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Increased neural and pupillary reactivity to emotional faces in adolescents with current and remitted major depressive disorder

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Abstract

This study combined multiple levels of analysis to examine whether disrupted neural and pupillary reactivity to emotional faces serves as a state- or trait-like marker of adolescent major depressive disorder (MDD). The study examined differences in pupil dilation and the event-related potential (ERP) late positive potential (LPP) component to emotional faces before and after a negative mood induction between 71 adolescents (age 11–18 years) with (i) a current diagnosis of MDD, (ii) a past episode of MDD currently in full remission and (iii) no lifetime history of any Axis I disorder. Relative to healthy control (HC) youth, adolescents with current or remitted MDD exhibited an enhanced LPP and pupillary response to all emotional facial expressions (fearful, happy and sad). This difference in reactivity between remitted depressed and HC adolescents persisted following the negative mood induction. Results also revealed that LPP and pupillary responses to emotional faces were significantly related, but only among the currently depressed adolescents. This study suggests that increased physiological and neural activation in response to social-emotional stimuli may not only characterize currently depressed adolescents, but also remains following MDD remission, potentially serving as a mechanism of risk for future depression relapse.

Key words: adolescence; major depressive disorder; emotional reactivity; pupil dilation; late positive potential

Adolescents with a previous history of major depressive disorder (MDD) are at extremely high risk of recurrence, with a cumulative probability of recurrence of 40% by 2 years and 70% by 5 years (Rao, 2006; Avenevoli *et al.*, 2015). This high rate of recurrence almost certainly reflects the presence of stable vulnerability factors. Discriminating between state- and trait-like markers of adolescent MDD may be the first step to help identify specific vulnerabilities for the illness.

One potential trait-like vulnerability marker for adolescent depression may involve disrupted processing of emotionally salient stimuli. Specifically, behavioral studies suggest that adolescent depression is characterized by an attention and interpretation bias for depression-relevant or negative stimuli (for a review, see Platt *et al.*, 2016). There is also some evidence that youth with MDD exhibit biased processing for positive stimuli at the behavioral level (Kyte *et al.*, 2005; Harrison and Gibb, 2015), though others have failed to replicate this finding (for a review, see Platt *et al.*, 2016). Notably, there is some indication that these emotion processing biases represent vulnerability factors implicated in adolescent MDD, as behavioral studies have shown that they are present in youth of depressed parents, a population at high-risk for developing MDD (Joormann

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et al., 2007; Gibb *et al.*, 2009; Kujawa *et al.*, 2011; Connell *et al.*, 2013), and among adolescents in full remission from MDD (Hankin *et al.*, 2010). When compared with behavioral findings, neural and psychophysiological measures can provide relatively objective measures of emotion processing and offer insight into the brain circuitry underlying vulnerability. There is also evidence that neural measures account for unique variance in predicting future behavior, including clinical status and response to treatment, above and beyond that accounted for by clinical and behavioral measures (Gabrieli *et al.*, 2015), raising the possibility that these measures may aid in identifying vulnerability factors implicated in adolescent MDD.

Researchers have utilized pupillometry, a psychophysiological measure capturing the assessment of changes in pupil dilation, to study emotion processing patterns and depression risk. The pupil dilates in response to stimuli requiring greater cognitive load and attentive responses and to stimuli of greater emotional intensity and remains dilated as long as processing persists, providing a peripheral index of brain activation in response to a specific stimulus (for a review, see Laeng *et al.*, 2012). Studies show that adults with current MDD exhibit increased pupil dilation to negative stimuli, compared with healthy controls (HCs) (Siegle *et al.*, 2001, 2003a). Other studies suggest that adults in remission from depression exhibit an enhanced pupillary response to negative words prior to a negative mood induction; however, following an instruction to think of a sad event in their lifetime, remitted depressed adults experienced a blunted pupillary response to negative words relative to HC adults (Steidtmann *et al.*, 2016). A heightened pattern of emotional reactivity has also been observed among adolescent populations deemed at high risk for depression by virtue of having a depressed mother. Specifically, greater pupil dilation to sad faces among these high risk youth prospectively predicted depression onset over a 2-year longitudinal follow-up period (Burkhouse *et al.*, 2015). No studies to date, however, have examined whether a similar pattern exists among adolescents in remission from MDD, representing a potential trait-like psychophysiological marker of risk for future depressive relapse.

Researchers have also utilized event-related potentials (ERPs) to capture emotion processing styles among clinical populations given their excellent temporal resolution and ability to capture multiple stages of emotional processing. The late positive potential (LPP) ERP component, in particular, is a promising measure of emotional processing, as it has been shown to be enhanced in response to emotional stimuli, including faces, pictures, and words (Hajcak *et al.*, 2010). Both an early and a late LPP component have been identified in previous studies, and it is suggested that the late portion may be better at distinguishing clinical groups (Hajcak and Olvet, 2008). Notably, in a sample of adolescents, females with current MDD exhibited a larger early and late LPP response to negative words, relative to nondepressed female youth (Auerbach *et al.*, 2015). Studies examining the LPP among youth of depressed mothers, however, tend to yield inconsistent patterns. Specifically, whereas one study showed that children of depressed mothers exhibit an enhanced LPP response to negative words (Speed *et al.*, 2016), others have shown that these high risk youth exhibit a blunted LPP response to emotional faces and images, regardless of valence (Kujawa *et al.*, 2012; Nelson *et al.*, 2015).

