Distribution Agreement

Meghan Hickey March 18, 2016

Can pupillometry dissociate fear and disgust? Trypophobia as a test case.

by

Meghan Hickey

Stella F. Lourenco
Adviser

Psychology Department

Stella F. Lourenco

Adviser

Roberto Franzosi

Committee Member

Scott O. Lilienfeld

Committee Member

2016

Can pupillometry dissociate fear and disgust? Trypophobia as a test case.

By

Meghan Hickey

Stella F. Lourenco

Adviser

An abstract of
a thesis submitted to the Faculty of Emory College of Arts and Sciences
of Emory University in partial fulfillment
of the requirements of the degree of
Bachelor of Arts with Honors

Psychology Department

2016

Abstract

Can pupillometry dissociate fear and disgust? Trypophobia as a test case.

By Meghan Hickey

Anxiety in response to objects with a cluster of holes has come to be known as trypophobia. Recent research suggests that such objects share low-level visual properties with evolutionarily threatening stimuli, namely snakes and spiders (Cole & Wilkins, 2013). The current consensus is that, like snakes and spiders, the discomfort associated with holes is rooted in fear. However, self-reports from individuals with trypophobia suggest that this anxiety may instead reflect a disgust reaction. Yet, fear and disgust are difficult to disambiguate behaviorally because both involve an avoidance response (Woody & Teachman, 2000). In the current study, we used pupillometry to test whether trypophobia is rooted in fear or disgust. We predicted that if trypophobic stimuli elicited fear, then they should invoke a sympathetic response, which is associated with pupil dilation; alternatively, if trypophobic stimuli elicited disgust, then they should invoke a parasympathetic response, which is associated with pupil constriction (Granholm & Steinhauer, 2004). To dissociate fear and disgust, in a first experiment participants passively viewed a slideshow of trypophobic, neutral, and threatening images. Next to control for the effect of spatial frequency, in a second experiment participants viewed a slide show of trypophobic images, threatening images, and images with low-level visual details similar to holes. Participants also completed emotional questionnaires and rated each of the images on fear, disgust, anxiety, and arousal to explore the connection between subjective and physiological responses to these stimuli. Pupillary responses across the image categories were compared for each experiment. In experiments 1 and 2, we found that holes elicited significantly more pupil constriction than threatening images, suggesting that the two are dissociable and trypophobia may be rooted in disgust. However, experiment 2 showed that the pupil responses to holes were not different from the low-level visual controls, suggesting that the difference between threat and holes is due to spatial frequency, not emotion. Lastly, correlations between image ratings and emotional questionnaires showed much overlap in the subjective reports of fear and disgust in the threatening and hole categories, suggesting that perhaps these anxiety responses are rooted in a combination of fear and disgust.

Keywords: anxiety disorders, trypophobia, fear, disgust, pupillometry, spatial frequency

Can pupillometry dissociate fear and disgust? Trypophobia as a test case.

By

Meghan Hickey

Stella F. Lourenco
Adviser

A thesis submitted to the Faculty of Emory College of Arts and Sciences
of Emory University in partial fulfillment
of the requirements of the degree of
Bachelor of Arts with Honors

Psychology Department

2016

Acknowledgements

Firstly, I offer a thank you to my advisor, Stella F. Lourenco, and all the members of the Spatial Cognition lab, namely Vlad Ayzenberg, for supporting me in doing this work. I appreciate all the suggestions and comments you each gave me throughout. Secondly, I would like to thank my two additional committee members, Roberto Franzosi and Scott O. Lilienfeld, for reading my thesis and attending my defense. Lastly, I would like to recognize my family and friends for supporting me while I pushed myself through the demanding moments in this research project. I am very proud to be presenting my thesis to the Faculty of Emory College of Arts and Sciences, and I am grateful for all these individuals that supported me in each step of the project.

Table of Contents

Can pupillometry dissociate fear and disgust? Trypophobia as a test case.

Anxiety disorders are the most common mental illness in the United States, affecting 18% of the population (NIMH, 2015). Individuals with anxiety disorders have irrational aversive reactions to situations that would otherwise be interpreted as nonthreatening (Barlow, 2002; Beck & Emery, 2005). Yet, the mechanisms underlying many of humans' basic anxieties are unclear (Cisler, Olatunji, & Lohr, 2009). Anxiety is the body's response to the overwhelming negative emotional and cognitive characteristics attributed to a particular situation or object (Beck & Emery, 2005). However, there are debates about which negative emotions contribute to the dysfunctional processing (Etkin & Wager, 2007) because in cases of animal phobias (e.g., arachnophobia, ophidiophobia) the driving emotion is fear (Sarlo, Palomba, Angrilli, & Stegano, 2002), but in other cases, such as blood-injection-injury type phobia, the driving emotion is disgust (Hermann et al., 2007). Although fear and disgust are both phobias and elicit general avoidance responses, they have distinct psychological and physiological underpinnings (Kreibig, 2010). Fear is a response to perceived danger, whereas disgust is an avoidance of contamination (Davey, Forster, & Mayhew, 1993; Woody & Teachman, 2000). Additionally, fear and disgust each elicit distinct autonomic nervous system responses. These different responses in turn cause pupil dilation in the case of fear and pupil constriction in the case of disgust. In the current study, we use pupillometry to investigate how these two emotions contribute to another phobia with more ambiguous emotional underpinnings, trypophobia (Cole & Wilkins, 2013).

The ambiguity between fear and disgust is especially apparent in the case of trypophobia because recent research suggests it is a fear response, whereas self-reports suggest it is a disgust response. Sufferers of trypophobia have adverse reactions to images containing holes (see Figure

1a), reporting anxiety and discomfort (Cole & Wilkins, 2013). Research suggests that such images share low-level visual properties with evolutionarily threatening stimuli, namely snakes and spiders, which may suggest that the anxiety response to holes is rooted in the same fear response (Cole & Wilkins, 2013). Yet, reports by self-described trypophobes in the same study, suggest that they may in fact be primarily experiencing disgust, describing the lotus seed pod as, for example, "uncomfortable or even repulsive to look at" (Cole & Wilkins, 2013). In spite of subjective reports, Cole and Wilkins (2013) proposed that the "fear" of holes arises from an aversion to the spectral qualities of configurations of holes that are processed similarly to fearful spider and snake images. Despite sharing these spectral qualities, people actually experience different emotional reactions to holes compared to spiders and snakes, suggesting the experienced anxieties could be rooted in different emotions. In the present study, we seek to better understand the trypophobic reaction using pupillometry, a documented measure of physiological reaction to different emotions (Bradley et al., 2008; Granholm & Steinhauer, 2004).

