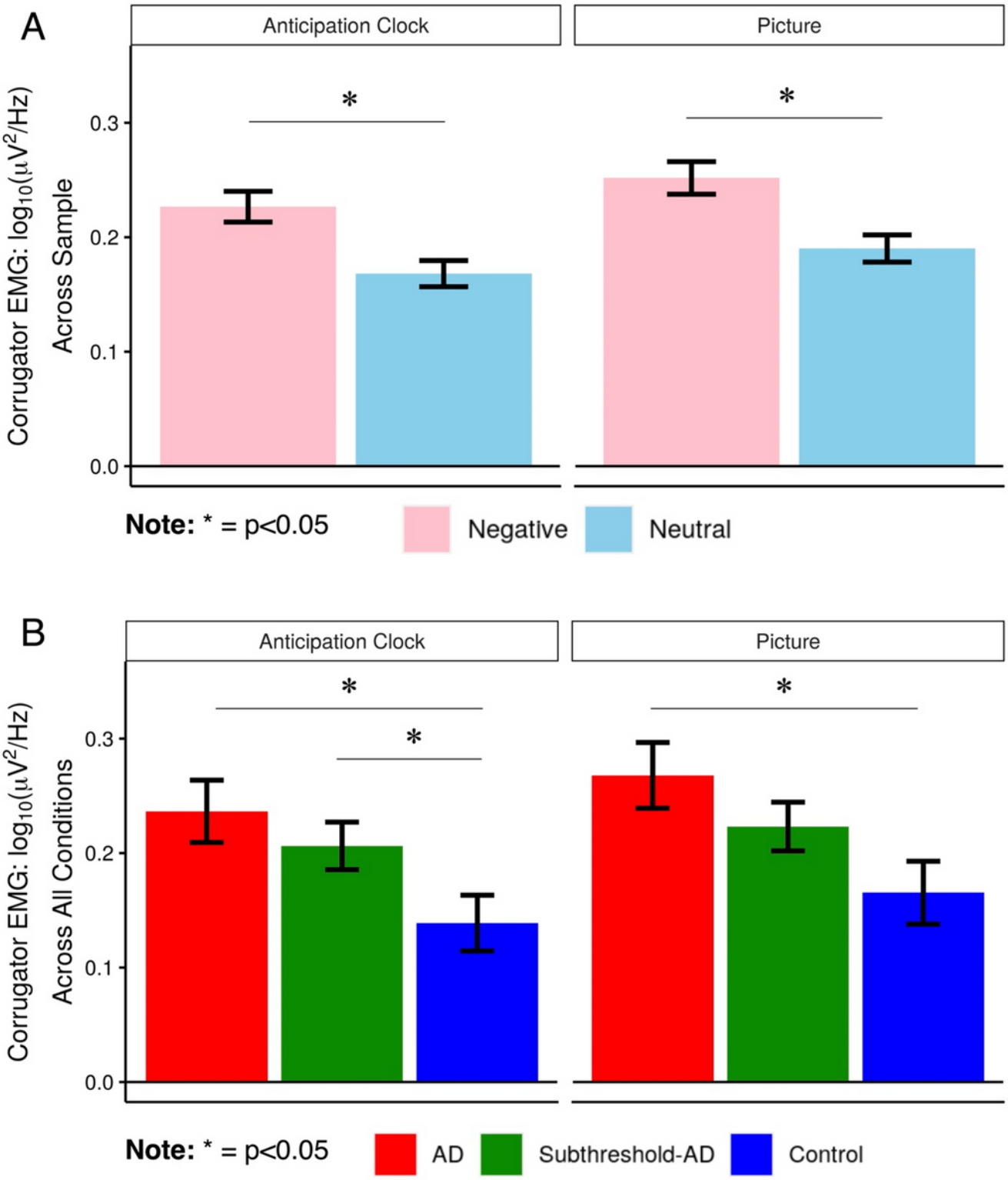

Figure 3

Corrugator magnitude results. Mean corrugator response magnitude during anticipation clock (left) and picture (right) periods across all subjects by valence. A significant main effect of valence (negative > neutral) was observed for both the anticipation clock and picture periods (p<0.05). C) Mean corrugator response magnitude during anticipation clock (left) and picture (right) periods across all subjects by group. During the anticipation clock periods, pathological anxiety (AD & subthreshold-AD) was associated with higher corrugator magnitude across all task conditions relative to controls (p<0.05). During picture viewing corrugator magnitude was increased in AD girls relative to controls (p<.05). Asterisks denote significant main effect, p<0.05. Bars represent mean +/- standard error.