Taken together, these previous studies suggest that differences in pupillary and neural (i.e. LPP) reactivity to emotional stimuli serve as a correlate of current depression in adolescents and adults, and can predict future depression onset among children deemed at high risk for depression. However, one critical unanswered question is whether these biases are also present in adolescents with remitted MDD (rMDD), a population at high risk for depressive relapse (Avenevoli *et al.*, 2015). In this study, we sought to examine whether disrupted neural and psychophysiological reactivity (measured via the LPP and pupil dilation, respectively) to emotional faces serves as a trait-like marker of adolescent MDD by examining these responses among youth with current MDD, rMDD, and no history of any psychiatric disorder.

Consistent with cognitive models of depression (Clark and Beck, 1999) suggesting that biases remain latent until activated by a negative stressor, this study examined pupillary and LPP responses to emotional faces before and after a standardized negative mood induction. Based on previous studies examining the relation between emotion processing and depression risk (Siegle *et al.* 2003a; Auerbach *et al.*, 2015; Burkhouse *et al.*, 2015; Speed *et al.* 2016), we predicted that, prior to the negative mood induction, the currently depressed adolescents would exhibit greater pupil dilation and an enhanced LPP response relative to rMDD and HC adolescents, and that this would be specific for negative (sad and fearful), *vs* positive (happy) faces. Consistent with disrupted physiological and electrocortical reactivity to emotional stimuli serving as a trait-like marker of adolescent MDD, we predicted that after, but not before, the negative mood induction, the rMDD adolescents would exhibit greater pupil dilation and an enhanced LPP response relative to HC adolescents, and that this would be specific for negative, *vs* positive, faces.

Finally, exploratory analyses were conducted to examine relations between pupil dilation and LPP responses prior to the mood induction. Although previous studies suggest that these two measures capture similar processes, only one study has directly tested this and it failed to find a significant relation between the two measures when healthy participants viewed natural scenes (Ferrari *et al.*, 2016). In this study, we examined relations between these two measures in response to emotional faces, and examined whether adolescents' depression status moderated the relation between pupil dilation and LPP responses to emotional faces.

Methods

Participants

Participants included 71 adolescents (18 with current MDD, 27 with rMDD, and 26 HCs) ranging in age from 11 to 18 years, recruited from the community. To qualify for the current MDD group, adolescents had to meet criteria for a current DSM-IV diagnosis of MDD. To qualify for the rMDD group, adolescents must have had a prior episode of MDD that was currently in full remission. To meet criteria for full remission from MDD, the MDD criterion A symptoms within the Schedule for Affective Disorders and Schizophrenia for School-Age Children—Present and Lifetime Version (K-SADS-PL; Kaufman *et al.*, 1997) interview must have been scored as absent in the past 2 months. Exclusion criteria for both the current and rMDD groups included a history of bipolar disorder, psychosis, substance abuse or dependence, or autism spectrum disorder. The HC group could not have any history of any DSM-IV Axis I disorder. The average age of adolescents in our sample was 14.14 years (s.d. = 1.93, range = 11–18), 56.6% were female, and 81.2% were Caucasian. Eight (44%) of the currently depressed adolescents and seven (26%) of the rMDD adolescents were taking current

Table 1. Demographic and clinical characteristics of participants

	Controls ($n = 26$)	Remitted ($n = 27$)	Current ($n = 18$)
Age (M, s.d.)	14.23[a] (1.75)	14.04[a] (2.23)	14.39[a] (1.88)
CDI (M, s.d.)	2.57[a] (2.48)	8.15[b], 6.06	22.33[c], 10.30
Family income (median)	$70 001–$75 000[a]	$35 000–$40 000[b]	$45 000-$50 000[b]
Gender (*n*, % Female)	11, 42%	15, 56%	14, 77%
Race (*n*, % Caucasian)	23, 88%	22, 82%	15, 83%

Note. Means and medians with different superscripts differ significantly. CDI, Children's Depression Inventory.

psychotropic medication. Table 1 displays participant characteristics separated by diagnostic status.

Measures

Diagnoses. The K-SADS-PL (Kaufman *et al.*, 1997) was used to assess for Axis I psychopathology. Among the 18 currently depressed adolescents, 9 had a current anxiety disorder, 5 had a current behavioral disorder and 5 had a history of recurrent MDD. Among the 27 rMDD adolescents, 9 had a current anxiety disorder, 7 had a current behavioral disorder, and 4 had a history of recurrent MDD. The average number of days since last MDD episode among the remitted depressed group was 593 (s.d. = 531, range = 67–2217). Interrater reliability for the K-SADS-PL, based on 20% of the sample interviews, was excellent ($\kappa = 1.00$).

Depressive and anxious symptoms. Adolescent's symptoms of depression and anxiety were assessed using the Children's Depression Inventory (CDI; Kovacs, 1981) and the Multidimensional Anxiety Scale for Children (MASC; March *et al.* 1997), respectively. In this study, both the CDI and MASC exhibited excellent internal consistency (α = 0.94 and 0.93, respectively).

Emotional faces task. Participants completed an emotional faces task where they viewed fearful, happy, sad and neutral youth faces presented one at a time on a computer screen. The stimuli in the paradigm were taken from the NIMH Child Emotional Faces Picture Set, comprised of emotional and neutral faces of youth aged 10–17-years old (Egger *et al.*, 2012). Participants were instructed to identify the emotional valence of the face by pressing a corresponding button on a keypad. For each trial of the task, participants first viewed a fixation cross for 1000 ms, followed by the presentation of the face for 5000 ms, followed by a fixation cross for 3000 ms. The inter-trial interval varied randomly between 500 and 750 ms. The task included 22 fearful, 22 happy, 22 sad and 22 neutral faces. Pupil dilation and EEG were recorded while participants completed the task.