Fear is defined as a basic emotional response to the perception of an imminent threat (Barlow, 1991; Craske, 1999). Previous research has established that the fear response involves activation of the sympathetic nervous system, commonly known as the "fight or flight" response (Gray, 1987; Bradley et al., 2008; Kreibig, 2010). This response includes an increase in overall cardiovascular function, as well as the release of norepinephrine and epinephrine, which facilitate this process (Folkow, 2000; Levenson, 1988). Together, this prepares the body to either fight or flee from a perceived threat. Though this reaction has an obvious adaptive function, it can also be maladaptive, if experienced in excess, or if applied to stimuli for which there is no imminent threat. The physiological response may be a symptom of many animal phobias such as fear of spiders and snakes (i.e., arachnophobia, ophidiophobia; Sarlo, Palomba, Angrilli, & Stegano, 2002).

In contrast to fear, Rozin and Fallon (1987) suggest that disgust is an emotional response to the threat of contamination or disease transmission. Although this may be analogous to the avoidance response in fear, there is a clear distinction with respect to the target of the response. In the case of fear, the response is to the threat of danger; in the case of disgust, the response is to the threat of contamination. Fear has evolutionary roots in danger avoidance and predator-prey interactions, whereas disgust may have developed in conjunction with the sensation of taste and smell, potentially allowing for the avoidance of sources of disease, such as in poisonous or rotten food (Woody & Teachman, 2000). Indeed, research suggests that the physiological response to disgust may be different from fear. In a review of autonomic nervous system activity in emotions, Kreibig (2010) suggests that general disgust elicits activation of the parasympathetic nervous system. Studies find that in reaction to facial expressions of disgust, participants exhibit decreased heart rate and skin temperature (Ekman, Levenson, & Friesen, 1983). Further, some common phobias, such as blood-injection-injury (BII) type phobia, are similarly rooted in a disgust response and also elicit parasympathetic activation (Hermann et al., 2007; Kreibig, 2010). Physiologically, this response pattern is the opposite of the “flight or fight” response and fear based anxieties, such that when experiencing a disgust reaction the body relaxes and withdraws from the environment by slowing overall cardiovascular functions (Kreibig, 2010).

The contrast present in the autonomic activation associated with fear and disgust is also seen neurally. Many studies have suggested the existence of specialized sub-cortical visual pathways for processing threats (e.g., snakes) through the superior colliculus, pulvinar, and amygdala (Ohman, Karlsson, Lundqvist, Ingvar, 2007; Van Le et al., 2013). These specialized neural responses to snakes, for example, suggest that they dissociate from other categories of anxiety provoking stimuli, such as disgust-based phobias. Further, processing of disgust has suggested the involvement of distinct neurological correlates from fear (Chapman & Anderson, 2012; Fitzgerald et al. 2004; Klucken et al., 2012; Schafer et al., 2009). Disgust stimuli seem to elicit activation in the insula, a region that has been shown to be involved in perceiving disgusting facial expressions (Phillips et al., 1998) or images of mutilation (Schienle et al., 2006; Wright et al., 2004), as well as experiencing aversive tastes or smells (Small, 2010; for review see, Chapman & Anderson, 2012). This evidence may reflect the differential behavioral demands of fear and disgust, where fear may require rapid shifts in attention for immediate response (Ohman et al., 2007), whereas disgust may not need to be time sensitive.

Researchers suggest that these distinct physiological and neurological processes for fear and disgust also lead to different behavioral responses. Previous studies have found observable attentional and perceptual biases in response to fearful stimuli such as spiders and snakes (Ohman, Flykt, & Esteves, 2001; Lobue & Deloache, 2010; Ayzenberg, Longo, & Lourenco, 2015). Thus, humans may have evolved specialized processes for evaluating and responding to threats efficiently and effectively. For example, when we detect any sort of animal or situation that may compromise our survival, we behave in a way that reduces this threat by either fleeing or attacking. On the contrary, disgust elicits a reliable withdrawal/avoidance behavioral pattern that protects the organism from contact with contaminated stimuli (Deacon & Olatunji, 2007; Olatunji & Sawchuk, 2005; Izard, 1993). This avoidance shares some similarity to the "fight or flight" response of fear in attempting to reduce the threat. However, there is no evidence of a disgust response eliciting attentional or perceptual biases, which further suggests that a disgust reaction is distinct from that of a fear reaction. Instead, disgust seems to elicit an involuntary facial grimace, nausea, and feeling of revulsion intended to prevent any further contact with the relevant stimulus, which has not been reported in a threat response (Rozin & Fallon, 1987).

Despite these differences, further investigation of fear and disgust is needed in order to better understand how they influence our perceptions and actions in the presence of emotional stimuli. In previous studies of trypophobia, researchers did not disambiguate the type of negative emotions experienced in the response. The present study seeks to specifically distinguish the emotional response by investigating the reaction to holes in comparison with animal phobias using pupillometry, a measure shown to accurately reflect physiological states (Sirois & Brisson, 2014; Granholm & Steinhauer, 2004). Previous studies have also used pupillometry to disambiguate pleasant and unpleasant emotional arousal from a neutral state (e.g., Bradley et al., 2008). Bradley and colleagues (2008) measured pupil diameter while participants viewed pictures differing on levels of emotional valence and arousal. They found that as the arousal level of positively and negatively valenced images increased, pupil diameter also increased. This response is consistent with increased arousal and therefore increased sympathetic nervous system activity, suggesting that emotional reactions can be disambiguated using pupillometry.

In the present study, we use pupillometry in an attempt to disambiguate fear and disgust in the context of trypophobia. Prior research has shown that threatening animals (e.g., spiders and snakes) elicit a fear response (Ohman, Flykt, & Esteves, 2001), such that corresponding pupil dilation associated with the sympathetic nervous system would be expected (Granholm & Steinhauer, 2004). If images of holes similarly elicit a fear response, as has been suggested by previous research (Cole & Wilkins, 2013), then the prediction is that there will be pupil dilation corresponding to a sympathetic response. However, if images of holes elicit a disgust response, then the prediction is that there will be pupil constriction corresponding to a parasympathetic response (Hermann et al., 2007; Kreibig, 2010). In a first experiment, we presented participants with trypophobic stimuli, which were images of objects with multiple holes and porous textures known to elicit discomfort in trypophobes (Cole & Wilkins, 2013). We also included images of evolutionary threatening (i.e., spiders and snakes) stimuli known to elicit fear responses (see Figure 1b). A third category of images was designed to be emotionally neutral and included images of neutrally valenced objects and textures (see Figure 1 c). In this experiment, we compared pupillary responses across the three categories. In a second experiment, we specifically controlled for the possibility that any changes in pupil size may be driven by the low-level visual properties of the image by including a low-level visual control category (see Figure 1d). Furthermore, we included self-report questionnaires in which participants rated stimuli on different emotional categories to explore the connection between subjective and physiological emotional responses.

Methods Experiment 1

Participants

Forty-one participants (30 females) were recruited from an undergraduate psychology course and participated for course credit. The average age of students was 19.84 years (*SD* = 1.21 years). All experimental procedures were approved by the local ethics committee.

