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Flashing a smile: Startle eyeblink modulation by masked affective faces

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Abstract

Affective faces are important stimuli with relevance to healthy and abnormal social and affective information processing. The aim of this study was to investigate the effect of brief presentations of affective faces on attention and emotional state across the time course of stimulus processing, as indexed by startle eyeblink response modulation. Healthy adults were presented with happy, neutral, and disgusted male and female faces that were backward masked by neutral faces. Startle responses were elicited at 300, 800, and 3,500 ms following stimulus presentation to probe early and late startle eyeblink modulation, indicative of attention allocation and emotional state, respectively. Results revealed that, at 300 ms, both face expression and face gender modulated startle eyeblink response, suggesting that more attention was allocated to masked happy compared to disgusted female faces, and masked disgusted compared to neutral male faces. There were no effects of either face expression or face gender on startle modulation at 800 ms. At 3,500 ms, target face expression did not modulate startle, but male faces elicited larger startle responses than female faces, indicative of a more negative emotional state. These findings provide a systematic investigation of attention and emotion modulation by brief affective faces across the time course of stimulus processing.

KEYWORDS
affective faces, masking, startle eyeblink

1 | INTRODUCTION

While affective faces are often used to study attentional and emotional processes in healthy and disordered populations (Cullen et al., 2015; Duval, Hale et al., 2013; Hess, Sabourin, & Kleck, 2007; Moser, Huppert, Duval, & Simons, 2008; Tseng et al., 2015), few studies have specifically examined the time course of attentional and emotional mechanisms involved in affective face viewing. The defense cascade model (Lang, Bradley, & Cuthbert, 1997) has been developed with respect to emotional scenes, and can be applied to affective faces to describe a timeline of emotional stimulus processing. This timeline begins with an orienting response, involving attention capture by the stimulus, followed by the emergence of an emotional state. Multiple studies have examined emotional scene processing over time, and report findings consistent with this model. Specifically, these studies have documented two response windows: the early window (~30–1,000 ms after stimulus presentation) when orientation of attention to an affective stimulus occurs, and the late window (~1,000 ms through the end of stimulus presentation), when a sustained emotional state occurs (Bradley, Codispoti, & Lang, 2006; Bradley, Cuthbert, & Lang, 1993).

Much of the work examining affective stimulus processing within the framework of the defense cascade model has used the startle eyeblink response to probe both attentional and affective processing during each of the response windows. Responses elicited during the early window index the amount of attention allocated to a stimulus via the process of

prepulse inhibition of startle (PPI; Blumenthal, 1999). PPI can be modulated by the amount of attention that is engaged by the prepulse, with greater attention resulting in increased PPI effect (DelPezzo & Hoffman, 1980; Elden & Flaten, 2002; Filion & Poje, 2003). By the end of this early window (500–1,000 ms lead intervals), PPI typically returns to baseline, suggesting that the attentional resources initially engaged by the emotional stimulus have by this time been disengaged (Bradley et al., 1993, 2006). During the late window, responses can be used to index a sustained emotional state (affective modulation of startle, AMS; Bradley et al., 1993, 2006; Cuthbert, Bradley, & Lang, 1996; Dichter, Tomarken, & Baucom, 2002). Startle responses are largest while viewing a stimulus with negative affective valence, smallest while viewing a stimulus with positive affective valence, and intermediate while viewing an affectively neutral stimulus.

While most of this work has been done with pictures of affectively positive, negative, and neutral scenes, comparable findings are emerging from studies using affective faces. Startle responses elicited between 3,000 and 6,000 ms, corresponding to the late time window, are larger while viewing negative, compared to neutral or positive, facial expressions (Anokhin & Golosheykin, 2010; Springer, Rosas, McGetrick, & Bowers, 2007). This effect may be limited by the gender of the depicted face, as Hess et al. (2007) found this effect only for male faces. The studies examining face processing typically examine startle response modulation only during the late time window. Thus, we conducted a study to investigate startle response modulation across the time course of processing (Duval, Lovelace, Aarant, & Filion, 2013). During the early window, face gender and expression interacted to modulate startle responses, such that more attention was allocated to angry compared to neutral male faces, and to happy and neutral compared to angry female faces, as indexed by greater amounts of PPI. During the late window, startle response magnitude was facilitated by affective (happy and angry) male faces compared to neutral male faces, while no modulation was observed for female faces. These results suggest that affective faces can elicit both attentional and affective responses in a manner consistent with the defense cascade model, and these responses can be effectively probed using the startle response. In addition, differences in reactivity to male and female faces may wash out some of the effects related to face expression. Thus, including face gender as a factor of interest in analyses of face processing is crucial.