Pupillometry recording and processing. A Tobii T60XL eye tracker was used to monitor pupil dilation. The eye-tracker consisted of a video camera and infrared light source that was used to track the location and size of participants' pupils during each task. Pupil was recorded at 60 Hz (every 16.7 ms). Blinks were identified as large changes in pupil dilation occurring too rapidly to signify actual dilation or contraction. Trials comprised of over 50% blinks were removed from consideration. Participants with >50% of rejected trials were removed from analyses. This resulted in the following number of subjects being removed pre-mood induction: HC = 1, rMDD = 1, current MDD = 0. Of the 51 rMDD and HC adolescents who had clean pre-mood induction pupil data, 9 subjects were removed for the post-mood induction data analysis due to poor data quality (HC = 4, rMDD = 5). The number of subjects removed from analyses due to excessive blinks was not significantly related to adolescents' diagnostic status (lowest $P = 0.42$). The average pupil diameter over the 333 ms preceding the onset of the stimulus was subtracted from pupil diameter after stimulus onset to produce stimulus-related pupil dilation. Consistent with previous studies (Silk *et al.*, 2008, 2009), average stimulus-related pupil dilation was calculated by taking the average early (2–5 s) and late (5–8 s) pupil response for each stimulus across all trials for each valence (fearful, happy, sad, neutral).

Electroencephalogramrecording and processing. Continuous electroencephalogram (EEG) was recorded using a custom cap and the BioSemi ActiveTwo system. The signal was preamplified at the electrode with a gain of 1600 and the EEG was digitized at 24-bit resolution with a sampling rate of 512 Hz. Recordings were taken from 34 scalp electrodes based on the 10/20 system. The electrooculogram was recorded from four facial electrodes. Data were processed offline using Brain Vision Analyzer software (Brain Products, Gilching, Germany). All data were re-referenced to the average of the left and right mastoid electrodes, and filtered with high- and low- pass filters of .1 and 30 Hz, respectively. Continuous EEG data were segmented beginning 100 ms before stimulus onset and continuing for 2000 ms after onset. Eyeblinks were corrected using the method by Gratton and colleagues (Gratton *et al.*, 1983) and semi-automated artifact rejection procedures removed artifacts with the following criteria: voltage step of more than 50 uV between sample points, a voltage difference of 300 uV within a trial, and a maximum voltage difference of <0.5 uV within 100 ms intervals. Participants were required to have a minimum of 12 artifact-free trials in each condition to be included in analyses (Moran *et al.*, 2013). This resulted in the following number of subjects being removed pre-mood induction: HC = 6, rMDD = 7, current MDD = 1. Of the 40 rMDD and HC adolescents who had clean pre-mood induction ERP data, 4 were removed for the post-mood induction data analysis due to poor data quality (HC = 2, rMDD = 2). The number of subjects removed from analyses due to excessive artifacts was not significantly related to adolescents' diagnostic status (lowest $P = 0.26$). Trials were baseline corrected using the 200 ms prior to stimulus onset and averaged across each face condition. Consistent with previous studies measuring LPP responses in youth (Kujawa *et al.*, 2012, 2015; Auerbach *et al.*, 2015) and based on visual inspection of the current data to determine where the LPP was maximal, the LPP was scored as the mean activity from 400 to 1000 ms (early segment) and 1000–2000 ms (late segment) at a cluster of parietal-occipital sites: P3, P4, PO3, PO4, Pz, O1, O2 and Oz.

Mood induction. The rMDD and HC adolescents completed a standardized negative mood induction in which the youth watched a 165 second clip from the movie *The Champ* (a scene in which a badly injured boxer summons his young son to the ring).

State mood ratings. A visual analog scale (VAS; Killgore, 1999) was used to measure state sadness before and after the

negative mood induction. Participants marked how they were feeling on a scale with anchors of very happy and very sad. The scale measured 100 mm and the distance from the left side of each scale was measured, with higher scores indicating greater state sadness.

Procedure

Informed consent and assent were collected upon arrival to the laboratory. Adolescents were administered the K-SADS-PL by a research assistant and then completed the CDI and MASC. Following this, adolescents completed the faces task. The rMDD and HC adolescents completed the negative mood induction and then repeated the faces task. The current MDD adolescents did not receive the negative mood induction or post-mood induction computer tasks. Participants completed the VAS before and after the negative mood induction. During this time, the adolescent's primary guardian was administered the K-SADS-PL. The University's Institutional Review Board approved all study procedures.

Analysis plan

To examine physiological and neural reactivity (pupil dilation and LPP) elicited during the faces task prior to the negative mood induction across all participants, two separate Emotion (fearful, happy, sad, neutral) × Segment [early (400–1000 ms for LPP, 2000–5000 ms for pupil dilation), late (1000–2000 ms for LPP, 5000—8000 ms for pupil dilation)] repeated measures ANOVAs were conducted, with LPP or pupil dilation serving as the dependent variables. Greenhouse-Geisser corrections were used in cases in which the sphericity assumption was violated.

To examine group differences in behavioral performance [accuracy and response time (RT)] during the faces task, Group × Emotion repeated measures ANOVAs were conducted with accuracy or RT serving as the dependent variables and separate analyses were conducted for responses before and following the mood induction.