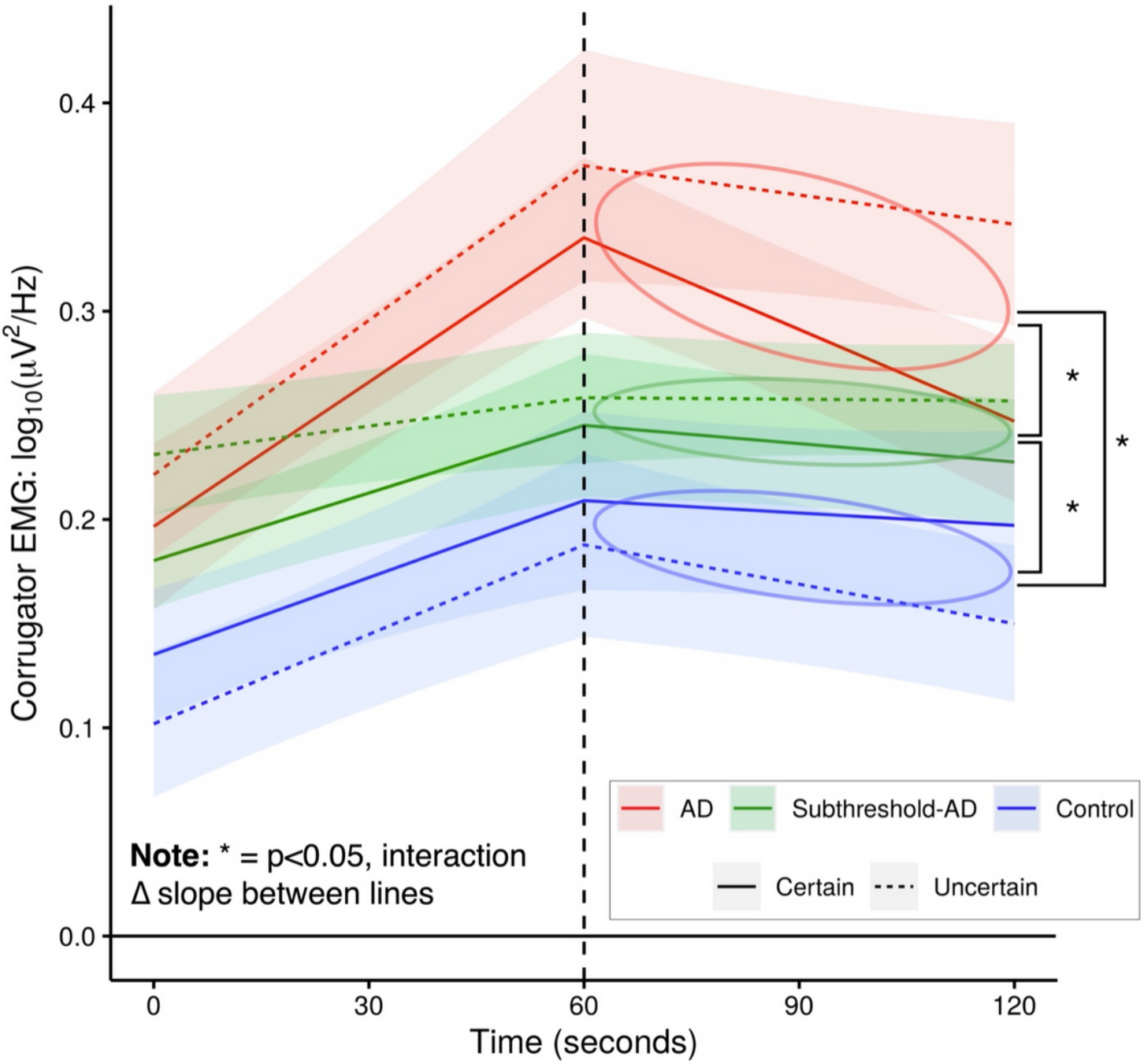

Figure 4

Negative condition corrugator response over time. Predicted estimates from the mixed effects linear spline model comparing the time course of corrugator response between uncertain negative vs. certain negative conditions by group. Shaded regions represent mean +/- standard error. The vertical black dashed line represents the knot point at 60 seconds that splits the response into splines that encompass the first and second minute of the corrugator response during the block. Ovals highlight the comparison of the corrugator trajectory between uncertain and certain blocks for each group during the 2nd half of the task blocks. Asterisks indicate 2nd half uncertainty by time effects for group contrasts that are significant. Results show that both AD and subthreshold-AD girls, compared to controls, demonstrate sustained responding in uncertain relative to certain conditions ($p<0.05$). Corrugator responses for uncertain, relative to certain, conditions for subthreshold-AD girls were significantly different from both AD and control girls ($p<0.05$).

Supplementary Files

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