Recent work examining affective stimulus processing has examined attention and emotional processes elicited by subliminally presented stimuli. Backward masking is a common technique that involves presenting a target stimulus at a short duration followed immediately by a masking stimulus (Esteves & Ohman, 1993). Many studies have found that subliminal fearful and positive scenes modulate physiological responses (ERPs, startle response, cardiac defense; Del Zotto

& Pegna, 2015; Liddell, Williams, Rathjen, Shevrin, & Gordon, 2004; Ruiz-Padial, Mata, Rodriguez, Fernandez, & Vila, 2005; Ruiz-Padial & Vila, 2007) and activity in brain regions involved in affective processing (e.g., the amygdala; Baskin-Sommers et al., 2015; Cui et al., 2014; Dannlowski et al., 2007; Killgore & Yurgelun-Todd, 2005; Whalen et al., 1998). Backward-masked face paradigms have also been used to understand social threat processing in multiple clinical populations, including posttraumatic stress disorder (Rauch et al., 2000), trait anxiety (Doty, Japee, Ingvar, & Ungerleider, 2013; Etkin et al., 2004), generalized anxiety disorder (Monk et al., 2008), and social anxiety disorder (Jusyte & Schönenberg, 2014). These findings demonstrate that masked faces can elicit affective responses similar to longer presentations of emotional stimuli and affective faces.

The purpose of the present study was to examine whether the time course of attention and emotional state elicited by affective faces occurs in response to subliminally presented affective faces. We specifically examined startle responses to masked affective faces at time points sensitive to attention allocation, attention disengagement, and sustained emotional state. Given the previously reported face gender effects (Hess et al., 2007), we examined the effect of face gender, along with face expression, on startle response magnitude. We elicited the startle response at lead intervals of 300, 800, and 3,500 ms during masked affective face presentations. We predicted that patterns of attentional engagement and emotional state elicited by masked faces would mirror those reported in other studies of affective stimulus viewing. Specifically, we expected that, during the early processing window (300-ms lead interval), attention allocated to masked faces would be modulated by face expression, such that masked affective faces will require more attentional resources than masked neutral faces, as indexed by significant increases in PPI to positive and negative compared to neutral faces. We predicted that, at the 800-ms lead interval, attention that had been allocated to the faces at 300 ms would be disengaged, resulting in a lack of PPI (consistent with prior research using nonmasked affective scenes; Bradley et al., 2006). We predicted that, during the late time window (3,500-ms lead interval), the gender of the face would play a role in modulating startle responses, such that male faces would be perceived as more negative than female faces and would elicit more robust AMS, in line with previous literature (Duval, Lovelace et al., 2013; Hess et al., 2007).

2 | METHOD

2.1 | Participants

Thirty-one undergraduate students participated in this study. Eight participants in total were excluded from the final analysis. One participant was missing more than 50% of the data

due to noisy EMG signal and spontaneous blinks within the scoring windows. Seven participants were defined as nonresponders (zero magnitude on more than 50% trials in any condition, despite robust spontaneous blinks). Response rates for nonresponders were low (range = 2–25%), compared to response rates for the participants we retained (range = 81–100%). Response rates did not differ between trial types ($p > .37$). The final sample consisted of 23 participants ranging in age from 18 to 25 years old ($M = 20.48$, $SD = 1.81$), with 18 (78.3%) women. Seventeen (74%) self-identified as White/Caucasian, 3 (13%) as Black/African American, 2 (9%) as Asian, and 1 (4%) as Hispanic.

