Research Article

SUSTAINED NEURAL ALTERATIONS IN ANXIOUS YOUTH PERFORMING AN ATTENTIONAL BIAS TASK: A PUPILOMETRY STUDY

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Background: *Biased attention patterns have been observed at early (16–500 ms poststimulus onset) and intermediate (1,500–4,000 ms post-onset) time points in anxious youth, but it is unclear whether a more sustained form of neural attentional bias, persisting well beyond the time frame of stimulus presentation and behavioral response, is also apparent. We investigated early, intermediate, and sustained forms of bias using behavioral measures and pupillary reactivity, an index of cognitive and affective load, to gain insight into potential neurocognitive targets for early intervention.* **Method:** *Twenty nonanxious youth and 74 youth with generalized anxiety disorder (GAD), separation anxiety disorder (SAD), and/or social phobia (SP) completed a dot-probe task, which requires participants to respond to a dot replacing either a neutral or fearful face. Emotional faces were presented for short/early (200 ms) or intermediate (2 s) intervals and followed by a sustained (up to 10.5 s) poststimulus interval. Pupil dilation, gaze direction, and reaction times (RTs) were measured during task completion.* **Results:** *Early and intermediate vigilance patterns in RTs and an avoidant pattern in gaze direction were observed in all participants irrespective of anxiety. Sustained pupil dilation in anxious youth was observed on trials in which the dot replaced fearful faces, along with an inflexible pattern of pupillary responding in comparison to controls.* **Conclusion:** *Sustained cognitive-affective load following emotional face viewing is altered and inflexible in anxious youth. These prolonged alterations extend well beyond the time frame of behavioral attentional bias and may indicate inflexible and insufficient sustained cognitive control. Early interventions targeting these alterations could improve long-term mental health trajectories. Depression and Anxiety 30:22–30, 2013.*

Key words: *pupil; anxiety; pediatric; attentional bias; dot-probe task*

INTRODUCTION

In anxious youth, attention toward threatening information appears to be systematically altered in ways that may contribute to the development and/or maintenance of anxiety disorders over time. When presented briefly with negative and neutral material, anxious adults and youth initially attend selectively to negative or threatening information.[1] In adults, some evidence suggests initial vigilance at early timepoints (16–500 ms poststimulus onset) is followed by avoidance of the negative or threatening information at intermediate timepoints (e.g. 1.5–4 s poststimulus onset,[2,3]) with a preliminary study showing the same effect in youth.[4] This vigilance-avoidance pattern[5] reconciles the early

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pattern of selective attention to threat seen during brief behavioral tasks with the chronic avoidance of anxiety-related cues that characterizes anxiety disorders in terms of their clinical presentation. In contrast to these early (vigilant) and intermediate (avoidant) forms of bias in anxiety, in depressed adults and youth, a late, sustained form of biased processing has been demonstrated in both functional Magnetic Resonance Imaging (fMRI)[6,7] and pupillary motility[8,9] (an index of cognitive load), suggesting persistent neurocognitive alterations that occur in the aftermath of negative stimulus presentation and extend well beyond both stimulus presentation and behavioral responses on traditional cognitive tasks. As such, these biases are not detectable by conventional behavioral measures of attention and are revealed primarily through indices sensitive to brain activity. For instance, in depressed adults, sustained emotional processing in the amygdala[6,7] and sustained decreases in regulatory regions of the prefrontal cortex (PFC)[7] persist for up to 10 s after a brief negative word presentation and are linked to clinically relevant phenomena such as rumination.[6] In depressed youth presented with brief negative words, sustained decreases in pupil dilation were observed 9–12 s after word presentation and were related to greater severity of depression, suggesting impaired sustained regulatory recruitment.[9]

Although attentional bias in anxiety is often characterized as an early process of initial vigilance and orienting,[10] previous studies have typically not examined whether more sustained forms of neural bias are also relevant—particularly within a developmental perspective focusing on children and adolescents. Given that pediatric anxiety is associated with increased risk of both anxiety and depression in adulthood,[11] it is possible that sustained forms of bias beginning in anxious children confer neurocognitive susceptibility to later development of depression, meaning that early interventions targeting these biases could alter life-long trajectories of mental health.