Measures and Materials

Stimulus presentation and Apparatus

Stimulus presentation consisted of 60 images (512 x 512 px): 20 holes from the trypophobia image database created by Cole and Wilkins (2013), 20 threatening animals, and 20 neutral objects. The trypophobic images included individual objects with multiple holes (e.g., sponge, lotus seed) as well as zoomed in displays of porous textures (e.g., honeycombs; see Figure 1 for example). Threatening animals consisted of images of spiders and snakes and were selected so that the entirety of the animal was visible and centered on uniform background (e.g., grass, branches). Neutral images were chosen to match the other two categories with respect to content. These images included individual objects (e.g., cup), zoomed in displays (e.g., pile of coffee beans), and neutrally valenced animals (i.e., butterfly, frog). Participants also viewed a neutral gray screen that served as the baseline. All images, including the baseline, were grayscaled using Adobe Photoshop, and equated for luminance using the SHINE toolbox for Matlab (Willenbockel et al., 2010; Mathworks).

Stimulus presentation was controlled with a custom Visual Basic Program and presented on a 22-inch computer monitor (1920 x 1080px; 75 Hz refresh rate). All images were centered on a uniform gray background. Pupil diameter was recorded using an Eyelink-1000 plus eye tracker (SR-Research) recording at 1000 Hz. Due to individual differences in eye shape, pupil size, and camera angle, pupil diameter was measured in arbitrary camera units with a possible resolution as small as 5 microns (0.005 mm; SR-Research).

Procedure

During stimulus presentation, participants were seated in a chinrest 60 cm from the screen. Eye gaze was first calibrated using a 5-point calibration routine. Participants were instructed that a series of pictures would be displayed and that they should look at the images for the entire duration of the study. Each trial began with the baseline phase (6 s) followed by the image phase (6 s; Bradley et al., 2008). The session consisted of 60 experimental trials, randomized for each observer. In total, the experiment lasted approximately 20 minutes.

Results

Pupil Data Reduction

For each participant, pupil data were extracted in a 2 to 6 second interest period after the start of each baseline and image phase. This was done in order to account for changes in pupil size due to the pupillary reflex in the first two seconds brought on by small changes in luminance and contrast between changing screens (Bradley et al., 2008). Pupil data were further pruned based on two criteria. First, a mean pupil size and standard deviation was calculated for each participant from their raw pupil size measurements. Pupil sizes that were more than 2.5 standard deviations away from the mean were considered outliers and removed for each participant (2.8% of the data). Next, to account for any trials during which participants were inattentive, we calculated the total looking times for each participant. Not surprisingly, we found that on average, participants monitored the screen significantly less during the baseline phase (M = 2.89 s, SD = 0.53 s) than during the image phase (M = 3.40 s, SD = 0.31 s), $t(40) = 10.04$, $p < 0.001$. For both the baseline and image phases, we excluded any trials for which total looking time was more than 2.5 standard deviations below these respective averages (7% of the data). Next, to enable comparisons across participants, the average pupil size during the 2 to 6 second interest period was converted to a percentage difference from baseline to image phase where a larger percentage indicates a larger decrease in pupil size from baseline.

Analysis of stimulus type

To examine the influence of stimulus type (holes, neutral, threatening) on pupillary response, we conducted a repeated measures Analysis of Variance (ANOVA) using percent change as the dependent variable. This analysis revealed a significant main effect of stimulus type, $F(1, 80) = 8.68$, $p < 0.001$, $\eta_p^2 = 0.178$. A linear contrast analysis revealed that pupillary responses increased linearly by image type in the hypothesized order: holes, neutral, threatening, $F(1, 40) = 12.56$, $p = 0.001$, $\eta_p^2 = .239$. Post-hoc comparisons (Bonferroni corrected) revealed that pupil size was smaller for the hole images compared with the threatening images, $t(40) = 3.5$, $p = 0.001$. There was also significantly smaller pupil size for hole images compared with neutral images, $t(40) = 3.94$, $p = 0.003$. There was no significant difference between pupillary changes for neutral and threatening images ($p = 1.00$; see Figure 2). Taken together these data suggest a difference in the pupillary response between holes and threatening images, with pupil constriction for holes within this sample of participants.

Discussion

Our results show that there was greater pupil constriction for the hole images than for the threatening and neutral images (Kriebig, 2010). This is consistent with the hypothesis that trypophobia elicits feelings of disgust, which leads to a parasympathetic response and corresponding pupil constriction. Further, this informs earlier work on trypophobia by showing that holes can be dissociated from images of evolutionary threatening animals (Cole & Wilkins, 2013), perhaps suggesting that trypophobia is a disgust- instead of a fear-based response.

Though these data suggest that the pupillary response to holes and threatening images is different, it is possible that this difference was driven by the low-level visual characteristics of the holes, not their emotional value. Specifically, it has long been known that the pupillary response to an image is correlated with the spatial frequency grating of that image, with higher spatial frequencies leading to smaller pupil sizes—the so-called pupil grating response (PGR; Barbur & Thomson, 1987; Cocker et al., 1994). As holes have high-spatial frequency, it is possible that pupil constriction was purely due to the PGR.

To test whether the difference between holes and threatening images in Experiment 1 was the result of the PGR, we conducted a follow up experiment in which we included images designed to match those of holes in terms of spatial frequency. In Experiment 2, we thus examined pupillary responses to hole images, images matched for high spatial frequency (i.e. PGR control), and, again, threatening images. If the pupillary response to holes stimuli in experiment 1 was simply due to a PGR, and not emotional content, then we would expect to find no significant difference in the pupillary responses between PGR control and hole images. However, if they do in fact elicit a disgust response and corresponding constriction over and above a PGR, we would expect to find pupil constriction that was significantly greater than PGR control images. Further, to confirm that these images elicit the expected emotion and to explore whether there may be a connection between physiological and subjective affective responses, we also had participants rate images on different emotion categories and had them complete validated emotional questionnaires.

Methods Experiment 2

Participants

Forty-four undergraduates (30 females) were recruited from an undergraduate psychology course and participated for course credit. The average age of students was 19.80 years (*SD* = 1.15 years). Two participants were removed due to a failure to fixate for at least half of the experiment. All experimental procedures were approved by the local ethics committee.

Measures and Materials

Stimulus presentation and apparatus

Similar to experiment 1, stimulus presentation consisted of 60 images (512 x 512 px): 20 holes from the trypophobia image database created by Cole and Wilkins (2013), 20 threatening animals (i.e., spiders and snakes), and 20 non-hole PGR control images. The hole and threatening images were identical to experiment 1.The non-hole PGR control images were matched to the trypophobic images as best as possible in content and level of detail. They included standalone objects with contrasting textures (e.g., geometric art) as well as zoomed in patterns (e.g., checkerboard, tiles). The apparatus was identical to Experiment 1.

Trypophobia Questionnaire (TQ)

The TQ was developed based on reports of various symptom types to assess severity level of a 'fear' of holes (Le, Cole & Wilkins, 2015). Nevertheless, it demonstrates a single construct. It asks participants to rank two trypophobic images, a lotus seedpod and honeycomb (see Figure 1a), on 17 symptoms (e.g., "I feel sick or nauseous"). Each symptom was rated on a 5-point Likert scale referring to the extent it was experienced: 1(Not at all), 2 (Slightly), 3 (Moderately), 4 (Considerably), and 5 (Extremely). Scores were summed to yield a composite score between 17 and 85. This questionnaire has high internal consistency and test-retest reliability (Le, Cole & Wilkins, 2015).

