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Sustained, not habituated, activity in the human amygdala: A pilot fMRI dot-probe study of attentional bias to fearful faces

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Abstract: The human amygdala consciously and nonconsciously processes facial expressions and directs spatial attention to them. Research has shown that amygdala activity habituates after repeated exposure to emotionally salient stimuli during passive viewing tasks. However, it is unclear to what extent the amygdala habituates during biologically relevant amygdala-mediated behaviors, such as the orienting of attention to environmentally salient social signals. The present study investigated amygdala habituation during a dot-probe task measuring attentional bias to backward masked fearful faces. The results suggest that across the duration of the 50 min (1,098 trial) task both attentional bias behavior and amygdala activity were sustained—rather than habituated. Thus, these initial findings indicate that when biologically relevant behavior is sustained, so too is amygdala activation.

Subjects: Neuroscience; Psychological Science; Attention; Emotion; Cognition & Emotion; Cognitive Neuroscience; Social Neuroscience

Keywords: amygdala; habituation; backward masking; attention bias; fear

The amygdala is located bilaterally within the anterior temporal lobe and is critically involved in various aspects of emotional and social processing (Adolphs, 2010). From early research, it has been shown that stimulation of the amygdala leads to unprovoked fear and/or aggression (Shealy & Peele, 1957).

ABOUT THE AUTHORS

Joshua M. Carlson is an assistant professor of Psychology and the Director of the Cognitive × Affective Behavior & Integrative Neuroscience (CABIN) lab at Northern Michigan University (NMU) in Marquette, MI. Millicent A. Weber, Kelly A. Morrow, Will S. Rizer, and Keara J. Kangas were students in the Psychology Department at NMU. The CABIN lab studies how social and/or affective stimuli such as facial expressions influence behavior and the neural circuitry underlying this influence on behavior. The results from this paper—suggesting that the tendency to direct our attention to the location of others’ fearful expressions is maintained for an extended period of time and is accompanied by sustained amygdala activation—builds upon these broader goals of the lab. The lab is currently researching the role of attention training in modifying the structure and function in the neural systems associated with emotional attention.

PUBLIC INTEREST STATEMENT

The amygdala is an important brain region involved in the detection of environmental salience (e.g. facial expressions) and our response to salient stimuli. Previous work indicates that the amygdala activates in response to threat, but habituates quickly when threat-related behaviors are not elicited. The results from this study suggest that when individuals are confronted with salient stimuli that signal potential danger, such as fearful facial expressions, our attentional bias to these stimuli is maintained for a prolonged period of time (i.e. 50 min) and the amygdala remains active during this time. Thus, when biologically relevant behavior is sustained, so too is the amygdala activation that underlies this behavior.
Damage to the human amygdala impairs the recognition of fearful facial expressions (Adolphs, Tranel, Damasio, & Damasio, 1994; Adolphs et al., 1999), learning new fear associations (LaBar, LeDoux, Spencer, & Phelps, 1995), the experience of fear (Feinstein, Adolphs, Damasio, & Tranel, 2011), and the preferential processing of threatening environmental stimuli (Anderson & Phelps, 2001; Bach, Hurlemann, & Dolan, 2014; Vuilleumier, Richardson, Armony, Driver, & Dolan, 2004). Functional neuro-imaging research indicates that the amygdala is reactive to fearful faces when processed at a conscious level (Breiter et al., 1996; Morris et al., 1996), and also when conscious awareness has been restricted by backward masking (Liddell et al., 2005; Morris, Öhman, & Dolan, 1998; Whalen et al., 1998; Williams et al., 2005), binocular rivalry (Williams, Morris, McGlone, Abbott, & Mattingley, 2004), and continuous flash suppression (Jiang & He, 2006) as well as in cases of cortical blindness (Morris, DeGelder, Weiskrantz, & Dolan, 2001; Morris, Ohman, & Dolan, 1999). One behavior that nonconscious amygdala activity has been linked to is the direction of spatial attention toward signals of environmental threat (Carlson, Reinke, & Habib, 2009). In particular, the amygdala appears to code for both the salience and spatial location of a stimulus (Peck, Lau, & Salzman, 2013). Through projections to visual cortex (Adolphs, 2004; Vuilleumier et al., 2004) and the prefrontal cortex (Amaral & Price, 1984; Carlson, Cha, Harmon-Jones, Mujica-Parodi, & Hajcak, 2014; Carlson, Cha, & Mujica-Parodi, 2013) the amygdala mediates the preferential processing of environmental threat.