To better understand the possibly interrelated role of early, intermediate, and sustained forms of attentional bias in pediatric anxiety, we combined pupilometry, an index of neural reactivity, with behavior during the dot-probe task, a widely used behavioral attentional bias task that has previously elicited altered reaction times (RTs)[12,13] and altered brain function[14–16] in clinically anxious youth. Both short (200 ms) and longer (2,000 ms) stimulus presentations were used to provide a snapshot of visual attention at multiple timepoints, allowing for behavioral detection of both early vigilance and intermediate avoidance effects in RTs.[2] Eyetracking data were collected to disambiguate RT patterns by providing a direct measurement of gaze patterns in relation to threat across the course of stimulus presentation.[3] Pupillary motility data were collected for an extended period (9–10.5 s) after stimulus offset. Due to the afferent inputs the pupil receives from both cognitive (e.g. frontal regions via a reticular formation pathway[17]) and affective (amygdala[18]) processing regions, pupilometry provides a summative index of activity in cognitive and emotional brain regions[19] capable of detecting sustained neural alterations.

RT, eyetracking, and pupilometry indices were collected in a sample of 74 treatment-seeking youth with generalized anxiety disorder (GAD), separation anxiety disorder (SAD), and/or social phobia (SP) and a control sample of 20 nonanxious youth. Consistent with previous behavioral findings, we hypothesized that anxious youth would show a pattern of early vigilance toward threat, as indicated by biased RTs following brief stimulus presentations and biased gaze patterns in early phases of trials. We expected a behavioral avoidance pattern would be evident in RTs following long stimulus presentations and in latter stages of gaze patterns. Furthermore, we hypothesized that across all trials, anxious youth would show sustained pupil dilation long after faces were gone and responses were made, indicative of prolonged processes of threat reactivity and/or regulatory attempts with unsuccessful abatement of brain responses.

METHODS

DOT-PROBE TASK

Seventy-four youth (aged 9–13 years old) with DSM-IV diagnoses of GAD, SAD, and/or SP and 20 youth with no lifetime DSM-IV disorders completed the dot-probe task (see Table 1 and Supporting information for further details). Informed consent and study procedures were approved by the University of Pittsburgh IRB. The task was administered individually in a quiet, moderately lit room on a monitor approximately 68 cm from the participant using Eprime software running on a PC. Following a standard method for discriminating early biases associated with vigilance from intermediate attentional biases associated with avoidance, after an initial fixation cross presented in the middle of the screen (500 ms), a fearful and a neutral face were presented simultaneously on the top and bottom of the screen for either a short (200 ms) or long (2000 ms) interval, followed by a probe (dot) replacing either the fearful face (henceforth, "congruent" trials) or the neutral face ("incongruent" trials). The dot remained on-screen for the remainder of the trial (8.8–10.6 s) in order to minimize changes in pupil dilation driven by a light reflex. Each trial lasted a total of 11.3 s, providing data on sustained post-face pupil dilation. Long neutral–neutral trials consisting of two blank ovals presented for 2,000 ms followed by a dot were included as a control condition.[1] Participants responded as quickly as possible to the probe, indicating its location on the screen by pressing a key for up or down, and were instructed to keep eyes on the screen for the duration of the task. Faces were grayscale conversions of the well-validated NimStim battery, half male and half female, with the same actor presented in both images in each pair. Hair was cropped from the images to reduce distraction from irrelevant information. Participants completed 16 randomly interspersed trials of each type (e.g. short congruent, short incongruent, etc.) for a total of 80 trials. Pupil/eyetracking data were collected during the task using a table-mounted RK-768 eye-tracker, consisting of a video camera and infrared light source pointed at a participant's eye and a device that tracked the location and size of the pupil and corneal reflection at

[1]Ovals were used instead of neutral faces given that neutral expressions may be interpreted as slightly negative or threatening by anxious individuals[20].

TABLE 1. Descriptive statistics for the anxious and nonanxious samples

	Anxious ($n = 74$)	Nonanxious ($n = 20$)
Age	10.6 (1.4)	10.5 (1.2)
Female (%)	49.	63.2
Caucasian (%)	95.7	84.2
Head of household education, median	Standard college degree	Standard college degree
Household income, median	$70–80,000	$70–80,000
Current diagnosis [a] (%)		
Separation anxiety disorder	26.1	0
Social phobia	24.6	0
Generalized anxiety disorder	68.1	0
Specific phobia	14.5	0
Major depressive disorder	1.4	0
Attention deficit disorder	5.8	0
Pediatric Anxiety Rating Scale	20.4 (4.3)	.63 (1.7)***

Note: Data presented as mean (*SD*) unless otherwise noted. No significant differences in demographic variables according to χ^2 and *t*-tests.
[a] Diagnostic groups are partially overlapping due to inclusion of comorbid patients. Primary/principle diagnoses were not designated, meaning that percentages for the three diagnostic inclusion groups will not sum to 100.
***$P < .001$.

