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Pupillary reactivity to negative stimuli prospectively predicts recurrence of major depressive disorder in women

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Abstract

There is a large body of research supporting the association between disrupted physiological reactivity to negative stimuli and depression. The present study aimed to examine whether physiological reactivity to emotional stimuli, assessed via pupil dilation, served as a biological marker of risk for depression recurrence among individuals who are known to be at a higher risk due to having previous history of depression. Participants were 57 women with a history of major depressive disorder (MDD). Pupil dilation to angry, happy, sad, and neutral faces was recorded. Participants' diagnoses and symptoms were assessed 24 months after the initial assessment. We found that women's pupillary reactivity to negative (sad or angry faces) but not positive stimuli prospectively predicted MDD recurrence. Additionally, we found that both hyper- and hypopupillary reactivity to angry faces predicted risk for MDD recurrence. These findings suggest that disrupted physiological response to negative stimuli indexed via pupillary dilation could serve as a physiological marker of MDD risk, thus presenting clinicians with a convenient and inexpensive method to predict which of the at-risk women are more likely to experience depression recurrence.

Descriptors: Depression recurrence, Pupillometry, Physiological reactivity, Biomarker, Emotion processing

Major depressive disorder (MDD) is the leading cause of disability worldwide with an estimated 350 million people affected (WHO Depression, 2016). Women are nearly twice as likely to experience MDD than men, thus representing a population that is particularly vulnerable to developing MDD (Kessler, McGonagle, Swartz, Blazer, & Nelson, 1993). Depression is a highly recurrent disorder, and up to 60% of individuals who experience an initial MDD episode are expected to relapse within 5 years following recovery (Bulloch, Williams, Lavorato, & Patten, 2014; Burcusa & Lacono, 2007; Solomon et al., 2000). Recurrent MDD is characterized by distinct genetic, neurobiological, and hormonal profiles (Admon et al., 2014; Holsen et al., 2013; Levinson et al., 2003), transmits a greater risk for more severe and chronic consequences (Burcusa & Lacono, 2007; Lewinsohn, Allen, Seeley, & Gotlib, 1999), and is less responsive to antidepressant medication (Kaymaz, van Os, Loonen, & Nolen, 2008). Thus, the identification of reliable and easily measurable markers of risk is critical for aiding clinicians in predicting which women who have recovered from an initial MDD episode are at greatest risk for relapse. This will allow clinicians and policy makers to channel limited resources to those most in need.

A rapidly growing body of research highlights the role of dysregulated neurophysiological reactivity to negative stimuli as a potential biological marker of depression risk (e.g., Price et al., 2016; Siegle, Granholm, Ingram, & Matt, 2001). Both hyper- and hyporeactivity in brain regions involved in visual emotive processing, including the anterior cingulate cortex (ACC), ventromedial prefrontal cortex (VMPFC), and the dorsolateral prefrontal cortex (DLPFC), in response to emotional stimuli in patients with MDD have been reported by neuroimaging studies (Bermpohl et al., 2009; Davidson, Irwin, Adnerle, & Kalin, 2003; Fales et al., 2009; Gotlib et al., 2005; Grimm et al., 2009; Guo, Nguyen, Hyett, Parker, & Breakspear, 2015; Keedwell, Andrew, Williams, Brammer, & Phillips, 2005; Kumari et al., 2003; Tao et al., 2012). These patterns of hyper- and hyporeactivity have also been linked to differential antidepressant treatment response (Williams et al., 2015). Specifically, Williams et al. found that nonresponders showed pretreatment hyperreactivity in the amygdala, followed by posttreatment hyporeactivity to sad stimuli, compared to controls. In contrast, responders showed hyporeactivity compared to controls at baseline, which normalized to levels similar to those observed in controls by the end of treatment, suggesting that both hypo- and hyperamygdala reactivity could be associated with MDD (Williams et al., 2015). Additionally, findings from studies that used measures of emotional reactivity other than fMRI provide support for both

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decreased and increased reactivity among participants with current MDD diagnosis and those who are at a greater risk for developing MDD (Bylsma, Morris, & Rottenberg, 2008; Cohen, Gunthert, Butler, O'Neill, & Tolpin, 2005; Kovacs, Rottenberg, & George, 2009; Kujawa, Hajcak, Torpey, Kim, & Klein, 2012; Lethbridge & Allen, 2008; Pine, Cohen, & Brook, 2001).