Habituation is an adaptive decrease in responsivity to conserve computational resources after repeated stimulus exposure(s) (Plichta et al., 2014). Although the amygdala responds to both consciously and nonconsciously viewed fearful faces, this response has been found to rapidly habituate during passive viewing conditions (Breiter et al., 1996; Whalen et al., 1998). On the other hand, during emotional decision-making (Tabert et al., 2001) as well as periods of uncertainty (Herry et al., 2007), amygdala remains activated. Thus, the amygdala rapidly and nonconsciously responds to environmental signals of threat and if the salience of these signals decreases, the amygdala response habituates.

As previously mentioned, a critical component of the amygdala response to threat is facilitated attentional processing at the location of potential threat (Carlson et al., 2009, 2013; Monk et al., 2008). However, the length of time for which this effect persists is unknown. Previous studies have shown that attentional bias to threat is sustained across two testing blocks (96 trials/block) in individuals prone to elevated attentional bias (Lonsdorf, Juth, Rohde, Schalling, & Öhman, 2014) and is sustained across two testing blocks (48 trials/block) when accompanied by unpredictable aversive sounds (Herry et al., 2007). Thus, attention bias behavior—and underlying amygdala activity—appear to persist or habituate slowly. Yet, the duration of time for which this sustained response persists is not well understood. Here, we used data from an existing study (Carlson et al., 2009), which measured attentional bias behavior and amygdala activity across 1,098 trials over a 50 min timespan, to test the degree to which behavioral and amygdala responses were habituated or sustained over an extended time-period. Given that amygdala activity appears to underlie attentional bias behavior, we hypothesized that the amygdala would remain active—provided attentional bias behavior was observed. However, the precise duration of these two effects has yet to be determined.

1. Methods

1.1. Participants
Seven male and five female, right-handed individuals, aged 18–35 participated in the study. Individuals were screened for prescription and recreational drug use, neurological and psychological histories, and for irremovable metal pieces prior to the study. Participants were given monetary compensation for participating in the study. All subjects gave informed consent and were treated in accordance to the guidelines of the Institutional Review Board of Southern Illinois University Carbondale. Due to a technical problem, one participant’s functional magnetic resonance imaging (fMRI) data was lost and therefore unavailable for fMRI analyses. MRI data from this sample has been previously reported (Carlson et al., 2009, 2012; Carlson, Reinke, LaMontagne, & Habib, 2011); however, all analyses included here are new.
1.2. Dot-probe task
Participants performed a dot-probe task, while event-related fMRI data was acquired. Each trial consisted of a fixation cue appearing for 1,000 ms, followed by two faces presented simultaneously for 33 ms to the left and right of the central fixation cue, and then immediately followed by the neutral face mask for 100 ms. A target dot appeared for 750 ms, behind either the right or left face. A randomized jittered inter-trial interval (ITI; 500–2,000 ms) concluded each trial. Subjects responded to the location of the dot using an MRI-compatible response pad. The right index finger was used to respond to left visual field (LVF) targets and the right middle finger was used for right visual field (RVF) targets (see Carlson et al., 2009 for more details).

Trials consisted of directed and undirected attention trials. Directed attention trials displayed two faces, one neutral and one fearful. Half of the directed attention trials were incongruent (target dot appeared opposite of the fearful face), and the other half of the trials were congruent (target dot appeared on same side as the fearful face). Faster reaction times on congruent compared to incongruent trials measure an automatic directing of attention to the fearful face. Half of the directed attention trials (=549) contained a LVF fearful face and the other half contained a RVF fearful face. Undirected attention trials (=549) showed faces of the same expression (i.e. fearful–fearful & neutral–neutral) and were considered to be independent of attentional bias because neither face demanded more attention than the other. The task was divided into 10 five-minute runs. Each run contained 162–170 trials (depending on the randomized jittered ITIs selected in a given run), which resulted in approximately 1,647 total trials across LVF, RVF, and undirected attention conditions.