60 Hz (every 16.7 ms). The resolution for a typical participant was better than 0.05 mm pupil diameter.

DATA PREPROCESSING

Pupil data were cleaned using previously described procedures;[21] see Supporting information for further details. Eye position was calculated based on the x- and y-coordinates of the recorded eye-gaze minus a corneal-reflection signal, which accounts for small head movements, and individually calibrated. Eye fixations were defined as eye positions stable within 1° of visual angle for at least 100 ms and were used to calculate the following gaze pattern indices: percentage of trials with initial fixations falling within regions of interest defined by the fearful and neutral face boundaries; and percentage of time spent fixating on fearful and neutral faces during each of four temporal subwindows during face presentation (0–500 ms; 500–1,000 ms; 1,000–1,500 ms; 1,500–2,000 ms; see Supporting information for further explication). Trials with incorrect responses (8.8% of all trials) were excluded prior to analysis. RT and pupil outliers were rescaled using a Winsorizing procedure (see Supporting information) and mean RTs were converted to harmonic means.

DATA ANALYSIS

Eyetracking data were analyzed for long trials only, as short trials were briefer than the standard time frame needed to make a single saccade (~300 ms).[22] For omnibus mixed-effects analysis of pupil data, data from each trial duration (short and long) at each timepoint were first subjected to principal components analyses (PCAs) to simplify the time dimension, yielding two time factors (see Supporting information). Mixed-effects analyses of factor loadings, in which subject was a random factor and group, condition (congruent versus incongruent), and time-factor (early versus late) were fixed factors, were then used to examine group and condition-related differences in the time course of pupil and eyetracking responses to the stimuli. Significant main and interaction effects were explored further in analyses comparing mean waveforms for each trial type at each timepoint along the waveform, holding waveform-wide Type I error at $P < .05$ as described previously;[19] see Supporting information.

RESULTS

EARLY AND INTERMEDIATE BIAS: RTs

For trials involving emotional faces, a 2 × 2 × 2 ANOVA with group (anxious versus nonanxious) as a between-subjects variable, dot-location (congruent versus incongruent) and stimulus duration (short versus long) as within-subjects variables, and harmonic mean RTs as the dependent measure revealed that, contrary to hypotheses, all subjects responded more quickly to congruent trials ($M = 803.9$, $SD = 290.3$) than to incongruent trials ($M = 827.1$, $SD = 292.3$; $F_{1,92} = 5.55$, $P = .02$, Cohen's d = .08) irrespective of stimulus duration, consistent with vigilance in all subjects (mean bias-to-threat score across both durations and groups = 23.14 ms, $SD = 105.7$). There was an additional main effect of duration, with short trials yielding shorter RTs ($F_{1,92} = 37.37$, $P < .001$). No main or interaction effects involving group were significant, and groups did not differ on RTs to neutral–neutral (oval) trials ($t_{92} = .04$, $P = .97$, d = .01; see Table 2 for mean RTs; see Supporting information for additional analyses of ovals trials, bias scores, and error rates).

EARLY AND INTERMEDIATE BIAS: EYETRACKING

Bias scores for eyetracking indices are presented in Fig. 1. A 2 (group) × 2 (valence) ANOVA examining percent of trials exhibiting initial fixations on each face type revealed no effects of valence, group, or valence × group interactions (*P*s > .53). Across the temporal subwindows during face viewing, all participants tended to spend a larger percentage of presentation time fixated on the neutral face ($M = 35\%$, $SD = 9.2\%$) than the fearful face ($M = 31\%$, $SD = 9.3\%$), an avoidance pattern

TABLE 2. Mean reaction times and eyetracking indices for the anxious and nonanxious samples