Snake Questionnaire (SNAQ)

The SNAQ is a measure used to assess the fear of snakes (Klorman, Weerts, Hastings, Melamed, & Lang, 1974). It is a 30-item self-report scale. Each item consists of a fearful or non-fearful statement related to snakes (e.g., "I avoid going to parks or on camping trips because there may be snakes about"). Participants rate each item as true (1) or false (0). Some items are reverse-scored. Scores are tallied by summing responses across the items; scores can vary from 0 to 30. This questionnaire has high internal consistency and test-retest reliability (Klorman et al., 1974).

Fear of Spiders Questionnaire (FSQ)

The FSQ is a measure used to assess the fear of spiders (Szymanski & O'Donohue, 1995). It consists of 18 items rated on a 1-7 scale (1 = totally disagree, 7 = totally agree) regarding their fear and avoidance of spiders (e.g., "If I saw a spider now, I would think it will harm me"). It is scored by adding up the scores from each of the 18 items. Total scores range from 0 to 126. Previous researchers have found this questionnaire to have excellent split half reliability and internal consistency, and good test-retest coefficients (Szymanski & O'Donohue, 1995).

Questionnaire for the Assessment of Disgust Sensitivity (QADS)

The QADS is a self-report instrument that assesses the individual disposition of disgust sensitivity (Schienele, Walter, Stark, & Waitl, 2002). It consists of 37 items rated on a 5-point Likert scale (0 = not disgusting, 4 = very disgusting) to be rated on how disgusting a statement is (e.g., "You touch a dead body"). All items are summed and scores range from 0 to 148. This questionnaire has proved to be objective and reliable (Schienele, Walter, Stark, & Waitl, 2002).

Spielberger's State-Trait Anxiety Inventory (STAI)

The STAI is a measure of state and trait anxiety (Spielberger et al., 1983). Only the trait version of the STAI was in the present study as to be consistent with the other questionnaires. Participants ranked 20 items on a 1-4 scale: (1 = not at all, 2 = somewhat, 3 = moderately so, 4 = very much so) regarding how they generally feel (e.g., "I feel nervous and restless"). Several items are reverse-coded and are summed, yielding a composite score between 20 and 80. This questionnaire has proved to be a reliable and sensitive measure (Spielberger et al., 1983).

Stimulus Ratings

Participants were shown each of the images from the stimulus slideshow and asked to answer four questions for each image in a randomized order. Three of the questions asked participants how fearful, disgusted, or anxious the images made them feel on a 0-7 scale (e.g., 0 = not at all fearful, 3 = moderately fearful, 7 = extremely fearful). A fourth question asked how they felt overall (valence/arousal) while viewing the stimulus on a 7-point scale (-3 = extremely negative, 0 = neutral, 3 = extremely positive). Images were presented in a randomized order.

Procedure

The stimulus presentation was completed first, where pupillary responses were recorded, and was identical to that of Experiment 1. Participants then completed computerized versions of all five self-report questionnaires in a randomized order. Finally, participants completed the computerized ratings for all stimuli. The experiment lasted approximately 45 minutes.

Results

Rating and Questionnaire Responses

We analyzed participant ratings by stimulus type to confirm that our image categories elicited the desired emotion. We conducted paired comparisons of mean affective ratings by image type. PGR control images were rated significantly lower on fear, disgust, anxiety, and arousal compared with threatening and hole images (ps < 0.001), which confirms that the PGR control images were more neutral in all emotional categories. Next, we sought to examine whether participants ranked holes as more disgusting or fearful. Paired comparisons showed that hole images were rated significantly more disgusting than they were fearful, $t(41) = -4.6$, $p < 0.001$, suggesting that the subjective trypophobic response is better matched by disgust than fear. For threatening images, however, paired comparisons revealed no difference in ratings of fear or disgust ($p > 0.966$); we were thus unable to distinguish the primary subjective emotional response to spiders and snakes. Since pupil size has been shown to scale with valence and arousal (Bradley et al., 2008), we investigated the order of valence/arousal for our stimulus categories. A linear contrast analysis revealed that arousal ratings increased linearly in the following order: PGR control, holes, threatening, $F(1, 41) = 62.30$, $p < 0.001$, $\eta_p^2 = .603$ (see Figure 3).

Participants' ratings for fear, disgust, and anxiety on images significantly correlated with their corresponding responses on the disgust, threat (i.e., snake, spider), and anxiety questionnaires (ps < 0.05). More specifically, participants' scores on the SNAQ and FSQ positively correlated with their ratings on fear and anxiety for threatening images (ps < 0.001). Additionally, participants' scores on the QADS positively correlated with their ratings on disgust and fear for threatening images, and disgust and anxiety for hole image types (ps < 0.05). Next, participant's scores on the TQ positively correlated with their ratings of fear, disgust, and anxiety for hole images (ps < 0.001). Lastly, participant's scores on the STAI-T positively correlated with their ratings on disgust for threatening images. Interestingly, participants' scores on the SNAQ and FSQ also positively correlated with their ratings on disgust for threatening images (ps < 0.001), suggesting fear and disgust are involved in both of these phobias. See Table 1 for a full summary.

Pupil Data Reduction

Pupil data were extracted as in Experiment 1. Pupil sizes that were more than 2.5 standard deviations were considered outliers and removed for each participant (2.2% of the data). Similarly, any trials in which the total looking time fell more than 2.5 standard deviations below the average for the baseline phase (M = 2.95 s, SD = 0.55 s) and the image phase (M = 3.42 s, SD = 0.32 s), were removed (4.2 % of the data).

Analysis of stimulus type

To examine the influence of stimulus type (holes, neutral, threatening) on pupillary responses, we conducted a repeated measures ANOVA using percent change as the dependent variable. This analysis revealed a significant main effect of stimulus type, $F(1, 82) = 7.35$, $p = 0.001$, $\eta_p^2 = 0.152$. Post-hoc comparisons (Bonferroni-corrected) revealed that there was significantly smaller pupil size for hole images compared to threatening images, $t(41) = -3.74$, $p < 0.001$, which replicates the effect in Experiment 1. The difference in pupil sizes between holes and PGR control images was not significant ($p > 0.1$; see Figure 3), which is perhaps unsurprising given that hole images have spatial properties similar to the PGR control images. Because hole and PGR control images elicit similar pupillary responses, there should be a difference between PGR control and threatening images, similar to the different found between holes and threat. However, the difference in pupil sizes between PGR and threat was not significant ($p > 0.1$; see Figure 3), suggesting that the difference in pupillary responses between holes and threatening images is not only accounted for by the spatial properties.