1.3. Functional MRI data acquisition and analysis
A 1.5T Phillips whole body scanner and head coil were used to collect T2* weighted EPI scans with the following parameters: TR = 2500 ms, TE = 50 ms, flip angle = 90°, matrix dimensions = 64 × 64, slices = 26, slice thickness = 5.5 mm, and gap = 0. Preprocessing included: image realignment corrections for head movement, slice timing corrections, normalization to standard 2 mm × 2 mm × 2 mm Montreal Neurological Institute space, and spatial smoothing with a Gaussian full-width-at-half-maximum 10 mm filter.

Given that only LVF directed attention trials were shown to produce behavioral and amygdala effects in our previous analysis of this data (Carlson et al., 2009), we restricted our new habituation analyses to LVF directed attention (=549 trials), as well as fearful–fearful and neutral–neutral undirected attention (=549 trials), trial-types (total trials used =1098). In SPM8, using a general linear model, first-level parameter maps were created for each condition across the 10 runs. A full factorial second-level model was created with trial-type (LVF, fearful–fearful, and neutral–neutral) and run (1–10) as within subjects factors. Left and right amygdala masks were created using the Masks for Regions of Interest Analysis software (Walter et al., 2003) and a search volume family-wise error (FWE) corrected (p < 0.05 with a 20 voxel extent threshold) region of interest (ROI) analysis was performed to look at common activity across all trial-types. Using this analysis of common activity, we reduced the likelihood of biasing isolated amygdala activity to any one of three trial-types. To test for habituation, temporal-gradients were generated for the right and left amygdala by extracting the first eigenvariate of each region for each condition and then analyzed in SPSS using a linear trend analysis.

2. Results

2.1. Behavioral habituation analysis
A 2 × 10 (LVF2 congruency: congruent vs. incongruent) × 10 (Run: 1–10) trend analysis for repeated measures was used to test for behavioral habitation of the congruency reaction time (RT) effect (i.e. congruent RT < incongruent RT). As can be seen in Figure 1, the interaction between congruency and run was not significant, F(1, 9) = 0.162, p = 0.697, η² = 0.018, suggesting that a behavioral level attentional bias to masked fearful faces was sustained over the 10 runs in this experiment.
2.2. Amygdala ROI habituation analysis
As displayed in Figure 2, the task elicited bilateral amygdala activity, Left: \( t(10) = 9.38, P_{\text{FWE}} < 0.05, k = 210, -32, -2, -12 \); Right: \( t(10) = 8.81, P_{\text{FWE}} < 0.05, k = 145, 28, 2, -16 \). Data were extracted from these clusters and a 2 (Hemisphere: left vs. right) × 3 (Condition: LVF directed attention vs. fearful–fearful vs. neutral–neutral) × 10 (Run: 1–10) trend analysis for repeated measures was used to test for left and right amygdala habituation for each of the three conditions. There was no evidence of overall amygdala habituation across runs, \( F(1, 9) = 0.036, p = 0.854, \eta^2_p = 0.004 \). Furthermore, the amygdala did not habituate as a function of the following potential interactions: Hemisphere × Run \( F(1, 9) = 2.904, p = 0.123, \eta^2_p = 0.244 \), Condition × Run \( F(1, 9) = 0.173, p = 0.687, \eta^2_p = 0.019 \), or Hemisphere × Condition × Run \( F(1, 9) = 0.159, p = 0.699, \eta^2_p = 0.017 \). In short, all linear contrasts were not significant—suggesting that neither the left nor right amygdala habituated over time. In contrast, significant Hemisphere × Run interactions were observed for eighth order (\( F(1, 9) = 9.22, \eta^2_p = 0.508 \)).
p = 0.01, \eta^2_p = 0.51) and ninth-order (F(1, 9) = 11.65, p = 0.008, \eta^2_p = 0.56) effects. Follow-up analyses indicate that the left amygdala displays an oscillatory pattern of the ninth order (F(1, 9) = 13.19, p = 0.005, \eta^2_p = 0.59), while the right amygdala displays an oscillatory pattern of the eighth order, F(1, 9) = 5.74, p = 0.04, \eta^2_p = 0.39. Thus, in contrast to a negative linear trend—indicative of habituation—amygdala activity oscillates in a non-linear nature over the extended 10 run scanning session.