2.2 | Stimuli

Male and female Caucasian faces with disgusted, happy, and neutral expressions were selected from a standardized face set (Matsumoto & Ekman, 1988). While many studies have used faces depicting anger, disgust expressions have also been found to elicit perceptions of physical or social threat (Burklund & Lieberman, 2007), eliciting similar or larger neural and startle responses compared to angry expressions (Burklund & Lieberman, 2007; Yartz & Hawk, 2002). Thus, we chose disgust faces in this study to probe reactivity to negative affective expressions. The intensity of the emotion depicted in the happy ($M_{male} = 5.4$, $SD_{male} = 0.04$; $M_{female} = 6.1$, $SD_{female} = .03$) and disgust ($M_{male} = 5.5$, $SD_{male} = 0.03$; $M_{female} = 5.4$, $SD_{female} = 0.28$) faces were matched based on the subjective ratings provided with the stimulus set ($ps > .15$).

2.3 | Procedures

Study procedures were approved by the University of Missouri–Kansas City Institutional Review Board, and all participants provided written consent prior to participating. Participants were then prepared for electromyography (EMG) recording. Their skin was cleansed and two reusable 4-mm electrodes were placed along the orbicularis occuli muscle, with two below the left eye (one directly below the pupil and the other ~1 cm lateral to the first), and one was placed over the mastoid bone behind the left ear to serve as a ground (Blumenthal et al., 2005). Data were collected in a sound-attenuating chamber using a Biopac MP150 data acquisition system with the EMG100C amplifier module (Biopac Systems, Inc., Goleta, CA), controlled by an IBM-compatible computer using the AcqKnowledge software version 3.8.1 for Windows (Biopac Systems, Inc.). Data were sampled at 2,000 Hz, amplified online by a factor of 5,000, filtered online using a band-pass filter (-3 dB low-pass cutoff frequency of 500 Hz and attenuation rate of 20 dB per octave; −3 dB high-pass cutoff frequency of 10 Hz and attenuation rate of 20 dB per octave), and integrated offline with a time constant of 10 ms (20 samples). The acoustic startle stimulus was a 105 dB SPL(A) broadband noise burst (50-ms duration, near-instantaneous onset and offset) presented binaurally through headphones (Telephonics, model TDH-49).

Participants were presented with pictures of 12 individuals, four displaying each of disgusted, happy, and neutral expressions (Matsumoto & Ekman, 1988). There were two male and two female faces in each affective category. Each trial began with a 2,000-ms fixation cross followed by presentation of an affective face for 17 ms, immediately replaced by a neutral face mask for 4,983 ms, which was a mirror image of the same model depicted in the target image. The duration of the mask was longer than in some prior studies using masked faces (Kim et al., 2010), but was consistent with studies using startle eyeblink to assess perception of unmasked affective scenes (Bradley et al., 2006) and faces (Anokhin & Golosheykin, 2010; Duval, Lovelace et al., 2013). The startle-eliciting stimulus was presented 300 ms, 800 ms, or 3,500 ms after the onset of the mask. Intertrial intervals (ITIs) ranged from 5 to 10 s (random, square distribution). Each of the 12 pictures was probed at one of the three lead intervals for a total of 36 startle responses elicited during face stimuli. An additional 12 startle probes were presented during the ITI period preceding a face stimulus (the following face stimulus was not probed on these trials to avoid excessive startle habituation) to obtain a baseline level of responding. The onset of the ITI startle probes was randomly determined, and no startle probe was presented within 1,000 ms of the beginning or end of the ITI window to ensure that the onset or offset of the pictures would not serve as a visual pre-pulse on these baseline trials. This resulted in a total of 48 startle probes across the paradigm. Trials were presented in a pseudorandom order such that no trial type (Lead Interval × Facial Expression) was presented more than two consecutive times. Trials were also divided into four blocks, with each trial type (Lead Interval × Facial Expression) appearing once during each block with an equal number of male and female faces to ensure even distribution of trials across the session. After the startle trials were concluded, participants were asked to report their subjective awareness of the masked faces with a series of questions from Kim et al. (2010). Participants were asked to first "describe the images you saw during the experiment," then "describe the expressions of the faces," and finally "report if you saw disgusted or happy faces." While all participants described seeing faces, only five indicated that they saw some semblance of affective expression, suggesting they were aware of the masked target faces. All analyses described below were run with and without these "aware" participants.