Due to the linear scaling of arousal by stimulus type, we sought to investigate whether pupil sizes would similarly scale linearly as shown in previous studies (Bradley et al., 2008; Partala & Surakka, 2003; Partala, Jokiniemi, & Surakka, 2000). However, a linear contrast analysis revealed that pupil size did not increase linearly with arousal rating ($p > .05$); instead, the relationship was better explained by a quadratic function, $F(1, 41) = 12.84$, $p = 0.001$, $\eta_p^2 =$ .0.239, with hole images eliciting the smallest pupil, followed by PGR control images, followed by threatening images (see Figure 3). This suggests that there is an additional component contributing to the apparent pupil constriction in response to the holes images, other than the arousal level.

Discussion

Our results show that pupillary responses to hole images were significantly greater than threatening images, thereby replicating the results of experiment 1. There was no evidence that pupillary responses to PGR control images differed from holes, consistent with the fact that the two image categories shared high-spatial frequencies. This may suggest that the difference between threatening and hole stimuli found in experiments 1 and 2 is the result of the PGR, and not emotion. If true, the pupil response to PGR control images and threatening images should similarly dissociate based on visual properties. However, there was no difference in pupillary responses between the threat and PGR category, suggesting that the difference seen between holes and threatening images is not strictly due to differences in spatial frequencies.

To better understand these data, it is important to look at the pupillary responses in comparison to participants' subjective responses. Previous research has shown that arousal and valence is positively and linearly correlated with pupil dilation (Bradley et al., 2008; Partalaa & Surakka, 2003; Partala, Jokiniemi, & Surakka, 2000). Participants rated PGR control images lowest on arousal, followed by holes, and then threatening images. Thus, based on ratings and past research one would have predicted that if our results were based solely on arousal we should have observed a linear effect of pupil size that scaled with arousal. However, our effects did not scale with arousal, suggesting that an emotion, such as disgust, may be contributing to additional constriction for hole images.

Participants' subjective reports help to further reveal the emotional content of the stimuli. An examination of the correlations between the image ratings and emotional questionnaires found much overlap in the subjective reports of fear and disgust in the threatening and hole categories. For threatening images, we were not able to identify the primary emotional response as participants rated snake and spider images similarly on disgust and fear. Further, both disgust and fear ratings were correlated with the snake and spider questionnaires. This may suggest there is a combination of both emotions involved in processing the threatening images, which would explain why we did not get more relative dilation for the threatening images. However, while trypophobic questionnaire scores correlated with both fear and disgust ratings of holes, our results show that participants nevertheless reported feeling more disgusted than fearful when viewing trypophobic stimuli. Taken together, these data suggest that although there was no difference in pupillary responses to PGR control and hole images, subjective ratings show that there is an emotional component involved in processing holes that might differ from the emotional processing of threatening stimuli. While on the other hand, ratings of PGR control images show that, as planned, participants find these stimuli emotionally neutral. Hole images seem to produce greater feelings of disgust, whereas threatening images may produce a combination of fear and disgust. The connection that these emotions have on the physiological responses needs to be further investigated.

General Discussion

Using pupillometry, a physiological measure shown to reflect emotional arousal (Bradley et al., 2008) and physiological states (Sirois & Brisson, 2014), we sought to disambiguate the emotional underpinnings of trypophobia, the adverse reaction to stimuli containing holes (Cole & Wilkins, 2013). This response has been suggested to result from visual properties shared with snakes and spiders, thus causing a fear reaction (Cole & Wilkins, 2013). However, anecdotal reports by self-described trypophobes suggests an alternative, namely that sufferers experience disgust instead of fear. In Experiment 1, we compared pupil responses across hole, threatening, and neutral images with the neutral category being an emotional control. In Experiment 2, we compared pupil responses across hole, threatening, and PGR control images with the PGR control category being both a control for high-spatial frequency characteristics and neutral emotion. We also had participants complete validated questionnaires and rate the images to investigate the relationship between the subjective and physiological response to our stimuli.

The physiological pupil response to hole images is significantly different from threatening images. Pupil responses to hole images were not different from PGR control images, which at first suggests that the physiological response we observed is caused by the high spatial-frequency of the stimuli. However, because pupil responses to PGR control images were also not different from threatening images, spatial frequency does not account for the difference between holes and threat found in both experiments 1 and 2. Additionally, pupil size did not scale with reported arousal levels by image type. In addressing both the effects of spatial frequency and arousal, these findings suggest there may still be an emotional component to the processing of hole images. Given that participants' subjective ratings indicated feelings of disgust more than fear when looking at hole images, this emotional component may be disgust. To summarize, hole stimuli may elicit a physiological disgust response, but it may not be fully dissociable from PGR.

This ambiguity in our data and the trypophobic reaction is paralleled by recent debates in the general field of phobias (Cisler, Olatunji, & Lohr, 2009; Olatunji, Cisler, McKay, & Phillips, 2010; Mason & Richardson, 2010). Investigations into the cognitive and psychophysiological reactions to affective stimuli have begun to question whether the emotion of fear is implicated in all phobias. Disgust, for example, may be implicated instead of, or perhaps in combination with, fear in certain phobias (Cisler, Olatunji, & Lohr, 2009, Davey, Forster, & Mayhew, 1993; Matchett & Davey, 1991). Indeed, research suggests that many anxiety disorders may in fact arise from the intersection of multiple negative emotions, where a disorder is marked emotionally by a weighting of, for example, fear and disgust (Sawchuk et al, 2002; Olatunji & Sawchuk, 2005). Sawchuk and colleagues (2002) examined spider phobics' and BII type phobics' reactions towards images of spiders and surgical operations, respectively. They found that although the spider phobics reported feeling primarily fearful and the BII type phobics reported feeling primarily disgusted, there was a high positive correlation between their fear and disgust ratings of the images, suggesting that these two emotions operate in tandem (Sawchuk et al., 2002). If true, then this would explain why we do not see greater pupil dilation for threatening images relative to neutral and PGR control images, as co-activation of sympathetic and parasympathetic nervous systems may be present in the responses to these stimuli (Kriebig, 2010).

This study has extended our knowledge on the physiological effects of emotion but there is still work to be done. Based on our findings, we suggest that trypophobia is not simply described by a fear-based reaction like spider and snake phobias, but instead, may be better described by a disgust reaction. However, it is important to continue to investigate this response by using other physiological measures (e.g., heart rate, skin conductance) to further elucidate the emotional origins of these anxiety disorders. In regards to pupillometry, although we controlled for visual properties including luminance, PGR, and color, future studies should more stringently control for the low-level visual properties of stimuli. Lastly, more research is needed to test whether pupillometry can be used to disambiguate emotions. One possible avenue of research could test stimuli known to elicit other, more clearly distinguishable emotions that have known corresponding sympathetic or parasympathetic nervous system responses to further validate pupillometry as a physiological measure. While there remain many open questions about the role of fear and disgust in anxiety disorders, the current study highlights one case, trypophobia, where the emotional response is more complex than previously believed.

References

Ayzenberg, V., Longo, M., & Lourenco, S. (2015). Evolutionary-based threat modulates perception of looming visual stimuli in human infants. *Journal of vision*, *15*(12), 797-797.