	Anxious ($n = 74$)	Nonanxious ($n = 20$)
Harmonic mean reaction times		
Short duration, congruent trials	775.3 (280.7)	717.5 (287.6)
Short duration, incongruent trials	800.4 (292.7)	767.4 (292.3)
Long duration, congruent trials	857.3 (331.3)	798.8 (264.5)
Long duration, incongruent trials	866.3 (320.4)	840.2 (251.8)
Long duration, ovals-only trials	815.5 (296.1)	818.5 (420.6)
Eyetracking indices (long duration trials only)		
percent of trials with initial fixation to fear[a]	46.9 (10.6)	47.2 (8.0)
percent of trials with initial fixation to neutral[a]	48.1 (11.2)	48.9 (8.8)
percent of window 1 spent fixating on fear	22.2 (8.9)	24.6 (7.7)
percent of window 1 spent fixating on neutral	23.9 (9.7)	23.8 (7.7)
percent of window 2 spent fixating on fear	31.3 (11.7)	34.3 (9.8)
percent of window 2 spent fixating on neutral	37.6 (11.6)	37.6 (9.1)
percent of window 3 spent fixating on fear	32.4 (12.3)	37.4 (10.5)
percent of window 3 spent fixating on neutral	38.0 (13.6)	39.6 (7.7)
percent of window 4 spent fixating on fear	34.3 (12.7)	38.7 (12.2)
percent of window 4 spent fixating on neutral	38.6 (13.9)	38.8 (9.3)

Note: Data presented as mean (*SD*). Congruent trials are those in which the dot replaces the fearful face in a pair; incongruent trials are those in which the dot replaces the neutral face in a pair. Window 1 = 0–500 ms post-face onset; Window 2 = 500–1,000 ms post-face onset; Window 3 = 1,000–1,500 ms post-face onset; Window 4 = 1,500–2,000 ms post-face onset.

[a] The remaining trials (approximately 5%) did not include a detectable face fixation during the 2,000 ms face presentation (e.g. gaze was directed off-screen).

[4 (time-window) × 2 (valence) × 2 (group) ANOVA; valence main effect: $F_{1,92} = 4.8$, $P = .03$; Cohen's d = .49]. Again, no main or interaction effects involving group were significant (*P*s > .22; see Table 2 for mean values; see Supporting information for analyses of additional eyetracking indices).

SUSTAINED BIAS: PUPIL DILATION

Pupillary reactions to each type of trial are shown in Fig. 2. Group (anxious, nonanxious) × dot-location (congruent, incongruent) × time (early versus late factor scores from PCA analysis) mixed-effects analyses of pupil dilation during short trials revealed that pupil dilation was increased in anxious youth when the dot replaced fearful faces, and decreased in anxious youth when the dot replaced neutral faces (group × dot-location $F_{1,356} = 10.6$, $P = .001$, between groups d = .59 for congruent, d = .61 for incongruent). Identical analyses for long-duration trials showed that pupil dilation was greater in the anxious group for congruent trials, but only during the latter portions of the trial (group × dot-location × time interaction $F_{1,366} = 4.3$, $P = .04$, between groups d = .54 for congruent). Simple effects analyses revealed that the nonanxious group showed a pupil pattern that varied according to dot-location, with greater pupil dilation during short incongruent trials (as compared to short congruent trials: $p < .001$, d = .72) and during late portions of long incongruent trials (as compared to late portions of long congruent trials: $P = .04$, d = .38). By contrast, the anxious group showed no variability as a function of dot-location ($P > .7$ for both durations). No group differences were observed during neutral–neutral (blank oval) trials (see Supporting information).

EXPLORATORY ANALYSES: RELATIONSHIPS BETWEEN EARLY, INTERMEDIATE, AND SUSTAINED BIAS, DIAGNOSTIC SUBGROUPS, AND SEVERITY

Relationships between individual differences in early, intermediate, and sustained bias indices (see Supporting information for bias score calculations) were interrogated in exploratory correlation analyses. Indices were uncorrelated with one another (*r*s < .18, *p*s > .1), with one exception: initial fixation bias and intermediate fixation bias (500–2,000 ms poststimulus onset) showed a small-to-moderate correlation ($r = .21$, $P = .046$). There was poor convergence across the RT and eyetracking indices of bias (early bias $r = -.05$, $P = .67$; intermediate bias $r = .04$, $P = .74$). As described in the Supporting information, eye position during faces was related to pupil dilation 8–10 s later, potentially consistent with posttrial adjustments and preparation for the subsequent trial. Also in the Supporting information, there was a suggestive but nonsignificant association of one eyetracking measure with diagnostic subtype, while there were no associations of bias with severity.