2.4 | Data scoring and analysis

Peak startle amplitude was defined as the largest integrated value within a window of 20–150 ms following the onset of

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the acoustic startle stimulus. These values were averaged for each participant within each experimental condition, including trials with no discernable blink as zero, to yield a measure of response magnitude. Trials with responses greater than 3 standard deviations from the mean for each stimulus type were excluded as outliers. Trials with invalid responses (response outside response window, spontaneous blinks interfering with response window, or significant noise in the EMG signal) were not scored. This resulted in a loss of 7% of the total trials.

Statistical effects were explored using a 3 (Lead Interval: 300, 800, 3,500) × 3 (Face Expression: happy, neutral, disgust) × 2 (Face Gender: male, female) repeated measures analysis of variance (ANOVA) on average response magnitude.[1] Additional two-way and one-way repeated measures ANOVAs were used to follow up significant main effects and interactions. Pairwise comparisons were submitted to Bonferroni correction in SPSS, and a corrected alpha level of .05 was used for all statistical comparisons. Greenhouse-Geisser corrections were used for all ANOVAs.

3 | RESULTS

Five participants endorsed the subjective awareness questions, indicating that they were aware of some aspect of the target faces. All following analyses were run with and without these five participants, and the response patterns remained in the same direction.[2] The analyses including all participants are reported here. As expected, the 3 (Lead Interval: 300, 800, 3,500) × 3 (Face Expression: happy, neutral, disgust) × 2 (Face Gender: male, female) ANOVA revealed a significant three-way interaction, $F(2.43, 48.61) = 6.15$, $p = .002$, $\eta_p^2 = .24$. In addition, we observed a Lead Interval × Face Gender interaction, $F(1.74, 34.83) = 7.71$, $p = .002$, $\eta_p^2 = .28$, and a main effect of lead interval, $F(1.84, 36.79) = 11.25$, $p = .000$, $\eta_p^2 = .36$.

To follow up the Lead Interval × Face Gender interaction, we conducted two ANOVAs to examine differences across lead intervals for each face gender condition separately. For male faces, the effect of lead interval revealed

larger startle responses at the 3,500-ms lead interval ($M = 189.6$) compared to the 800-ms ($M = 121.0$; $p = .002$) and 300-ms ($M = 101.99$; $p = .000$) lead intervals. There was no difference in startle response magnitude between the 800-ms and 300-ms lead intervals ($p = .197$). For female faces, startle magnitude only differed between the 3,500-ms ($M = 151.45$) and 300-ms ($M = 104.84$; $p = .022$) lead intervals, with no difference between the 800-ms lead interval and the other two lead intervals ($ps > .191$).

To follow up the significant three-way interaction, we conducted 2 (Face Gender: male, female) × 3 (Face Expression: disgust, neutral, happy) ANOVAs at each lead interval. Additional one-way ANOVAs with Bonferroni-corrected pairwise comparisons were used to follow up significant effects.

3.1 | Early window (300-, 800-ms lead intervals)