There is evidence that the pupil dilates in response to stimuli associated with cognitive load and emotional intensity (Hess & Polt, 1960; Siegle, Steinhauer, Carter, Ramel, & Thase, 2003), providing a peripheral measure of cognitive and emotion processing. Pupil dilation has also been linked to activity in brain regions associated with the processing and regulation of emotion, including DLPFC, VMPFC, amygdala, inferior frontal gyrus (IFG), superior frontal gyrus (SFG), and ACC, such that greater blood oxygen level-dependent (BOLD) activity in those areas was associated with greater pupil dilation (Siegle, Steinhauer, Stenger, Konecky, & Carter, 2003; Urry et al., 2006). Specifically, modulation of negative affect corresponded to changes in pupil diameter, such that participants who displayed higher activation in the left IFG and SFG clusters when they were instructed to decrease negative emotions evidenced higher pupil dilation (Urry et al., 2006). Additionally, activity in ACC, which is thought to be related to tasks that require heightened control due to emotional and cognitive interference, was reported to be positively associated with pupil dilation during the Stroop task, and higher magnitude of ACC activation predicted increased pupil dilation during error-related trials (Critchley, Tang, Glaser, Butterworth, & Dolan, 2005). There is also evidence that pupil dilation can serve as a reliable index of activity in the locus coeruleus-noradrenergic (LC-NA) neuromodulatory system, which is essential for a broad range of cognitive and emotional processes (Gilzenrat, Nieuwenhuis, & Cohen, 2010; Hermans, Henchens, Roelofs, & Fernández, 2013; Murphy, O'Connell, O'Sullivan, Robertson, & Balsters, 2014; Murphy, Robertson, Balsters, & O'Connell, 2011). Both hypo- and hyperactivity of the LC-NA system are thought to be associated with emotion dysregulation and affective disorders, including depression (Aston-Jones, Rajkowski, & Cohen, 1999). Moreover, disrupted LC-NA transmission is associated with depressionlike behaviors, including learned helplessness and insomnia, in animals (Pavcovich & Ramirez, 1993).

Notably, pupillometry is a relatively inexpensive and accessible measure of cognitive-affective processing that could be easily used in clinicians' offices for assessing risk. Importantly, there is a small but growing body of research suggesting that pupil dilation to emotional stimuli may be associated with depression risk. Importantly, there is evidence for the association of both increased and decreased pupillary reactivity to negative emotional stimuli and depression diagnosis, treatment success, and risk. For example, there is evidence that individuals with current MDD evidence increased pupil dilation to negative stimuli compared to never-depressed participants (Siegle, Steinhauer, Carter et al., 2003). Pretreatment sustained pupil dilation in response to negative stimuli, in combination with initial symptom severity, has also been shown to predict remission rates following cognitive-behavioral therapy among patients with MDD (Siegle, Steinhauer, Friedman, Thompson, & Thase, 2011). Specifically, the findings suggested that remission was associated with low sustained pupil dilation to negative stimuli and high initial severity of depression. Decreased pupil dilation to emotional stimuli has been reported among never-depressed individuals who are at a higher risk for MDD due to having a parent with MDD (Bistricky, Ingram, Siegle, & Short, 2014). Lastly, increased pupil dilation to sad faces has been found to prospectively predict depression onset among children of mothers with a history of MDD (Burkhouse, Siegle, Woody, Kudinova, & Gibb, 2015). Despite the strengths of these studies, it is still unclear whether pupil dilation in response to negative information predicts MDD recurrence risk in adults.