3. Discussion
In this pilot study, we utilized an existing data-set (Carlson et al., 2009) where attentional bias behavior and amygdala activity were measured over an extended period of time to assess the degree to which these measures habituate during this prolonged timespan. At the behavioral level, there was a clear capture of attention by masked fearful faces—faster responses on congruent relative to incongruent trials—which was maintained from start-to-finish in a dot-probe task. Similarly, at the neural level, task-related amygdala activity oscillated throughout the 1,098 trial attention task and remained sustained relative baseline. Thus, our preliminary findings suggest that behavioral and neural measures of attentional bias to fearful faces do not habituate, but remain sustained, for at least 50 min (1,098 trials).

The current results add to previous literature indicating that threatening or emotionally salient stimuli capture observers’ attention. This capture of attention by emotionally significant stimuli occurs both when the emotional stimulus is processed consciously and nonconsciously (Armony & Dolan, 2002; Beaver, Mogg, & Bradley, 2005; Blanchette, 2006; Carlson & Mujica-Parodi, 2015; Carlson & Reinke, 2008; Carlson et al., 2009; Cooper & Langton, 2006; Fox, 2002; Koster, Crombez, Verschuere, & De Houwer, 2004; Mogg & Bradley, 1999, 2002; Öhman, Flykt, & Esteves, 2001; Pourtois, Grandjean, Sander, & Vuilleumier, 2004). Several studies have previously examined habituation of attentional bias at the behavioral level. Generally, these studies indicate sustained attentional bias behavior that does not rapidly habituate (Herry et al., 2007; Lonsdorf et al., 2014), although not all studies have reported this pattern (Cohen, Eckhardt, & Schagat, 1998). Our behavioral results extend this understanding and demonstrate that even after 1,098 trials attention bias behavior persists for backward masked fearful faces in the dot-probe task. Furthermore, a relatively recent finding shows that attentional bias can be conceptualized both in terms of a stable trait-like bias as well as a dynamic state-like bias, which alternates between momentary biases toward and away from threat (Iacoviello et al., 2014; Naim et al., 2015). The fluctuation in dynamic state-like biases has been termed “attention bias variability,” which is essentially calculated as the standard deviation of congruent—incongruent reaction time differences throughout the dot-probe task. Our finding of sustained attentional bias (across 10 five minutes runs) suggests that although there may be moment-to-moment variability in attentional bias, a strong bias towards threat is present and sustained for at least 50 min (1,098 trials).

As mentioned in the introduction, the amygdala has been found to respond to a variety of threatening, or salience-related stimuli, despite whether these stimuli are processed consciously (Breiter et al., 1996; Morris et al., 1996) and nonconsciously (Liddell et al., 2005; Morris et al., 1998; Whalen et al., 1998; Williams et al., 2005). Early work using passive viewing of emotional facial expressions in block designs found that—under these circumstances—the amygdala activates to salient environmental stimuli, but this response rapidly habituates (Breiter et al., 1996; Whalen et al., 1998). Other passive viewing studies of fear learning have reported similar effects using both block (Tabert et al., 2001) and randomized event-related designs (Greenberg, Carlson, Cha, Hajcak, & Mujica-Parodi, 2013) leading to the general acceptance of the idea that the amygdala habituates to signals of threat. However, our results add to other research indicating that—for some behaviors—the amygdala remains active over an extended period of time (Herry et al., 2007; Tabert et al., 2001). Indeed, our findings indicate that amygdala activity was sustained over 1,098 trials in a 50 min time period during which attentional behavior was also sustained in the dot-probe task. Other reports of sustained amygdala activity have also been accompanied by sustained behavioral engagement including attentional bias under conditions of uncertainty (Herry et al., 2007) and emotional decision-making (Tabert et al., 2001). Thus, it appears that in circumstances where emotional information processing aids or modulates
cognitive processing the amygdala remains active—presumably contributing to this cognitive bias. The available evidence suggests that this is at least true for emotion-related influences on decision-making and attention allocation. On the other hand, a dip in amygdala activity from run one to run two was observed. Studies not collecting additional data/runs may mistake this temporary dip in amygdala activity—which appears to be part of an oscillatory pattern (see Figure 2)—as habituation. Furthermore, given that we used a randomized event-related design, which results in uncertainty regarding the onset and nature of a given trial, it could be argued that this element of uncertainty is driving the observed sustained amygdala activation observed in our experiment. However, previous reports have found habituated amygdala activation in event-related designs (Greenberg et al., 2013) and sustained activation in block designs (Tabert et al., 2001) and therefore, the event-related nature of our design does not seem to be a sufficient factor in eliciting sustained amygdala activation. Although the current study assessed amygdala habituation to fearful faces, the amygdala appears to be more generally involved in processing abstract dimensions of a stimulus such as its salience, ambiguity, unpredictability, and general biological importance (Cunningham & Brosch, 2012; Pessoa, 2010; Pessoa & Adolphs, 2010). Additional research is needed to fully understand all the circumstances and stimuli for which the amygdala habituates or, on the other hand, remains active.