Figure 1, left and center, present average magnitude of startle responses probed at 300 and 800 ms, respectively. A 2 (Face Gender: male, female) × 3 (Face Expression: disgust, neutral, happy) factorial ANOVA on mean startle magnitude elicited at 300 ms revealed a significant main effect of face expression, $F(1.89, 41.47) = 5.00$, $p = .013$, $\eta_p^2 = .19$, and a significant Face Gender × Face Expression interaction, $F(1.50, 33.03) = 6.54$, $p = .008$, $\eta_p^2 = .23$. To follow up this interaction, we conducted ANOVAs to examine differences across face expression for each face gender condition separately. For male faces, the effect of face expression revealed attenuated startle responses to disgust ($M = 70.7$) compared to neutral ($M = 134.6$; $p = .017$) faces. No other comparisons were significant ($ps > .14$). For female faces, the effect of face expression revealed attenuated startle responses to happy ($M = 68.3$) compared to disgust ($M = 143.6$; $p = .010$) faces, and a trend toward attenuated responses to happy compared to neutral ($M = 102.6$; $p = .072$) faces. The difference between disgust and neutral faces was not significant ($p = .265$).

As expected, a 2 (Face Gender: male, female) × 3 (Face Expression: disgust, neutral, happy) factorial ANOVA revealed no significant main effects or interactions for average startle response magnitude at the 800-ms lead interval (all $ps > .08$).

3.2 | Late window (3,500-ms lead interval)

A 2 (Face Gender: male, female) × 3 (Face Expression: disgust, neutral, happy) factorial ANOVA revealed a significant main effect of face gender on startle response magnitude at the 3,500-ms lead interval, $F(1, 21) = 12.25$, $p = .002$, $\eta_p^2 = .37$ (see Figure 1, right). Startle response magnitudes
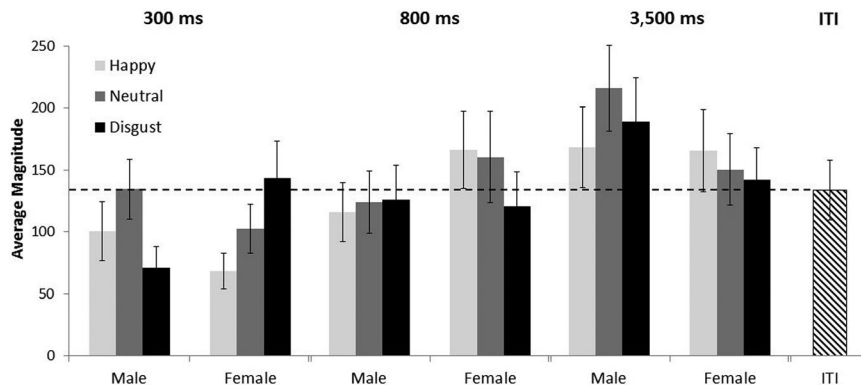
FIGURE 1 Average startle response magnitude for each lead interval (300, 800, 3,500 ms) during happy, neutral, and disgusted male and female facial expressions. Error bars indicate standard error of the mean. The striped bar and dotted line represent the average baseline startle response during inter-trial intervals

were greater while viewing male ($M = 190.29$) compared to female ($M = 147.68$) faces, $t(22) = 4.41$, $p = 002$. No other main effects or interactions at this lead interval were significant (all $ps > .13$).

4 | DISCUSSION

The purpose of this study was to examine the time course of attention allocation and emotional state in response to masked affective faces. We specifically examined the effects of face expression and face gender on startle responses elicited during early and late processing windows to probe attention and emotion, consistent with the defense cascade model (Lang et al., 1997).

Results revealed that at the 300-ms lead interval, face gender interacted with face expression, such that more attention was allocated to masked happy compared to disgusted female faces, and more attention was allocated to masked disgusted compared to neutral male faces. This supports the hypothesis that masked affective faces modulate attention differently for faces of different genders. These findings are partially consistent with our previous study examining the time course of responding to unmasked happy, angry, and neutral faces (Duval, Lovelace et al., 2013). While our previous findings are consistent with the current findings for male faces—greater attention allocation to negative (disgust and angry) compared to neutral faces—the patterns for female faces were slightly different between the studies. For example, while the current results demonstrate greater attention allocation to happy compared to disgusted female faces, our previous study found greater attention allocation to both happy and neutral compared to angry female faces. It is possible that the use of disgusted instead of angry faces resulted in slightly different patterns of modulation. However, based on the pattern of our results, and given that male faces are typically perceived as more negative than female faces, regardless of expression (Becker, Kenrick, Neuberg,

Blackwell, & Smith, 2007), our specific findings could be interpreted to suggest that the greatest amount of attention was allocated to the most positive (happy females) and most negative (disgusted male) faces. At the 800-ms lead interval, face expression and face gender had no effect on startle response magnitude, suggesting that any attentional resources allocated at 300 ms had been disengaged by this time (Bradley et al., 1993).