In this study, we sought to examine whether pupil dilation to emotional stimuli prospectively predicts depression risk in a group of individuals known to be at heightened risk: those with previous history of MDD. Given that women are at a higher risk for MDD than men, (Kessler et al., 1993), we focused exclusively on women. Based on previous research suggesting that the association between depression and changes in pupil dilation was stronger for negative stimuli, compared to positive information (Burkhouse et al., 2015; Siegle et al., 2011), we hypothesized that pupillary reactivity to negative (sad or angry faces) but not positive stimuli would prospectively predict MDD recurrence. We also sought to examine the potential role of hyper- versus hyporeactivity to emotional stimuli. In addition to examining whether high versus low pupillary reactivity predicted risk for MDD recurrence, we also examined whether both high and low pupillary reactivity may be a marker of risk. Given evidence that emotion dysregulation and depression are characterized by both increased and decreased activity in DLPFC, VMPFC, and ACC, as well as hypo- and hyper-LC-NA activation, all of which are indexed by pupil dilation, we hypothesized that both hyper- and hypopupillary reactivity would predict risk for MDD recurrence.

Method

Procedure

Potential participants were recruited from the community through a variety of means (e.g., newspaper and TV advertisements) as part of a larger study of intergenerational transmission of depression. Women responding to the advertisements were screened over the phone, and eligible participants were invited to participate with their children. Upon arrival to the laboratory, participants provided informed consent, were administered the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I), and completed computer-based tasks. Follow-up assessment occurred every 6 months for 24 months (five assessments in total), during which participants were administered the SCID-I to assess for new episodes of MDD. Of the 57 participants who completed the Time 1 assessment, 51 (89.5%) completed Time 2, 48 (84.2%) completed Time 3, 41 (71.9%) completed Time 4, and 38 (66.6%) completed Time 5. If a participant missed an appointment, the following appointment covered any diagnoses that occurred since their last completed assessment. Importantly, our use of survival analysis accommodates differing duration of follow-up by focusing both on whether and when the event (diagnosis) occurred during follow-up. Participants who did not experience an MDD recurrence during the follow-up, regardless of the duration of the follow-up for that individual, were treated as right censored in the analysis (cf. Tabachnick & Fidell, 2007). For example, for participants who dropped out after Time 4 assessment, we coded the time variable as 18 months, signifying that those participants did not relapse during the participation in our study.[1]

Participants who completed all of the assessments did not differ significantly from those who missed one or more assessments on

1. The findings of the study were maintained when we only focused on participants who completed Time 5 assessment.

Table 1. *Means (Standard Deviations) for the Emotional Faces Paradigm*

Components	Angry faces	Happy faces	Sad faces
Peak pupil dilation (mm)	.025 (.018)	.025 (.019)	.046 (.031)
Detection accuracy	.998 (.007)	.995 (.014)	.971 (.057)
Response time (ms)	1,882 (703)	1,663 (792)	2,000 (663)

any of the baseline demographic, clinical, or pupil variables (lowest $p = .07$), except annual family income. Participants with missing data had significantly lower annual family income ($35,001–$40,000), compared to participants with no missing data ($55,001–$60,000). Participants were compensated $275 for their participation in all five assessments. All study procedures were approved by the Binghamton University's Institutional Review Board.

Participants

Participants were 57 women with a past history of MDD recruited from the community. The average age of women in the study was 39.21 ($SD = 6.99$, range = 24–55). The majority of participants were Caucasian (85.5%) and the rest were African American (3.6%), American Indian (1.8%), multiracial (3.6%), or from other racial/ethnic groups (5.5%). The median annual family income was $40,001–$45,000.

Measures

Lifetime histories of psychiatric disorders were assessed using the SCID-I (First, Spitzer, Gibbon, & Williams, 2002). A subset of 20 SCID-I interviews was coded by a second interviewer, and inter-rater reliability for diagnoses of MDD was excellent ($\kappa = 1.00$). Exclusion criteria included the presence of schizophrenia, a history of alcohol or substance dependence within the last 6 months, or lifetime history of bipolar disorder. All of the participants had a past history of one or more episodes of MDD. None of the participants had current MDD, psychotic, obsessive-compulsive, or eating disorders. Twenty-one percent of the participants ($n = 12$) met criteria for current anxiety disorder. During the follow-up, 19 women met criteria for a new MDD episode.