Barbur, J. L., & Thomson, W. D. (1987). Pupil response as an objective measure of visual acuity*. *Ophthalmic and Physiological Optics*, *7*(4), 425-429.

Barlow, D. H. (1991). Disorders of emotion: Clarification, elaboration, and future directions. *Psychological Inquiry, 2,* 97-105.

Barlow, D. H. (2000). Unraveling the mysteries of anxiety and its disorders from the perspective of emotion theory. *American Psychologist*, 55, 1247−1263.

Beck, A. T., Emery, G., & Greenberg, R. L. (2005). *Anxiety disorders and phobias: A cognitive perspective*. Basic Books.

Bradley, M. M., Miccoli, L., Escrig, M. A., & Lang, P. J. (2008). The pupil as a measure of emotional arousal and autonomic activation. *Psychophysiology*,*45*(4), 602-607.

Chapman, H. A., & Anderson, A. K. (2012). Understanding disgust. *Annals of the New York Academy of Sciences*, *1251*(1), 62-76.

Cisler, J. M., Olatunji, B. O., & Lohr, J. M. (2009). Disgust, fear, and the anxiety disorders: A critical review. *Clinical psychology review*, *29*(1), 34-46.

Cocker, K. D., Moseley, M. J., Bissenden, J. G., & Fielder, A. R. (1994). Visual acuity and pupillary responses to spatial structure in infants. *Investigative ophthalmology & visual science*, *35*(5), 2620-2625.

Cole, G. G., & Wilkins, A. J. (2013). Fear of holes. *Psychological science*, 1-6.

Craske, M. H. (1999). *Anxiety Disorders: Psychological approaches to theory and treatment.* Boulder, CO: Westview Press.

Davey, G. C. L., Forster, L., & Mayhew, G. (1993). Familial resemblances in disgust sensitivity and animal phobias. *Behavior Research and Therapy, 31*, 41-50.

Deacon, B., & Olatunji, B. O. (2007). Specificity of disgust sensitivity in the prediction of behavioral avoidance in contamination fear. *Behavior research and therapy*, *45*(9), 2110-2120.

Ekman, P., Levenson, R. W., & Friesen, W. V. (1983). Autonomic nervous system activity distinguishes among emotions. *Science*, *221*(4616), 1208-1210.

Etkin, A., & Wager, T. D. (2007). Functional neuroimaging of anxiety: a meta-analysis of emotional processing in PTSD, social anxiety disorder, and specific phobia. *American Journal of Psychiatry*.

Fitzgerald, D. A., Posse, S., Moore, G. J., Tancer, M. E., Nathan, P. J., & Phan, K. L. (2004). Neural correlates of internally-generated disgust via autobiographical recall: a functional magnetic resonance imaging investigation. *Neuroscience letters*, *370*(2), 91-96.

Folkow, B., 2000. Perspectives on the integrative functions of the 'sympatho-adrenomedullary system'. *Autonomic Neuroscience*, 101–115.

Granholm, E., & Steinhauer, S. R. (2004). Pupillometric measures of cognitive and emotional processes. *International Journal of Psychophysiology*, *52*(1), 1-6.

Gray, J. A. (1987). *The psychology of fear and stress.* (2nd ed.). New York: Cambridge University Press.

Hermann, A., Schäfer, A., Walter, B., Stark, R., Vaitl, D., & Schienle, A. (2007). Diminished medial prefrontal cortex activity in blood-injection-injury phobia. *Biological psychology*, *75*(2), 124-130.

Izard, C. E. (1993). Four systems for emotion activation: cognitive and noncognitive processes. *Psychological review*, *100*(1), 68.

Klorman, R., Weerts, T. C., Hastings, J. E., Melamed, B. G., & Lang, P. J. (1974). Psychometric description of some specific-fear questionnaires. *Behavior Therapy*, *5*(3), 401-409.

Klucken, T., Schweckendiek, J., Koppe, G., Merz, C. J., Kagerer, S., Walter, B., & Stark, R. (2012). Neural correlates of disgust-and fear-conditioned responses. *Neuroscience*, *201*, 209-218.

Kreibig, S. D. (2010). Autonomic nervous system activity in emotion: A review. *Biological psychology*, *84*(3), 394-421.

Le, A. T., Cole, G. G., & Wilkins, A. J. (2015). Assessment of trypophobia and an analysis of its visual precipitation. *The Quarterly Journal of Experimental Psychology*, *68*(11), 2304-2322.

Levenson, R.W., 1988. Emotion and the autonomic nervous system: a prospectus for research on autonomic specificity. In: Wagner, H.L. (Ed.), *Social Psychophysiology and Emotion: Theory and Clinical Applications*. Wiley, Chichester, pp. 17–42.

LoBue, V., & DeLoache, J. S. (2010). Superior detection of threat-relevant stimuli in infancy. *Developmental science*, *13*(1), 221-228.

Mason, E. C., & Richardson, R. (2010). Looking beyond fear: The extinction of other emotions implicated in anxiety disorders. *Journal of Anxiety Disorders*, *24*(1), 63-70.

Öhman, A., Carlsson, K., Lundqvist, D., & Ingvar, M. (2007). On the unconscious subcortical origin of human fear. *Physiology & Behavior*, *92*(1), 180-185.

Öhman, A., Flykt, A., & Esteves, F. (2001). Emotion drives attention: detecting the snake in the grass. *Journal of experimental psychology, 130*(3), 466.

Olatunji, B. O., & Sawchuk, C. N. (2005). Disgust: Characteristic features, social manifestations, and clinical implications. *Journal of Social and Clinical Psychology*, *24*(7), 932-962.

Olatunji, B. O., Cisler, J., McKay, D., & Phillips, M. L. (2010). Is disgust associated with psychopathology? Emerging research in the anxiety disorders. *Psychiatry research*, *175*(1), 1-10.

Partala, T., Jokiniemi, M., & Surakka, V. (2000). Pupillary responses to emotionally provocative stimuli. In *Proceedings of the 2000 symposium on Eye tracking research & applications* (pp. 123-129). ACM.

Partala, T., & Surakka, V. (2003). Pupil size variation as an indication of affective processing. *International journal of human-computer studies*, *59*(1), 185-198.

Phillips, M. L., Young, A. W., Scott, S., Calder, A. J., Andrew, C., Giampietro, V., & Gray, J. A. (1998). Neural responses to facial and vocal expressions of fear and disgust. *Proceedings of the Royal Society of London B: Biological Sciences*, *265*(1408), 1809-1817.

Rozin, P., & Fallon, A. E. (1987). A perspective on disgust. *Psychological review*, *94*(1), 23.

Sarlo, M., Palomba, D., Angrilli, A., & Stegagno, L. (2002). Blood phobia and spider phobia: two specific phobias with different autonomic cardiac modulations. *Biological psychology*, *60*(2), 91-108.

Sawchuk, C. N., Lohr, J. M., Westendorf, D. H., Meunier, S. A., & Tolin, D. F. (2002). Emotional responding to fearful and disgusting stimuli in specific phobics. *Behavior research and therapy*, *40*(9), 1031-1046.