DISCUSSION

EARLY AND INTERMEDIATE BIAS

Contrary to previous findings in several studies of anxious youth,[1,12,13,23,24] the current study found no

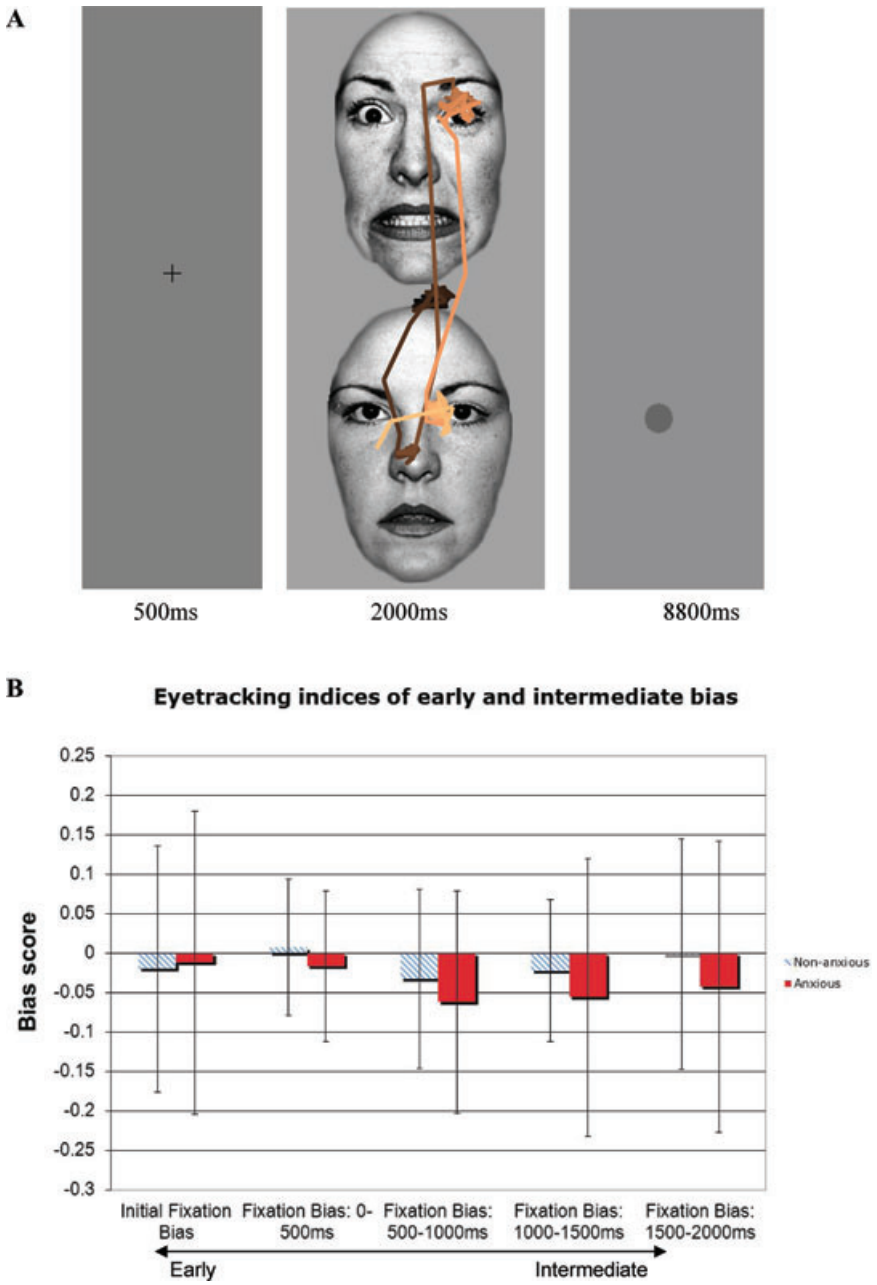

Figure 1. (A) Representative long duration, congruent trial showing avoidant fixation pattern during face presentation. Color gradient depicts the passage of time, moving from darker (black) to lighter (nude) color as the trial progresses. Subject begins with a fixation at the screen center, shows an initial fixation to the neutral face, then fixates on the fearful face, and finally returns to the neutral face. (B) Bias scores for eyetracking indices. Bias calculated for each individual as a difference score: percent of trials (for initial fixation) or percent of time during a given window (for fixation bias) with fixations to fearful face minus percent of trials/time with fixations to neutral face. Positive scores indicate vigilance, negative scores indicate avoidance. Error bars represent *SD*.

evidence in RT data for increased early attentional bias toward threat in anxious youth, instead revealing a generalized pattern of vigilance across all subjects at both short and long trial durations. An avoidance pattern of gaze was present at early-to-intermediate timepoints (~500–2,000 ms poststimulus onset; Fig. 1B), but again, it was evident in the full sample and not moderated by anxiety. Although meta-analytic findings support early vigilance in anxious youth,[1] studies of attentional bias in anxious youth have been fairly equivocal,[25,26] and, consistent with our findings, some have argued that an early bias toward the processing of threat-related information characterizes all children irrespective of anxiety,[27] particularly in younger samples with a similar age range