To examine group differences in LPP and pupillary reactivity, consistent with previous research (Kujawa *et al.*, 2012; Kappenman *et al.*, 2013), difference scores were first calculated to reflect each participant's reactivity to each emotional stimulus compared with neutral (i.e. emotion—neutral). Following this, Group × Emotion × Segment repeated measures ANCOVAs were conducted, with LPP or pupil dilation serving as the dependent variables. Given the significant group difference in family income, this variable was included as a covariate in all analyses. To examine group differences in LPP and pupillary reactivity following the negative mood induction, similar ANCOVAs as described earlier were conducted with the addition of Time (pre- and post-MI) as a within-subjects factor, and the Group factor now including only remitted depressed and control adolescents. Bonferroni procedures were used to correct for multiple comparisons. Specifically, an adjusted *P*-value of 0.0125 was used to interpret significant group effects, and *post-hoc* comparisons were conducted using Bonferroni correction in SPSS.

Finally, to examine relations between pupil dilation and the LPP to emotional faces prior to the mood induction, linear mixed modeling was used with an autoregressive (AR1) covariance structure. Data were included for LPP responses for each emotion at each segment, with subject treated as a random effect and emotion and segment treated as repeated measures. LPP served as the dependent variable, and predictors in the analysis were depression group, pupil dilation, emotion, segment and all interactions.

Results

Preliminary analyses

Demographic and clinical characteristics separated by diagnosis are presented in Table 1. Preliminary analyses were conducted to examine LPP and pupil responses to the faces task prior to the mood induction across all participants. Results from the repeated measures ANOVAs are presented in Table 2. We should also note that the groups did not differ in their LPP or pupillary response to neutral faces at pre- or post-mood induction (lowest $P = 0.29$) justifying the use of difference scores to examine differences in LPP and pupil dilation to emotional stimuli.

Pre-mood induction

Behavior—faces. Participants displayed high detection accuracy on the task overall ($M = 92.5$, s.d. $= 4.47$). No significant group main effects or interactions were observed for accuracy (lowest $P = 0.35$) or RT (lowest $P = 0.20$).

Late positive potential. Results revealed a significant main effect of depression group, $F(2, 53) = 5.71$, $P < 0.01$, $n_p^2 = 0.18$, with no other significant main effects or interactions (lowest $P = 0.15$). *Post-hoc* pairwise comparisons revealed that, across emotions, the current ($M = 1.30$, SE $= 1.34$, $p = 0.04$, Cohen's $d = 0.64$) and remitted ($M = 2.33$, SE $= 1.20$, $P < 0.01$, Cohen's $d = 0.86$) depressed adolescents exhibited a greater LPP response across emotional expressions, relative to HC adolescents ($M = -3.24$, SE $= 1.14$). The current and remitted depressed adolescents did not differ significantly ($P = 1.00$; Cohen's $d = 0.20$). The scalp topographies and waveforms depicting this finding are presented in Figure 1. The group difference remained significant after statistically controlling for the influence of children's anxiety symptoms (MASC) and diagnoses ($P = 0.04$), suggesting that the findings were at least partially independent of adolescent's current and past anxiety. This finding was also maintained when statistically controlling for the influence of adolescents' psychotropic medication status (highest $P = 0.04$).

Pupil dilation. When examining the influence of depression status on adolescents' pupillary response to emotional faces, results revealed a main effect of group, $F(2, 64) = 3.91$, $P = 0.01$, $n_p^2 = 0.12$. *Post-hoc* pairwise comparisons revealed that, across emotions, the HC adolescents ($M = -0.002$, SE $= 0.002$) exhibited significantly less pupil dilation than remitted depressed adolescents ($M = 0.004$, SE $= 0.002$, $P = 0.01$; Cohen's $d = 0.61$). The difference between the HC and currently depressed adolescents was a non-significant trend ($M = 0.003$, SE $= 0.002$, $P = 0.08$; Cohen's $d = 0.41$) and there was no difference between current and remitted depressed adolescents ($P = 1.00$; Cohen's $d = 0.10$). This finding is illustrated in Figure 2. The main effect of group was maintained when statistically controlling for the influence of children's current anxiety symptoms and diagnoses and psychotropic medication status (highest $P = 0.03$). None of the two- or three-way interactions with group were significant (lowest $P = 0.10$).

Post-mood induction

State mood ratings. To examine the influence of depression status on adolescents' ratings of state sadness following the mood induction, we conducted a Group (rMDD, HC) × Time (Pre- *vs* Post-mood induction) repeated measures ANOVA with state

Table 2. Repeated measures ANOVA results and means and s.d. of LPP and pupil dilation responses to emotional faces prior to the mood induction across all participants

	Fear M (s.d.)	Happy M (s.d.)	Sad M (s.d.)	Neutral M (s.d.)	Emotion F (P-value)	Segment F (P-value)	Emotion × Segment F (P-value)
LPP	6.80[a] (0.99)	7.40[a] (0.99)	7.40[a] (0.98)	6.23[a] (0.97)	2.41 (0.16)	200.74 (<0.001)	1.43 (0.24)
Early	10.82[a] (1.07)	10.69[a] (1.05)	10.84[a] (1.06)	8.92[a] (1.05)			
Late	2.78[a] (1.11)	4.11[a] (1.11)	3.79[a] (1.11)	3.55[a] (1.05)			
Pupil	0.010[a] (0.002)	0.007[b] (0.001)	0.009[a,b] (0.002)	0.007[b] (0.001)	4.45 (0.04)	57.15 (<0.001)	1.05 (0.37)
Early	0.017[a] (0.003)	0.013[a] (0.002)	0.016[a] (0.002)	0.015[a] (0.002)			
Late	0.003[a] (0.001)	−0.001[a] (0.001)	0.001[a] (0.001)	−0.001[a] (0.001)			

Note: Means with different superscripts differ significantly. LPP, late positive potential. LPP early = 400–1000 ms, LPP late = 1000–2000 ms, Pupil early = 2000–5000 ms, Pupil late = 5000–8000 ms.