Schäfer, A., Leutgeb, V., Reishofer, G., Ebner, F., & Schienle, A. (2009). Propensity and sensitivity measures of fear and disgust are differentially related to emotion-specific brain activation. *Neuroscience letters*, *465*(3), 262-266.

Schienle, A., Schäfer, A., Hermann, A., Walter, B., Stark, R., & Vaitl, D. (2006). fMRI responses to pictures of mutilation and contamination. *Neuroscience letters*, *393*(2), 174-178.

Schienle A, Walter B, Stark R, Vaitl D: Ein Fragebogen zur Erfassung der Ekelempfindlichkeit (FEE) [A questionnaire for the assessment of disgust sensitivity]. *Zeitschrift für Klinische Psychologie und Psychotherapie: Forschung und Praxis (2002)*, 31:110-120.

Sirois, S., & Brisson, J. (2014). Pupillometry. *Wiley Interdisciplinary Reviews: Cognitive Science*, *5*(6), 679-692.

Small, D.M. 2010. Taste representation in the human insula. *Brain Struct. Funct.* 214: 551–561.

Szymanski, J., & O'Donohue, W. (1995). Fear of spiders questionnaire. *Journal of behavior therapy and experimental psychiatry*, *26*(1), 31-34.

The National Institute of Mental Health. (2016, March). Anxiety Disorders. Retrieved March 16, 2016, from http://www.nimh.nih.gov/health/topics/anxiety-disorders/index.shtml

Van Le, Q. V., Isbell, L. A., Matsumoto, J., Nguyen, M., Hori, E., Maior, R. S., & Nishijo, H. (2013). Pulvinar neurons reveal neurobiological evidence of past selection for rapid detection of snakes. *Proceedings of the National Academy of Sciences of the United States of America*, 110(47), 19000-19005.

Willenbockel, V., Sadr, J., Fiset, D., Horne, G. O., Gosselin, F., & Tanaka, J. W. (2010). Controlling low-level image properties: the SHINE toolbox. *Behavior research methods*, *42*(3), 671-684.

Woody, S. R., & Teachman, B. A. (2000). Intersection of disgust and fear: Normative and pathological views. *Clinical Psychology: Science and Practice*, *7*(3), 291-311

Wright, P., G. He, N. Shapira, *et al*. 2004. Disgust and the insula: fMRI responses to pictures of mutilation and contamination. *Neuroreport* 15: 2347–2351.

Figures

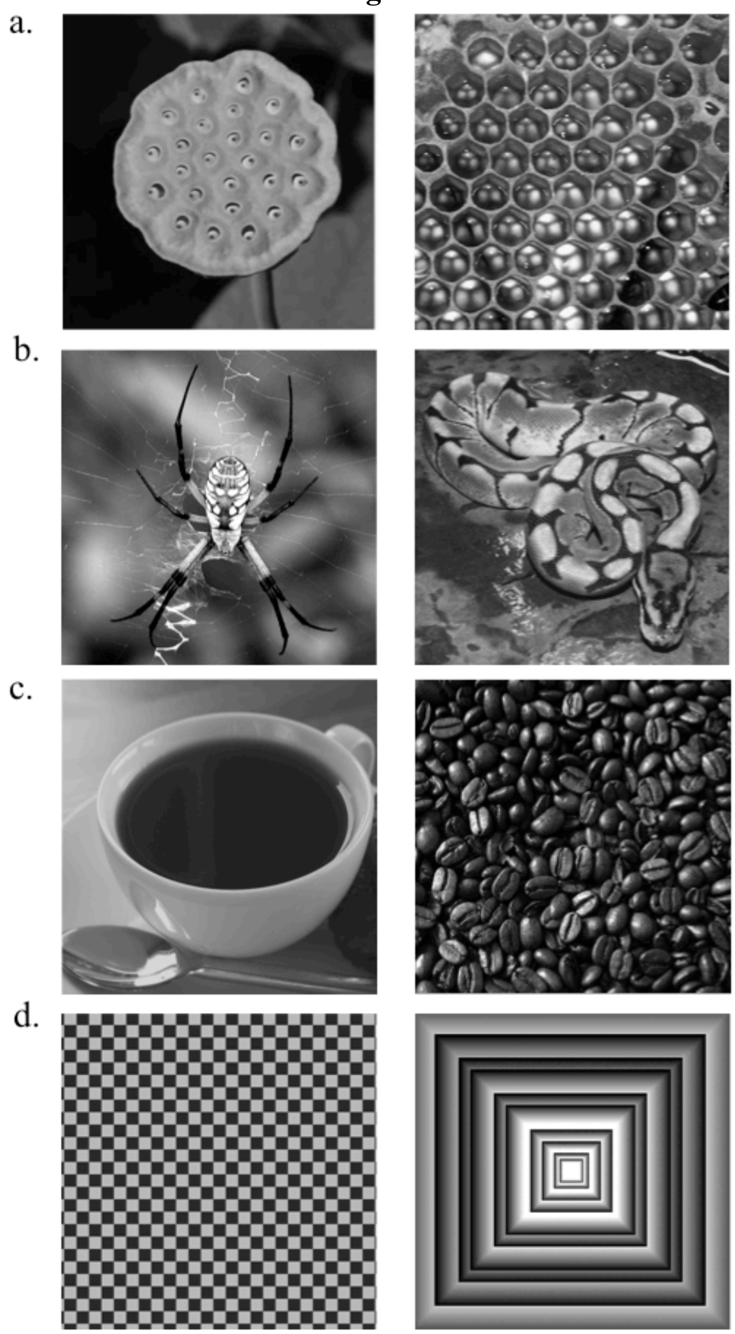

Figure 1. a. Two images from the trypophobia category: lotus seed pod (left) and honeycomb (right). *b*. Two images from the threatening category: spider (left) and snake (right). *c*. Two images from the neutral category: coffee mug (left) and coffee beans (right). *d.* Two images from the low-level visual control category: checkerboard (left) and geometric graphic (right).