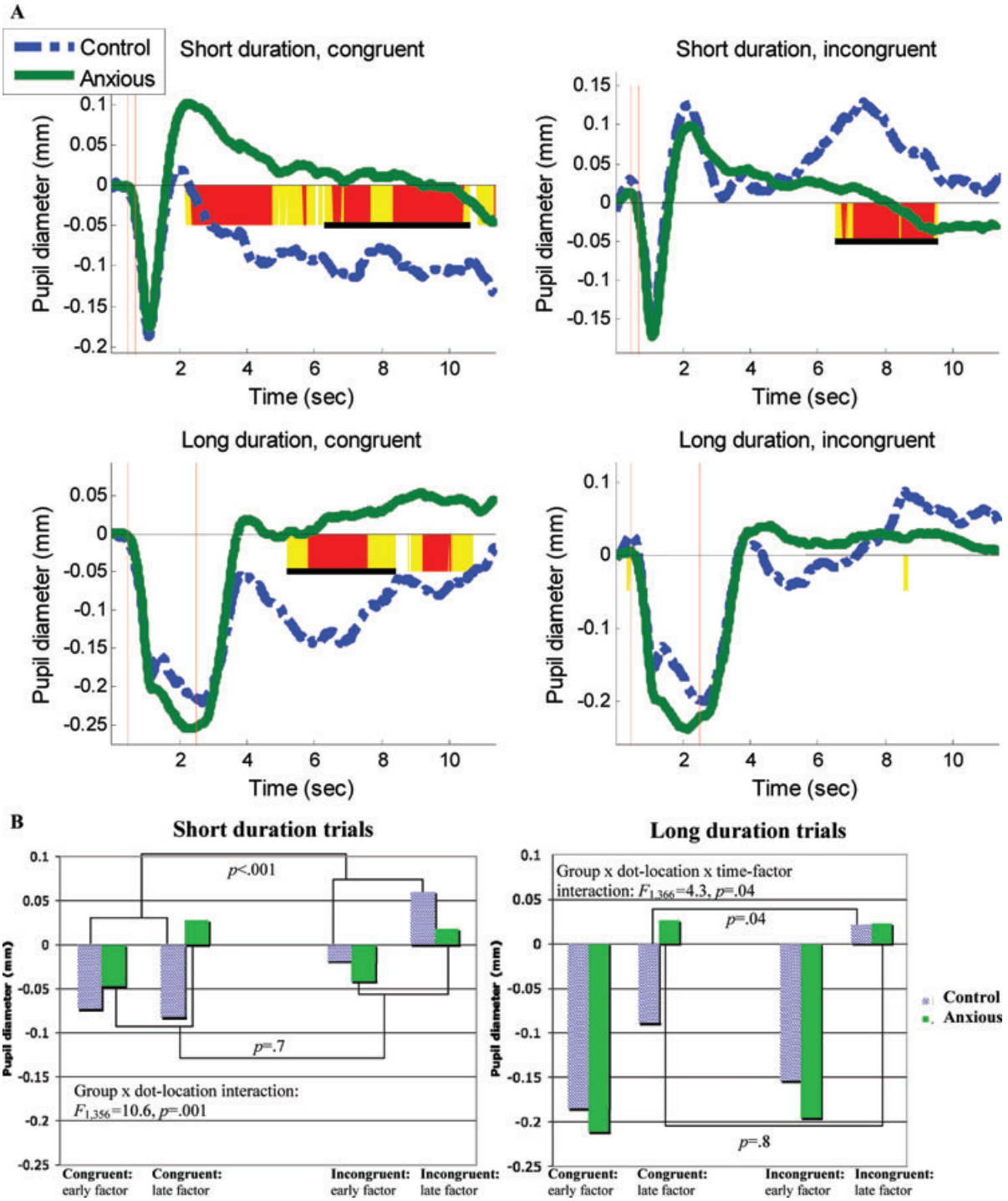

Figure 2. (A) Differences in pupillary response to dot-probe trials as a function of group. Pupil diameter represents change from baseline. Initial constrictions are related to pupillary light reflex. Regions of statistically significant differences are highlighted along the *x*-axis (red = $P < .05$, yellow = $P < .1$). Underlined area along the X-axis indicates that the length of statistically significant contiguous *t*-tests exceeded the contiguity threshold needed for multiple comparisons correction. Vertical red lines represent onset of faces (first line) and offset of faces/onset of dot (second line). (B) Mean pupil diameter as a function of trial duration, dot-location, and group during the "early" and "late" time periods defined on the basis of pupil principal components analysis (PCA; see Supporting information). The early factor (approximately 0.5–1.5 s after stimulus onset on short trials and 0.5–3 s after stimulus onset on long trials) relates largely to pupillary light reflex in response to face presentation, while the late factor (approximately 2–10 s after stimulus onset on short trials and approximately 3–11 s after stimulus onset in long trials) represents sustained pupil dilation. Diagram highlights the within-group simple effects of dot-location, which are significant in the nonanxious group only.