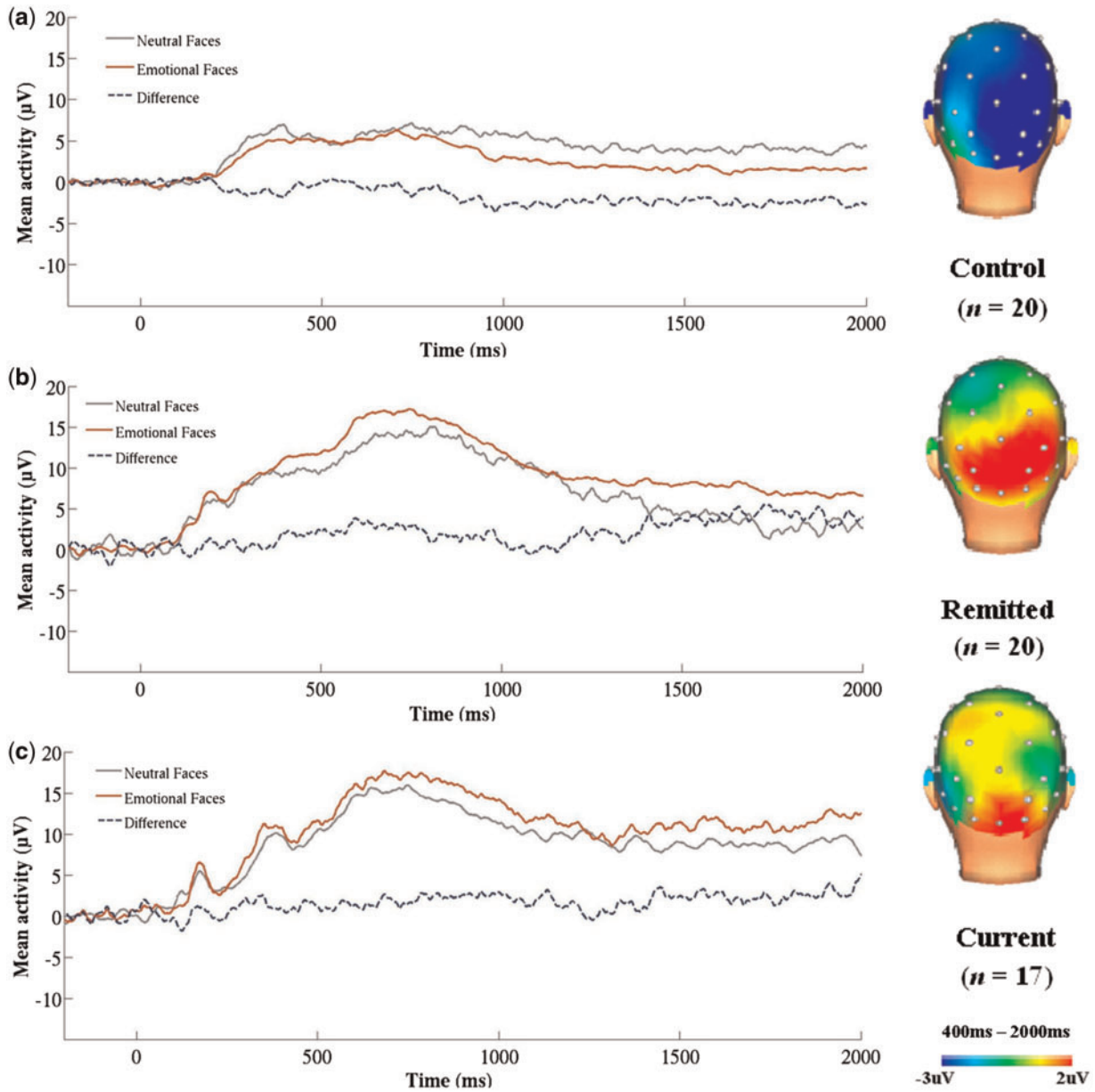

Fig. 1. Waveforms and scalp topographies depicting the LPP in response to emotional facial expressions (average of fearful-neutral, happy-neutral, sad-neutral) from 400 to 2000 ms following stimulus onset for the **(a)** control ($n = 20$), **(b)** remitted depressed ($n = 20$) and **(c)** current depressed ($n = 17$) groups.