Participants' current symptoms of depression and anxiety were assessed via the Beck Depression Inventory-II (BDI-II; Beck, Steer, & Brown, 1996) and the Beck Anxiety Inventory (BAI; Beck & Steer, 1993), respectively. In this sample, both measures showed excellent internal consistency (BDI-II: $\alpha = .91$; BAI: $\alpha = .92$).

Pupil dilation was assessed in a moderately lit room using Tobii T60 and T60XL eye trackers[2] (Falls Church, VA), while participants viewed full-color images of actors displaying various emotions (angry, happy, sad, neutral) taken from a standardized stimulus set (Matsumoto & Ekman, 1988). The stimuli consisted of emotional and neutral photographs from each actor, morphed to form a continuum of 10% increments between the two photographs. Each emotion is represented by four continua (two male and two female actors), for a total of 12 continua. Eleven morphed images were used from each continuum, representing 10% increments of the two emotions ranging from 100% neutral (0% target emotion) to 100% target emotion (e.g., 90% neutral, 10% sad; 80% neutral, 20% sad; and so on). Each trial started with a fixation cross, presented for 500 ms. The pictures were then presented, one at a time in random order, in the middle of the screen for 3 s, and the participant was asked to indicate which emotion was being displayed (angry, happy, sad, neutral) by pressing a corresponding button on a keypad. The intertrial interval randomly varied between 750 ms and 1,000 ms. The stimuli were 16.51 cm high and 20.32 cm wide, and participants sat 65 cm from the screen, yielding a visual angle of 14.48° × 17.77°. Participants completed 264 trials (88 trials per emotion) with a break after 132 trials. To provide an adequate number of trials for pupillary analyses within each morph level, images were binned into three separate morph conditions for analyses: low (0%, 10%, 20%, 30%), medium (40%, 50%, 60%), and high (70%, 80%, 90%, 100%). Because our previous findings from children in this study suggested that the strongest pupillary effects are observed at the highest morph level (Burkhouse, Siegle, & Gibb, 2014; Burkhouse et al., 2015), the present study focused solely on images at the highest morph level (29.33 trials).[3] Descriptive statistics for participants' responses to each facial emotion type at the highest morph level are shown in Table 1. Change in pupil dilation in response to angry, sad, and happy faces is depicted in Figure 1. During this task, pupil size was recorded using the eye trackers, which took measurements every 16.7 ms (60 Hz) for 3 s following the onset of each facial stimulus. Data were cleaned using Siegle et al.'s (2008) recommended procedures. Trials comprised of over 50% blinks were omitted, which resulted in the exclusion of an average of 10% of trials per participant (range 0–41%; $SD = 11\%$). Following standard procedures, linear interpolation was used to replace blinks throughout the data set, and the data were smoothed using a 10-point weighted average filter. The total number of rejected trials and the percentage of pupil data that was replaced with linear interpolations were not significantly correlated with women's depressive symptoms at baseline or with their likelihood of developing a depressive episode during the follow-up (lowest $p = .20$). The relative luminance of the images of angry faces (120.33 arbitrary units [AU]) was significantly higher than the relative luminance of the images of happy (110.15 AU) and sad (114.84 AU) faces. The relative luminance of the images in the latter two groups also differed significantly. Effects associated with a light reflex that were independent of stimulus type were removed by subtracting the mean waveform across all three valences from the average waveform for each valence (Franzen, Buysse, Dahl, Thompson, & Siegle, 2009). 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. Peak stimulus-related pupil dilation was calculated by taking the maximum pupil response on average across all trials for each valence (angry, happy, sad). No outliers were identified using box plots for peak pupil dilation to angry, happy, or sad faces. Peak pupil dilation to angry faces was correlated with peak pupil dilation to sad, $r = .26$, $p = .05$, and happy faces, $r = .31$, $p = .02$, with no correlation between peak pupil dilation to happy and sad faces, $r = .04$, $p = .76$.