to our sample (e.g. age 8–12;[26,28,29]), potentially due to age-normative deficits in executive control over attentional allocation. Furthermore, to our knowledge, no previous study has demonstrated a vigilance-avoidance pattern of RTs in pediatric anxiety, although a single study found a vigilance-avoidance eyetracking pattern in youth with SAD.[4] Adult anxiety studies also have not been fully consistent in finding support for the vigilance-avoidance hypothesis, e.g.[30] potentially due to sources of measurement error that influence RT findings across the lifespan.[31]

In addition to age group, several methodological features of the current study may explain the discrepancy with previous findings, particularly given the well-documented fragility of dot-probe findings.[23,25,31] We selected fearful rather than angry face stimuli because they evoke greater amygdalar responses than angry faces[32] (a relevant neural substrate of attentional bias[15]), have been shown to elicit equivalent RT and eyetracking bias as angry faces in anxious adults performing the dot-probe,[33] and have transdiagnostic relevance to fear perception and the implication that a generic, unspecified threat is present. However, as angry rather than fearful faces have been used in the majority of facial dot-probe studies to date, early attentional bias in anxious youth may be specific to angry faces. The atypical timing of our stimulus presentations may also have affected results. Although dot-probe presentation times in the literature have varied from very brief (e.g. 17 ms)[15] up to 2,000 ms as in our study,[34] presentation times in the range of 500–1,500 ms have most typically been associated with vigilance in RTs,[12,13,23,24,35] while in the single previous study showing a vigilance-avoidance effect in the gaze patterns of anxious youth,[4] avoidance effects did not emerge until 3–4 s post-onset. The vertical rather than horizontal positioning of stimuli may also impact dot-probe effects.[36] Finally, the long duration of our trials (designed to allow measurement of sustained neural-attentional bias) may have affected results by limiting the total number of trials presented (thus limiting power) and/or by creating unanticipated effects on attention (e.g. boredom/fatigue). Relatedly, RTs in the current study were longer than typical dot-probe RTs (400–600 ms, e.g.[23,24]) for this age group, potentially reflecting boredom/fatigue, atypical population characteristics, or unique features of pressing the number-keypad used for recording responses.

SUSTAINED BIAS

As hypothesized, pupil data revealed evidence of increased sustained cognitive-affective load following threatening stimulus presentation in anxious youth. These alterations persisted long after a behavioral response was made, potentially indicative of increased depth of residual threat processing and/or increased sustained attempts at effortful emotion regulation in anxious youth. Notably, these increases were specific to trials in which a dot replaced a fearful rather than a neutral face, suggesting that visual attention must be drawn to the location where the threat previously appeared in order to elicit this sustained elevated response.

Unexpectedly, interactions between dot location and group in the pupil data were driven primarily by changes across trial type in the nonanxious group (Fig. 2). While pupil dilation waveforms in the anxious group remained fairly consistent across dot-location conditions, the nonanxious group showed a variable pattern characterized by increased pupil diameter during incongruent trials. This resulted in significantly greater sustained pupil dilation in the nonanxious group during late phases of short incongruent trials—a reversal of the pattern of group differences observed during congruent trials. Studies combining fMRI with pupilometry have highlighted the pupil's ability to track prefrontal regulatory processes (e.g. dorsolateral prefrontal and anterior cingulate activity) during cognitive and affective tasks,[19,37,38] suggesting this pattern in control participants may reflect greater regulatory control brought on-line in response to trials requiring attention to be disengaged from the location of threat. Unlike congruent trials, these trials required reversal of the general pattern of vigilance to threat observed in RT data across all participants, perhaps prompting increased sustained cognitive control. Alternately, pretrial preparatory mobilization of executive regions (e.g. dorsolateral PFC[39]) could have decreased the need for cognitive resource expenditure during subsequent congruent trials in the nonanxious group, as suggested by the association of pupil dilation late in the trial with previous eye position (potentially consistent with posttrial adjustments and preparation for the subsequent trial; see Supporting information). Failure of anxious participants to match this pattern of pupil modulation by trial type may indicate a deficit in flexible responding to changing circumstances in anxious youth, consistent with psychophysiological models of anxiety emphasizing autonomic inflexibility[40] and cognitive inflexibility.[41]