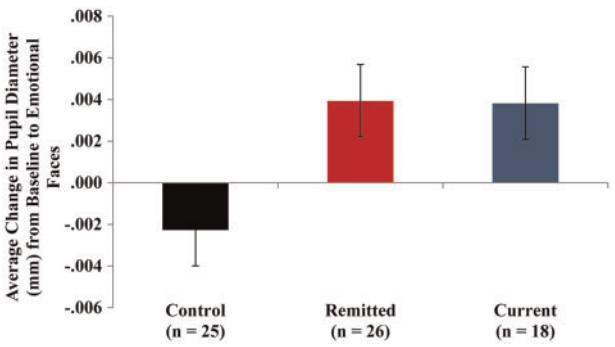

Fig. 2. Differences in average pupil dilation to emotional facial expressions (average of fearful-neutral, happy-neutral, sad-neutral) between control ($n = 25$), remitted depressed ($n = 26$) and current depressed ($n = 18$) groups.

sadness ratings as the dependent variable. Results revealed a main effect of time, $F(1, 46) = 7.71$, $P < 0.001$, $n_p^2 = 0.52$, with participants exhibiting a significant increase in sadness from baseline ($M = 3.04$, SE = 0.24), to after the mood induction, ($M = 5.17$, SE = .30). The Group × Time interaction was nonsignificant, $F(1, 46) = 0.09$, $P = 0.76$, $n_p^2 = 0.01$.

Behavior—post-mood induction. Neither the main effects of group or time, nor the Group × Time interaction was significant in predicting accuracy (lowest $P = 0.43$) or RT (lowest $P = 0.26$) during the faces task.

LPP—post-mood induction. When examining the influence of group and the mood induction on adolescents' LPP response to emotional faces, results revealed a significant main effect of group, $F(1, 34) = 7.71$, $P = 0.01$, $n_p^2 = 0.19$. Across both time points, the remitted depressed adolescents exhibited a greater LPP response to emotional faces ($M = 1.35$, SE = 0.97) relative to HC adolescents ($M = -1.70$, SE = 0.86; Cohen's $d = 0.50$). The scalp topographies and waveforms depicting this finding are presented in Figure 3. The main effect of group was maintained when statistically controlling for the influence of children's current anxiety symptoms, history of anxiety diagnoses, and psychotropic medication status (highest $P = 0.04$). In contrast, there were no significant main effects of time or emotion or any significant interactions (lowest $P = 0.09$).

Pupil dilation—post-mood induction. When investigating the influence of diagnostic status on adolescents' pupillary response to emotional faces, results revealed a significant main effect of group, $F(1, 40) = 10.90$, $P < 0.01$, $n_p^2 = 0.22$. Across both time points, remitted depressed adolescents ($M = 0.001$, SE = 0.001) exhibited greater pupil dilation to emotional faces relative to HC adolescents ($M = -0.005$, SE = 0.001; Cohen's $d = 0.70$). This effect remained significant after statistically controlling for the influence of children's current anxiety symptoms, history of anxiety diagnoses and psychotropic medication status (highest $P = 0.02$). In contrast, there were no significant main effects of time or emotion or any or significant interactions (lowest $P = 0.10$).

Exploratory analyses

Follow-up analyses were conducted to determine whether any of the group findings described earlier were moderated by children's age, gender or family income. None of these analyses were significant (lowest $P = 0.09$). We also conducted additional follow-up analyses to determine if the pre-mood induction results replicated when excluding subjects who did not have post-mood induction pupil or LPP data. All of the significant effects were maintained and the pattern of results was identical.

Relation between LPP and pupil dilation

Finally, a linear mixed model was conducted to examine the extent to which LPP and pupil dilation were correlated prior to the mood induction, and whether this would be moderated by adolescents' depression status. Results from the LMM with LPP as the dependent variable revealed significant main effects of group, $F(2, 106) = 4.43$, $P = 0.01$, and segment, $F(1, 261) = 39.76$, $P < 0.001$, and a significant group × pupil interaction, $F(2, 404) = 2.79$, $P = 0.03$. Further examination of the group × pupil interaction revealed that, among currently depressed adolescents, pupil dilation and LPP were significantly and positively related, $t(119) = 2.96$, $P < 0.01$. However, this relation was not significant among the rMDD, $t(147) = 1.30$, $P = 0.55$ or HC, $t(135) = 0.88$, $P = 0.83$, adolescents. These findings are depicted in Figure 4. None of the other main effects or interactions were significant (lowest $P = 20$).

Discussion

The goal of this study was to examine whether disrupted physiological (pupillary) and neural (LPP) reactivity to emotional stimuli serves as a state- or trait-like marker of adolescent depression. First, we examined differences in pupillary reactivity and LPP responses to emotional faces between adolescents with current MDD, rMDD or no history of any psychiatric disorder. The current and remitted depressed adolescents exhibited an enhanced LPP and pupillary response to all emotional facial expressions (fearful, happy, and sad) relative to HCs. Notably, this finding was at least partially independent of adolescent's current levels of anxiety, history of anxiety disorders, differences in family income, and use of psychotropic medication.

Second, we examined whether pupillary and LPP responses to emotional faces in remitted and never depressed adolescents changed following a negative mood induction. Although adolescents in both groups exhibited a significant increase in sadness from baseline to after the mood induction, this increase in sadness was equivalent across groups and there were no significant changes in physiological or electrocortical reactivity for either group. Although the precise reason for this finding remains unclear, one possibility is that the remitted depressed adolescents exhibited a ceiling effect in their reactivity to emotional stimuli prior to the negative mood induction (i.e. these biases were already apparent prior to the negative mood induction). On the other hand, a second, alternative explanation could be that the movie clip used to induce a negative mood may have not been salient enough to elicit changes in physiological or neural activity despite the significant increase in state sadness, as it focused predominately on the parent–child relationship. That is, during adolescence, there is a shift in social and affective dynamics of parent–child interactions (Nelson *et al.*, 2005; Crone and Dahl, 2012), as youth orient more towards peers and individualization. Thus, a negative mood induction that elicits social exclusion among peers may be more successful in changing physiological and neural responses in adolescent populations.