Results

We conducted a survival analyses to assess whether peak pupil dilation to emotional stimuli assessed at baseline predicted MDD

2. There were no significant differences in pupil findings across the two eye trackers.

3. There were no significant linear or quadratic effects of peak pupil dilation to angry, happy, or sad faces at low and medium morph levels (lowest $p > .12$).

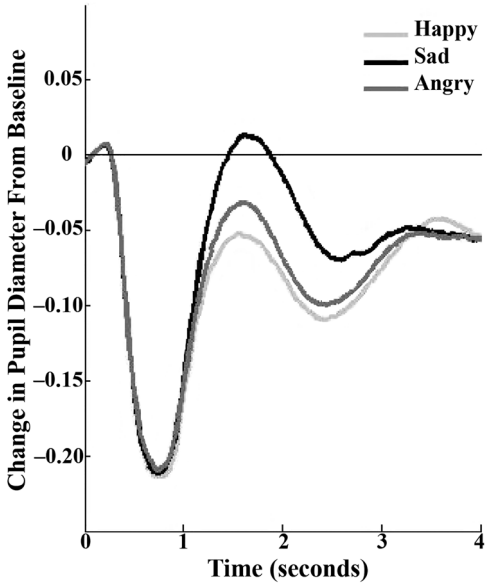

Figure 1. Participants' change in pupil diameter in response to angry, happy, and sad faces.

recurrence during the 2-year follow-up period. As noted earlier, we examined both linear and nonlinear effects of peak pupil dilation to emotional faces at high levels of morph. Specifically, in the first step of the model, we entered the linear effect of peak pupil dilation. To examine nonlinear effects, we added a quadratic peak pupil dilation variable in the second step of the Cox regression model for survival analysis, which allowed us to determine whether this quadratic effect explained significant unique variance in MDD risk beyond that accounted for by the linear effect. Focusing first on the predictive validity of peak pupil dilation to sad faces, we found that the linear effect of peak significantly predicted women's time to MDD recurrence, $\beta = -1.66$, Wald = 3.78, $p = .05$. This suggests that women who evidenced decreased peak pupil dilation to sad faces experienced MDD recurrence sooner during a 2-year follow-up, compared to women who exhibited moderate or

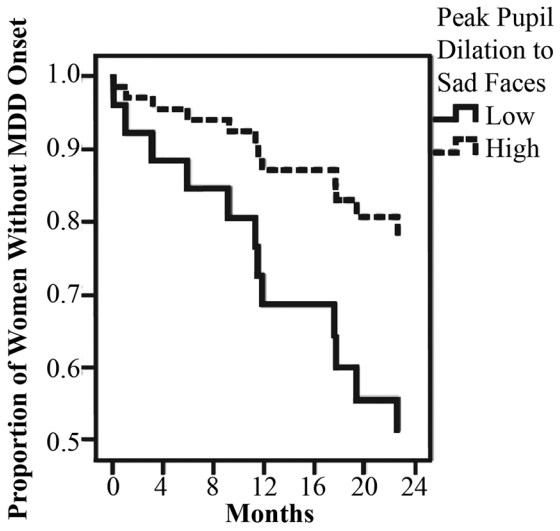

Figure 2. Results of the survival analysis using Cox regression model predicting prospective onset of MDD based on peak pupil dilation to sad faces.

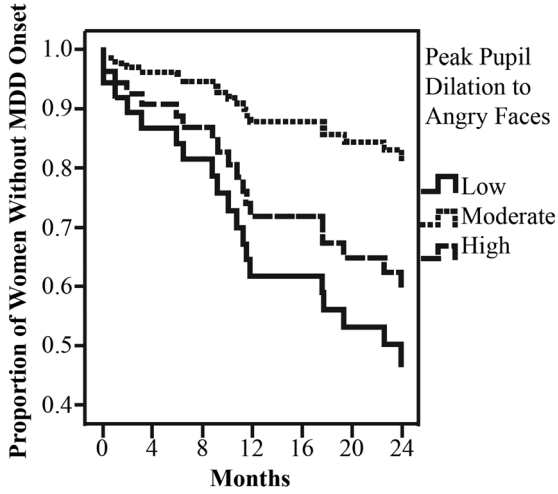

Figure 3. Results of the survival analysis using Cox regression model predicting prospective onset of MDD based on peak pupil dilation to angry faces.