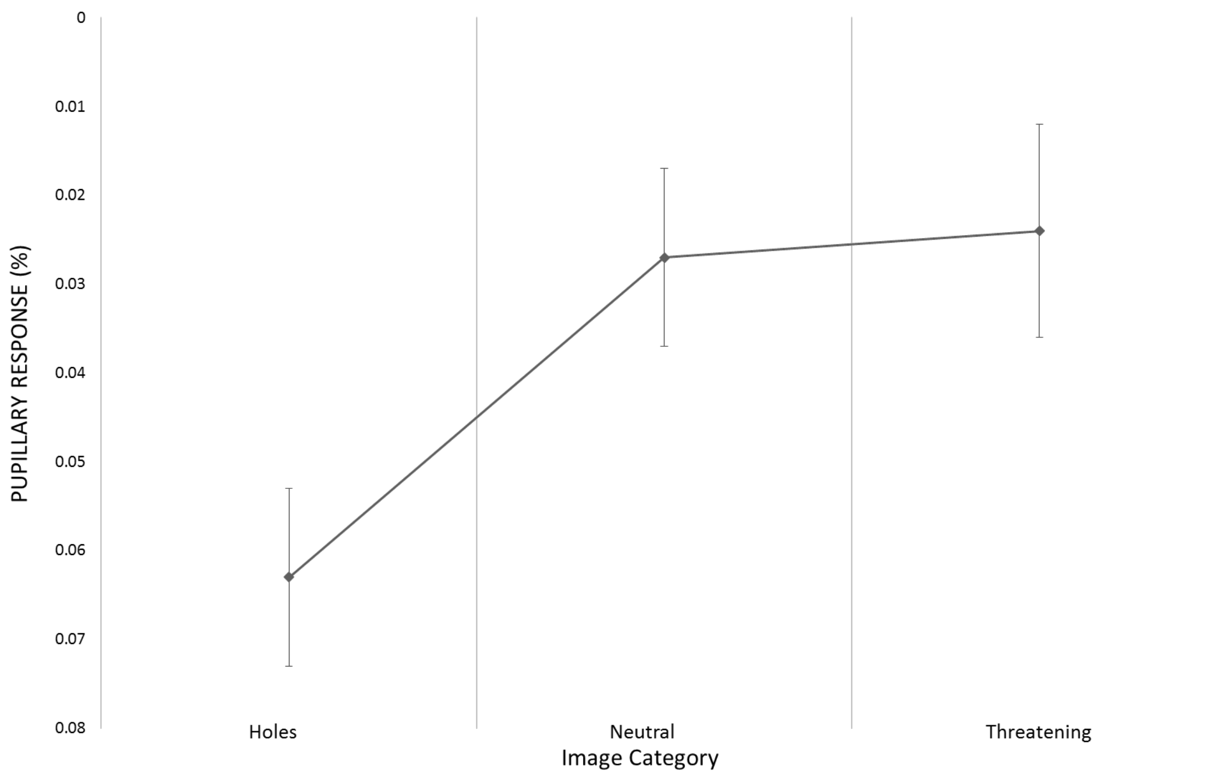

Figure 2. Mean pupillary response representing the percent decrease from the baseline to image phase for holes (*M* = 0.063, *SE* = 0.01), neutral (*M* = 0.027, *SE* = 0.01), and threatening (*M* = 0.024, *SE* = 0.012) images. The left axis is reversed for clarity, with a higher value/lower position representing a smaller pupil size and thus more constriction. Error bars represent standard error of the mean. Pupil constriction for hole images was significantly greater than for threatening and neutral images ($p = 0.001$).

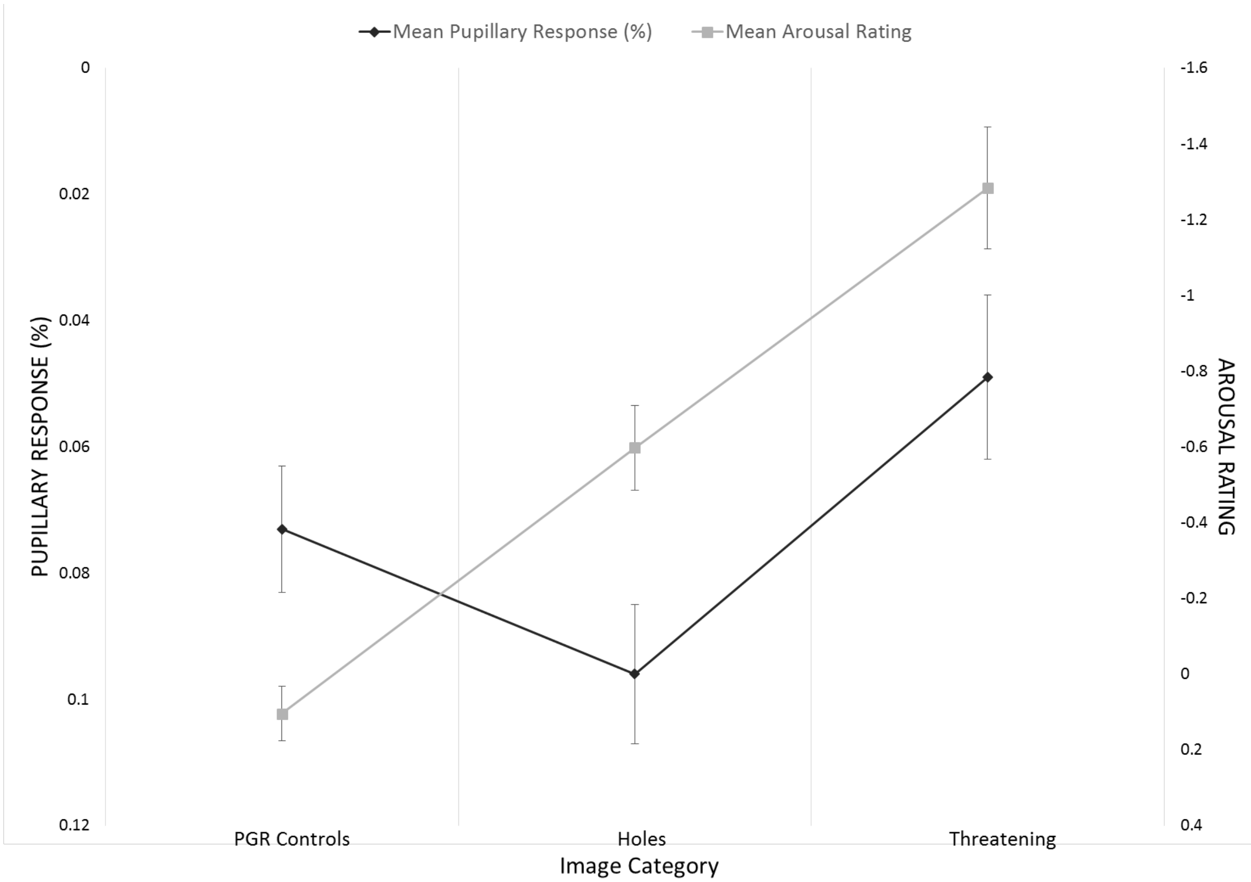

Figure 3. The left axis and black line represents mean pupillary response for PGR control images ($M = 0.027$, $SE = 0.01$), hole images ($M = 0.063$, $SE = 0.01$), and threatening images ($M = 0.024$, $SE = 0.012$). The axis is also reversed for clarity, with a larger value/lower position representing a smaller pupil size and thus more constriction. The right axis and grey line represents mean reported arousal for PGR control images ($M = 0.10$, $SE = 0.072$), hole images ($M = -0.60$, $SE = 0.11$), and threatening images ($M = -1.3$, $SE = 0.16$). The axis is reversed for clarity with a higher point representing higher negatively valenced arousal. Error bars represent standard error of the mean. Pupil size did not scale with arousal. Pupil constriction for hole images was significantly greater than for threatening images ($p < 0.001$).