During the late lead interval (3,500 ms), startle response magnitude was facilitated in response to male faces compared to female faces. This finding suggests that male faces were perceived as more aversive than female faces, regardless of expression, consistent with previous reports that male faces are associated with more negative affect compared to female faces (Becker et al., 2007). However, this is not consistent with other evidence that startle responses are modulated by affective expression at the late lead interval (Anokhin & Golosheykin, 2010; Duval, Lovelace et al., 2013; Hess et al., 2007). It is important to note that, since target and mask pairs depicted the same gender, any effects of gender cannot be attributed to the target face, and are most likely the result of perception of the mask. The lack of modulation by facial expression could indicate that brief presentations of masked faces do not impact emotional state occurring during the late time window. It is also possible that the effect of face expression on startle response modulation is less robust than the effect of other types of emotional stimuli. In fact, previous studies do report that face stimuli are less arousing and elicit blunted affective responses, when compared directly to emotional scenes (Wangelin, Bradley, Kastner, & Lang, 2012).

A number of limitations should be considered when interpreting the findings. First, due to a relatively small and unbalanced sample, we were unable to investigate differences related to participant gender. Given previous findings that participant gender impacts responses to affective faces (Anokhin & Golosheykin, 2010), future studies should

investigate differences between male and female participants in response patterns to masked affective male and female faces. Second, based on our assessment of target face awareness, it appears that the masking paradigm used in this study may not have resulted in truly subliminal presentations for all participants. While the removal of the "aware" participants did not alter the patterns of results, it is important to interpret the findings reported here accordingly. Third, the faces we chose in this study may limit the generalizability of our findings to other face expressions. While many studies use angry facial expressions, we chose to use disgust faces because of their potential applicability to disorders like social anxiety. It is important to refrain from drawing conclusions about reactivity to all negative facial expressions based on the results of this study. In addition, because there were multiple variables of interest (lead interval, face expression, face gender), the number of trials per condition was low. While the paradigm used reduced the likelihood of excessive habituation to the startle probes and face stimuli, it may have resulted in less reliable averages across conditions and a more stringent threshold for removing participants due to missing data. While other similar studies examining startle reactivity to affective stimuli also reported using a small number of trials per condition (Bradley et al., 2006), they used unmasked images with highly arousing content (e.g., International Affective Picture System, IAPS). Lastly, the current investigation was limited to the use of startle eyeblink response modulation as a measure of attention allocation and emotional state. To accommodate the range of startle probe onset latencies across early and late windows, the mask was presented much longer than in traditional masked stimulus paradigms. This could have impacted attentional and emotional responses, making it difficult to make direct comparisons between the current findings and other studies reporting on the time course of unmasked stimulus processing. It will be interesting in future studies to replicate these findings using ERPs and fMRI to further elucidate the time course and affective reactivity elicited by affective faces.

The findings of the current study are the first to demonstrate startle response modulation by masked affective faces across the time course of stimulus processing, allowing for assessment of both attention allocation and emotional state in the same paradigm. Specifically, with respect to the defense cascade model (Lang et al., 1997), attention was modulated by both the expression and gender of masked faces in the early time window, while emotional state was modulated by face gender during the late time window. These patterns of results replicate previous findings that very brief presentations of affective faces, outside of subjective awareness, can influence attentional engagement during early processing. These effects dissipate after about 800 ms, and the effects of

facial expression do not extend to the later time points when affective modulation occurs (although face gender did modulate emotional state). These findings lend support to the use of masked affective faces to probe early processes, like attention allocation, and present a more detailed timeline of the impact of subliminally presented faces on emotional processing.

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