increased peak pupil dilation. Specifically, the odds of participants with low peak pupil dilation to sad faces experiencing MDD recurrence were approximately five times higher than participants with higher peak pupil dilation to sad faces. When the quadratic effect was added in the second step, it was not significant, $\beta = -5.43$, Wald = 1.88, $p = .17$. To visually depict the significant linear effect, we plotted the results for women in the upper and lower quartiles of pupillary reactivity to sad faces. As shown in Figure 2, women who evidenced lower, compared to higher, peak dilation to sad faces experienced a shorter time to MDD recurrence over the follow-up. To examine the robustness of these findings and the predictive validity of peak pupil dilation beyond baseline symptoms, we tested whether the effect would be maintained when we statistically controlled for the influence of baseline levels of depression and anxiety. Although these results were maintained when we statistically controlled for the impact of anxiety symptoms (BAI), $\beta = -1.82$, Wald = 4.16, $p = .04$, they were reduced to a nonsignificant trend when we statistically controlled for the baseline depressive symptoms (BDI-II), $\beta = -1.50$, Wald = 2.78, $p = .10$.

Focusing next on the predictive validity of peak pupil dilation to angry faces, we found that the linear effect of participants' pupil peak dilation to angry faces was not significant in the first step of the survival analysis, $\beta = -.90$, Wald = .37, $p = .54$. However, when the quadratic effects of peak pupil dilation were added in the second step, it was significant, $\beta = 16.32$, Wald = 8.60, $p = .003$. To visually depict these findings, we plotted the results for women in the upper and lower tertiles of peak pupil dilation to angry faces, as well as those at the median. As can be seen in Figure 3, women exhibiting either high or low peak pupil dilation to angry faces, compared to those exhibiting more moderate peak pupil dilation, were at greatest risk for MDD recurrence during the follow-up. This result was maintained when we statistically controlled for the impact of current depressive, $\beta = 12.34$, Wald = 9.81, $p = .002$, or anxiety, $\beta = 9.22$, Wald = 5.55, $p = .02$, symptoms, suggesting that it was at least partially independent of participants' current mood at the baseline assessment. Given the significant results observed for both the linear effect of peak pupil dilation to sad faces and the quadratic effect of peak pupil dilation to angry faces, we included both in the same survival analysis (as well as the linear

effect of peak pupil dilation to angry faces) to determine if both were uniquely predictive of MDD risk. In this analysis, the quadratic effect of peak pupil dilation to angry faces remained significant, $\beta = 15.65$, Wald = 8.49, $p = .004$, but the linear effect of peak pupil dilation to sad faces was reduced to nonsignificant, $\beta = -1.61$, Wald = 3.30, $p = .07$, suggesting that peak pupil dilation to angry faces was a stronger predictor of MDD risk in this sample. In regard to participants' peak pupil dilation to happy faces, the results showed no significant linear, $\beta = .69$, Wald = .44, $p = .51$, or quadratic, $\beta = -5.74$, Wald = 1.27, $p = .26$, effects on MDD recurrence.