In combination, the current results suggest that adolescents with current or past MDD exhibit similar levels of pupillary and neural reactivity to facial displays of emotion. To the extent that emotion processing biases are evident in formerly depressed adolescents even in the absence of a negative stressor,

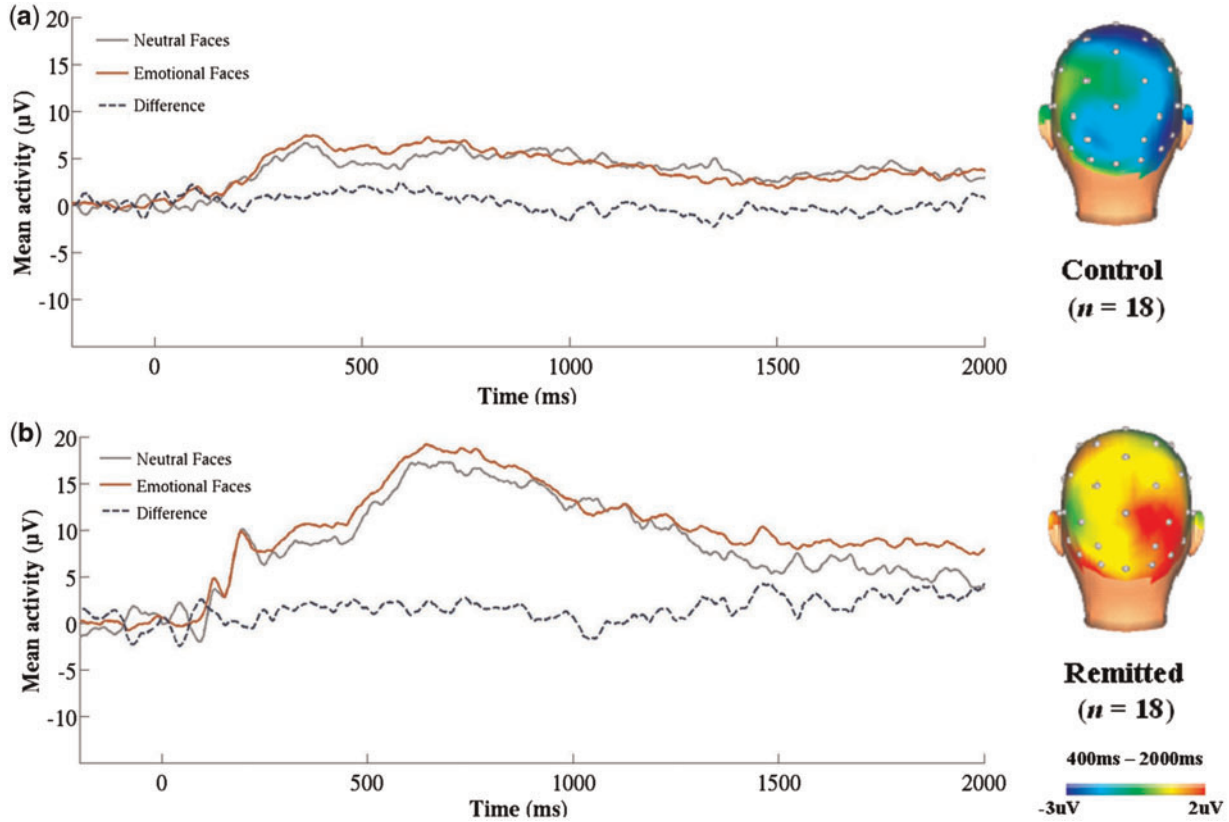

Fig. 3. Waveforms and scalp topographies depicting the LPP in response to emotional faces (average of fearful-neutral, happy-neutral, sad-neutral) from 400 to 2000 ms following stimulus onset for the **(a)** control ($n = 18$) and **(b)** remitted depressed ($n = 18$) groups after the negative mood induction.

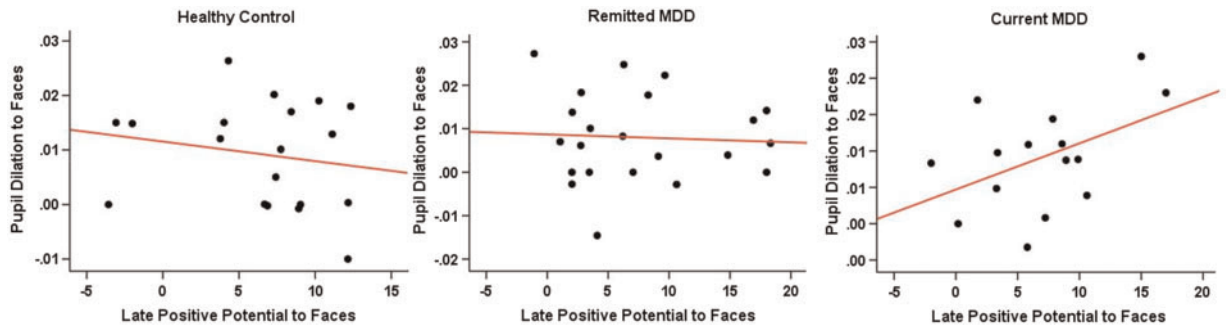

Fig. 4. Relation between average pupillary and LPP responses to emotional faces (average of happy, fearful, neutral, and sad faces) among the control, remitted and currently depressed adolescents.

increased physiological and neural activation in response to emotional faces may be a trait-like marker of risk for depressive relapse among this population. Although, because of the cross-sectional design of this study, we could not determine whether this reactivity actually increases risk for first onsets or recurrence of MDD, there is evidence from previous prospective studies showing that increased pupil dilation to negative faces predicts future depressive episodes among children of depressed mothers (Burkhouse *et al.*, 2015). Thus, heightened physiological and neural activation to emotional facial expressions may be a predictor of future depressive relapse among adolescents.