All studies inherently have their strengths and weaknesses. Our pilot study had a relatively small sample size, compromising the detection of small effects. Although, this limitation was, at least partially, overcome by using a data-set in which amygdala activity was known to be present (Carlson et al., 2009), these preliminary findings need to be replicated using a larger sample. Another limitation of our pilot study is the sole comparison of fearful to neutral facial expressions. Future research including different categories of emotional stimuli is needed to determine whether the effects observed here are specific to threat (fearful faces) or more generally observed for emotional or salience-related stimuli. The primary strength of our study was the extended length of time (50 min) and number of trials (549/condition) utilized in our task, which is considerably longer than the typical fMRI experiment (15 ± 5 min, 30–40 trials/condition). This strength allowed us the ability to test amygdala habituation over an extended time-period. In sum, although not without limitation, the current results provide preliminary evidence that attentional bias behavior and amygdala activity are sustained during the dot-probe task when using fearful faces.

**Notes**

1. Note that previous behavioral dot-probe studies using masked threatening faces have also found only left visual-field (LVF) effects (Fox, 2002; Mogg & Bradley, 2002), which were interpreted by these authors as evidence of a right-hemisphere (RH) bias for negative emotion processing (Davidson, 1992). Thus, the current LVF/RH only effects are in-line with this bias for negative emotion processing. However, a majority of the dot-probe studies conducted by our lab have produced effects across both visual fields (Carlson et al., 2012, 2013, 2014; Carlson, Fee, & Reinke, 2009; Carlson & Mujica-Parodi, 2015; Carlson, Mujica-Parodi, Harmon-Jones, & Hajcak, 2012; Carlson & Reinke, 2008, 2010, 2014; Carlson, Torence, & Vander Hyde, 2016), which weakens this claim. It is possible that the unique testing environment of the MRI scanner (i.e. lying on their back, loud scanner noise, and a potential visibility limiting head coil) could have contributed to the LVF only effects. For example, previous research has shown that leaning forward biases left hemisphere mediated approach motivated behavior, while a reclined posture biases right hemisphere withdrawal motivated behavior (Price & Harmon-Jones, 2015).

2. Note that previous analysis of this data found that congruent trials were faster than incongruent trials, but only for LVF trials. Thus, across the 10 runs of the experiment masked fearful faces captured attention. Given that RVF trials did not capture attention in this sample, the habituation analysis was restricted to LVF trials.
3. This experiment contained 6,039 trials per condition (549 trials per condition per participant × 11 participants). A large number of trials per participant increases the reliability (i.e. decreases the statistical error) of each measure. Given this increased precision, the need for a larger sample is reduced. Using the standard 40 trials per condition per participant, it would take approximately 150 participants to obtain the same number of trials per condition used in the current study. Thus, although the number of participants used in the current study was relatively small, the total number of amygdala data points per condition was very large.

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