To examine the unique contribution of peak pupil dilation to each of the three emotions, we conducted two additional survival analyses, one that included linear effects for each emotion type (to examine the robustness of our finding for pupil dilation to sad faces) and one that included both linear and quadratic effects of peak pupil dilation to all three emotions (to examine the robustness of findings for pupil dilation to angry faces). We found that, when only linear effects of peak pupil dilation were included in the survival analysis, decreased peak pupil dilation to sad faces was the only significant predictor of shorter time to MDD recurrence ($\beta = -1.74$, Wald = 3.71, $p = .05$), but not peak pupil dilation to angry ($\beta = -.72$, Wald = .21, $p = .65$) or happy ($\beta = 1.24$, Wald = 1.33, $p = .25$) faces. However, when quadratic effects of peak pupil dilation to all three emotions were included in the second analysis, the quadratic effect of peak pupil dilation to angry faces was the only significant predictor of time to depression recurrence ($\beta = 15.31$, Wald = 7.14, $p = .008$), but not the quadratic effects of peak pupil dilation to happy ($\beta = -8.85$, Wald = 2.26, $p = .13$) or sad ($\beta = -5.13$, Wald = 1.58, $p = .21$) faces. We should also note that, once the quadratic effects were added to the model, the significant linear effect of peak pupil dilation to sad faces was reduced to nonsignificant ($\beta = -1.30$, Wald = 1.12, $p = .29$). To provide a more practical example of the predictive validity of women's peak pupil dilation to angry faces, a total of 47.4% of women who evidenced low peak pupil dilation to angry faces (lowest tertile) and 36.8% of women who evidenced high peak pupil dilation to angry faces (highest tertile) experienced MDD recurrence during the 2-year follow-up, compared to 15.8% of women who evidenced moderate peak pupil dilation to angry faces (middle tertile).

Discussion

In this study, we examined whether pupillary reactivity to negative stimuli could be used to determine which women with a prior history of MDD are at greatest risk for recurrence. Building from evidence that MDD and MDD risk may be associated with both hyper- and hyporeactivity to negative stimuli, we examined both linear and nonlinear effects of peak pupil dilation. We found that, among women with a history of prior MDD, risk of MDD recurrence was greatest among those who exhibited either hyper- or hypopeak pupillary reactivity angry facial stimuli, compared to those who exhibited a more moderate peak pupillary response during a 2-year follow-up. These findings were maintained when we statistically controlled for the influence of depressive and anxiety symptom levels at baseline, suggesting that peak pupil dilation to angry faces contributes unique risk for MDD recurrence beyond current symptom elevations. It was also maintained when we statistically controlled for the influence of peak pupil dilation to sad faces, suggesting that it is uniquely predictive of MDD risk. Although we also found that decreased peak pupil dilation to sad stimuli prospectively predicted MDD recurrence, this finding appeared to be less robust. Specifically, although the findings were maintained when we statistically controlled for baseline anxiety symptoms, it was reduced to a nonsignificant trend when we statistically controlled for baseline depressive symptoms and when we controlled for peak pupil dilation to angry faces. Future research is needed, therefore, to determine the role of pupillary reactivity to sad faces in (women's) risk for MDD recurrence. Finally, consistent with previous research (Burkhouse et al., 2015) and our prediction, peak pupil dilation in response to viewing happy faces was not a significant predictor of depression onset.

The current findings extend previous research in several important ways. First, the findings are consistent with research showing that depression is associated with dysregulated physiological reactivity to negative stimuli (e.g., Mayberg, 2003). Although the precise neural mechanisms underlying these effects cannot be determined because the pupil is enervated by the connections from multiple brain regions (Critchley et al., 2005; Siegle et al., 2011; Urry et al., 2006), they are consistent with the a priori hypothesis that both hypo- and hyperreactivity in DLPFC, VMPFC, ACC, and the LC-NA system would be associated with depression. Specifically, findings from neuroimaging studies reported that both hypo- (Fales et al., 2009; Ritchey, Dolcos, Eddington, Strauman, & Cabeza, 2011; Siegle, Thompson, Carter, Steinhauer, & Thase, 2007) and hyperactivity (Bermpohl et al., 2009; Elliott, Rubinsztein, Sahakian, & Dolan, 2002; Grimm et al., 2009) in the DLPFC was linked to MDD. Findings from the studies that examined activity in VMPFC were also similar and suggest that MDD is associated with both hyper- (Keedwell et al., 2005) and hypoactivity (Guo et al., 2015) during emotive processing. Similarly, both hypo- (Davidson et al., 2003; Kumari et al., 2003) and hyperactivity (Gotlib et al., 2005; Tao et al., 2012) in the ACC were associated with MDD. Additionally, previous studies have suggested that both hypo- and hyper-LC-NA activity was associated with depression (Ressler & Nemeroff, 2000). Relying on previous research that employed pupil dilation as a peripheral marker of LC-NA system functioning (Gilzenrat et al., 2010; Murphy et al., 2014), the current report provides initial support that, to the extent that pupil dilation reflects LC-NA functioning and activation of DLPFC, VMPFC, and ACC, both hypo- and hyperactivity in response to angry facial stimuli prospectively predict risk for MDD relapse among at-risk individuals.