While anxious participants' regulatory responses appeared deficient for incongruent trials, their overactive brain responses during congruent trials are convergent with previous neuroimaging studies of the dot-probe task in anxious youth suggesting hyperreactivity of the amygdala[15] and ventrolateral PFC (VLPFC),[14] a region implicated in top-down affect regulation.[42] A recent MEG study was also highly convergent with our findings, demonstrating increased VLPFC activity in anxious youth specifically during the poststimulus (dot presentation) period of congruent trials, but not incongruent trials,[16] again suggesting our pupil findings may reflect alterations in regulatory prefrontal regions. The long trial durations in the current study extend this literature by suggesting that hyperactive responses in anxious youth are quite enduring and can be sustained up to 10.5 s following stimulus offset (Fig. 2). The converse pattern of decreased pupil dilation observed during incongruent trials resembles findings from depressed youth, who exhibit decreased sustained pupil dilation following

negative word presentations compared to healthy youth.[9] Patterns in youth with both disorders may reflect decreased flexible regulatory control, although the deficit is elicited in anxious youth only when they are asked to direct attention away from the previous location of threat.

RELATIONSHIPS AMONG INDICES

Early, intermediate, and sustained bias were largely unrelated in the current sample, suggesting dissociable constructs. Although dot-probe RTs are frequently interpreted as an index of the overt location of visual attention just prior to dot onset, in the current study RTs and gaze patterns were discordant. An RT pattern of vigilance to threat was present across all subjects and both trial durations, in conjunction with an intermediate avoidance gaze pattern, suggesting that dot-probe RTs have a complex relationship to gaze in children and may be influenced by processes other than gaze direction, such as alterations in prefrontal cognitive control occurring after face offset.[16]

STRENGTHS AND LIMITATIONS

The current study focuses on a maturational window that represents a period of vulnerability for developing affective disorders.[43] Mechanistic studies of this age group are therefore uniquely well suited to inform early intervention. To our knowledge, this is the first study of attentional bias to incorporate pupilometry and one of the first child anxiety studies to incorporate eyetracking and allow for measurement of both vigilance and avoidance components of attentional bias. The current study used a well-characterized, treatment-seeking sample of youth with clinical anxiety disorders and incorporated multiple indices of cognition and attention, allowing for a more complete investigation into the nature and time course of threat processing and its brain reactivity correlates. The study was limited by modest sample size in the control group, which constrained power in between-group analyses, and by the unbalanced and overlapping (comorbid) distributions across diagnostic subgroups, which limited our ability to examine the question of diagnostic specificity of findings. Our failure to identify robust effects of diagnosis or clinical severity (see Supporting information) limits conclusions regarding clinical relevance of findings. Generalizability may be limited due to the primarily Caucasian, relatively affluent sample. Finally, although pupilometry offers an inexpensive and noninvasive index of brain reactivity with strong temporal resolution, it lacks specificity in the spatial domain. Future studies should build on these findings using concurrent pupilometry and neuroimaging to simultaneously maximize spatial and temporal resolution, with a particular focus on identifying the neuroanatomical substrate of the sustained processing alterations identified here.

CONCLUSIONS

In summary, the present study identified sustained neural alterations, as measured by pupillary motility, in anxious youth long after behavioral responses to emotional stimuli were made, possibly reflecting 1) sustained attempts at regulatory control following attention to threat and 2) not enough flexible up-regulation of regulatory control following directed attention away from threat. Early and intermediate RT indicators of vigilance to threat and an intermediate avoidant gaze pattern were present in all participants irrespective of anxiety. Late pupil alterations suggest sustained, effortful processes in response to threat that are inflexible and likely maladaptive. As these sustained responses to threat may promote development of longer term maladaptive patterns, early intervention strategies targeting these features may hold promise in the treatment and/or prevention of anxiety as well as other affective disorders including depression. For instance, given recent interest in direct modification of attentional bias as a method of reducing anxiety vulnerability,[36] our findings suggest that careful consideration of altered brain reactivity occurring long after the time course of targeted behavioral biases is warranted, as these sustained patterns may require a targeted intervention of their own.

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