When compared with previous adolescent depression studies (e.g. Auerbach *et al.*, 2015), this study found limited evidence for enhanced physiological and neural reactivity being specific to negative stimuli. Instead, adolescents with current and rMDD exhibited increased reactivity to both positive and negative facial expressions. Notably, the Auerbach *et al.* (2015) study utilized self-referential words, rather than faces, with a sample of females only. Thus, there may be important factors (e.g. gender, stimulus type) that moderate the relation between emotional reactivity and youth depression risk. Moreover, although behavioral studies have suggested that adolescent depression is characterized by an attention bias specifically for negative stimuli, this finding has not been consistently replicated across depression risk studies, and the direction of this negative bias (i.e. preferential attention *vs* attentional avoidance) has also been inconsistent across studies (for a review, see Platt *et al.*, 2016). This may be a result of the low reliability for behavioral measures of attentional biases across studies (Schmukle, 2005;

Kappenman *et al.*, 2015). Future studies that utilize multiple levels of measurement (e.g. eye-tracking, pupil dilation, ERPs) with larger samples are needed to help shed light on the direction and specificity of these biases for youth depression risk.

It is also noteworthy that although enhanced pupillary and LPP responses to facial expressions appear to be evident among adolescents with current and rMDD, these reactivity measures were only significantly correlated among the currently depressed adolescents. This could be the result of schematic interconnectedness (Dozois, 2014), which suggests that a negative cognitive schema is activated among individuals with current depression and, as a result, their emotional and attentional biases can be observed and connected at multiple levels of analysis. According to this theory, relations among multiple measures of reactivity to emotional stimuli are less likely to be observed among individuals with lower depressive symptoms, as their negative cognitive schema is not currently activated. It is also possible that we did not observe relations among these measures for the remitted depressed and HC adolescents due to their underlying neural circuitry. Studies show that the LPP is correlated with activity in the occipital and parietal neural regions (Keil *et al.*, 2002; Sabatinelli *et al.*, 2007), and it has been suggested that the increased occipital activation, in particular, may be a result from projections from the amygdala (Bradley *et al.*, 2003). Therefore, enhanced LPP responses may reflect sustained attention to emotion and index downstream processes resulting from hyperactivation of the amygdala (Hajcak *et al.*, 2010). On the other hand, pupil dilation has been less reliably linked with amygdala, and instead linked to activity in brain areas associated with the processing and regulation of emotion, including the dorsolateral prefrontal cortex and ACC (Siegle *et al.*, 2003b; Urry *et al.*, 2009). Moreover, there is evidence that pupil dilation is a reliable index of activity in the locus coeruleus-noradrenergic neuromodulatory system, which is essential for a broad range of cognitive and emotional processes (Murphy *et al.*, 2014). Thus, although both pupil dilation and LPP represent areas of emotion processing, the LPP may be more tightly linked with sustained attentional responses, whereas pupil dilation may be more broadly associated with the regulation of emotions. Future studies using neuroimaging are needed to investigate the underlying neural circuits associated with these enhanced physiological and neural responses to emotional facial expressions among adolescents with depression.

This study benefited from several strengths, including the use of diagnostic interviews to assess adolescent depression history and the multi-method assessment of reactivity to emotional stimuli. This study also extends prior research by examining trait-like markers of adolescent depression, and benefited from the inclusion of adolescents with current and rMDD. Despite these strengths, there were also limitations. First, prospective designs are needed to determine if increased physiological and neural activation in response to emotional facial expressions in adolescents in remission from MDD is present prior to the first onset of MDD, thereby representing a putative vulnerability factor, or is the result of a previous episode of depression, representing a potentially scar effect. Second, the remitted depressed adolescents had a high rate of psychiatric comorbidity though this may have resulted in a more generalizable sample given the high rates of comorbidity among adolescents with MDD (Costello *et al.*, 2003). Third, the sample size of the current study may have precluded the detection of important moderators (e.g., gender, age). Given the gender difference that emerges during the adolescent period with females being twice as likely to experience MDD compared with males (Hankin *et al.*, 1998), future studies with larger sample sizes are needed to examine whether neural and physiological activation to emotional faces assessed prior to puberty may help to identify which adolescents are at greatest risk for developing depression and help to explain the emergence of gender differences in depression during this time.

Despite these limitations, this study suggests that increased physiological and neural activation in response to social-emotional stimuli may serve as a trait-like marker that remains following remission of MDD, which could be one mechanism by which these adolescents are at such increased risk for depression in the future. To the extent that this is true, it may have important clinical implications. For example, pupillometry is an inexpensive tool that could be administered in clinical settings, such as pediatricians' offices. In addition, compared to other neural measures, such as fMRI, EEG measures are more economical, easily administered, and easily transportable to clinical settings to inform prevention and treatment planning. Thus, the current findings could lead to targeted prevention and intervention efforts among adolescents.

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