The current findings also suggest that pupillary reactivity to negative stimuli (perhaps specifically angry faces) may be a promising biomarker of risk for MDD recurrence among women with a history of MDD. Specifically, in the current study, peak pupil dilation in response to angry faces was a more robust predictor of depression onset than peak pupil dilation to sad faces. Most studies that found differences in pupillary reactivity in individuals with and without depression employed negative words without assigning them to sad or angry categories (e.g., Steidtmann, Ingram, & Siegle, 2010). Studies that used images of faces found that pupillary reactivity specifically to sad faces predicted depressive symptoms and onset among at-risk children (Burkhouse et al., 2015). However, recent research on attention biases among individuals with remitted depression found evidence for selective attention specific to angry faces (Sears, Newman, Ference, & Thomas, 2011; Woody, Owens, Burkhouse, & Gibb, 2015). Thus, together with previous research, our findings suggest that, among individuals with a history of MDD, physiological reactivity to stimuli that evoke feelings of guilt or indicate interpersonal rejection (e.g., angry faces) may be a better prediction of recurrence than physiological reactivity to

self-referential stimuli (e.g., sad faces). If replicated, these findings could aid clinicians in identifying which individuals with a prior MDD history are at a greater risk of recurrence using an inexpensive and convenient methodology. This could help focus the limited clinical resources and direct aid to the population who are at the highest risk of MDD recurrence.

The present study had several strengths, including the longitudinal design and focus on a readily accessible and easily measurable physiological marker (Siegle, Steinhauer, Stenger et al., 2003). Although firm conclusions must await replication, the current findings provide promising initial evidence that peak-to-negative stimuli could serve as a biological marker of depression risk in adult women with a history of MDD and may help identify which of those individuals are at greatest risk for MDD recurrence. Despite these strengths, the current study has a number of limitations, which could provide direction for future research. First, the current study focused only on risk for MDD relapse and did not examine whether pupillary reactivity may also predict first onsets. Thus, future studies are needed to determine whether peak pupil dilation to negative stimuli may be a biomarker of risk for both recurrence and first onsets of MDD. Second, only 19 women experienced MDD recurrence during follow-up in our sample, and the findings need to be replicated by future studies using larger samples. Third, the current sample was primarily Caucasian, middle-class women, and future research is needed to determine whether the results will generalize to other populations. Additionally, the images were not equated on luminance, and there were significant differences in relative luminance based on emotion category. However, the effects associated with light reflex were removed prior to analyses. Moreover, the main research question of this study was focused on individual differences in peak pupil dilation to images within each emotion category. Lastly, given that the pupil is enervated by connections from multiple brain areas, we are unable to clarify a specific brain mechanism underlying the findings. Additionally, it remains unclear whether the same brain areas are involved in the association between both hypo- and hyperpeak pupil reactivity and MDD recurrence or whether different brain areas underlie the link between hypo- versus hypopeak pupil reactivity to angry faces and MDD recurrence.

In summary, the current findings extend previous research and suggest that pupillary hypo- or hyperreactivity to angry faces stimuli may be a promising biomarker of risk for MDD recurrence in at-risk populations. This is the first study that has examined whether disrupted physiological reactivity to negative stimuli prospectively predicted depression recurrence among women with a prior history of MDD. If replicated, these findings suggest that pupillary reactivity could be used as a biomarker to determine which at-risk individuals are at greatest risk for recurrence so that limited intervention resources could be targeted to those most at need.

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