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Fear bradycardia and activation of the human periaqueductal grey

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ABSTRACT

Animal models of predator defense distinguish qualitatively different behavioral modes that are activated at increasing levels of predation threat. A defense mode observed at intermediate threat levels is freezing: a cessation of locomotion that is characterized by a parasympathetically dominated autonomic nervous system response that causes heart rate deceleration, or fear bradycardia. Studies in rodents have shown that freezing depends on amygdalar projections to the periaqueductal grey (PAG). In humans, freezing-like behaviors are implicated in development and maintenance of psychopathology, but neural mechanisms underlying freezing or its characteristic autonomic response profile have not been identified. Here, we combined event-related blood oxygenation level-dependent functional MRI (BOLD-fMRI) with autonomic response measures in a picture viewing paradigm to probe activity and interconnectivity within the amygdala–PAG pathway and test for an association with parasympathetic as opposed to sympathetic activation. In response to negatively arousing pictures, we observed parasympathetic (bradycardia) and sympathetic (pupil dilation) autonomic responses, BOLD responses in the amygdala and PAG, and effective connectivity between these regions. Critically, BOLD responses in the PAG to negative pictures correlated on a trial-by-trial basis with bradycardia but not pupil dilation. This correlation with bradycardia remained significant when partialling out pupil dilation. Additionally, activity in regions associated with motor planning and inhibition mirrored the PAG response. Thus, our findings implicate the human PAG in a parasympathetically dominated defense mode that subserves a state of attentive immobility. Mechanistic insight into this qualitatively distinct defense mode may importantly advance translational models of anxiety disorders.

Introduction

The coevolution of predators and prey has spawned a repertoire of qualitatively different behavioral defense strategies that animals deploy at various levels of predator threat (Blanchard et al., 2001; Eilam, 2005; Fanselow, 1994). A passive defense mode activated at intermediate threat levels is freezing: a state of attentive immobility that serves to avoid detection by predators (Lang and Davis, 2006; Öhman and Wiens, 2002). It is well known that freezing behavior in both animals and humans is associated with heart rate deceleration, or fear bradycardia (Lang and Davis, 2006). This parasympathetically dominated autonomic response contrasts with the sympathetically dominated fight-or-flight response activated during imminent predation threat (Fanselow, 1994). In humans, freezing and its concomitant attentional focus on threat-related information is thought to contribute to a vicious cycle whereby emotional disturbance triggers an attentional bias for threat-related information, and vice versa (Fox et al., 2001; Hagenaars et al., 2012; Holmes et al., 2004; Marks, 1987; Roelofs et al., 2010). However, relatively little is known about the neural regulation of freezing in humans.

Rodent studies have shown that the amygdala plays a key role in orchestrating defensive behavior and transitioning between defensive modes (Davis and Whalen, 2001; Fendt and Fanselow, 1999; Gozzi et al., 2010; Haubensak et al., 2010). For instance, stimulation of the central nucleus of the amygdala produces freezing, bradycardia, and pupil dilation (Applegate et al., 1983), whereas lesions block both autonomic and behavioral manifestations of fear (Fendt and Fanselow, 1999; Kapp et al., 1979). Autonomic responses are mediated by downstream connections to the lateral hypothalamus, which controls sympathetic responses, and to medullar nuclei that control parasympathetic effects through vagal efferents (Schwaber et al., 1982). Behavioral manifestations of predator defense, however, depend on the periaqueductal grey (PAG; Ledoux et al., 1988), a midbrain region implicated in a host of homeostatic processes including fear, pain, and analgesia (Linnman et al., 2012; Neugebauer et al., 2009). In particular, lesions of the ventral (Liebman et al., 1970; Lyon, 1964), but not the dorsal (Kim et al., 1993)

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PAG disrupt freezing. Human research on amygdala lesioned patients has produced findings that are roughly consistent with the animal literature (Bechara et al., 1995; Funayama et al., 2001; LaBar et al., 1995), but the anatomical specificity of this work is limited. Neuroimaging studies in humans have moreover shown that activity in amygdala–PAG circuitry varies with threat proximity (Mobbs et al., 2007, 2009, 2010). However, activity in these circuits has not been associated specifically with freezing or its accompanying parasympathetic autonomic response.

A well-established paradigm to study the autonomic psychophysiology of defense behaviors in humans is exposure to pictorial stimuli taken from the International Affective Picture System (Lang et al., 2005). Studies using this paradigm have shown that autonomic responses to affective stimuli, which vary on dimensions of valence and arousal, closely parallel autonomic responses that accompany defensive behaviors in rodents (Lang and Davis, 2006; Lang et al., 1998). For instance, negatively valenced and highly arousing pictures elicit sympathetic changes such as galvanic skin responses (Lang and Davis, 2006) and pupil dilation (Bradley et al., 2008). Notably, however, numerous studies have demonstrated that such stimuli also induce marked bradycardia (Bradley et al., 2008; Hermans et al., 2007; Lang and Davis, 2006) that may be associated with sustained attentional processing of such stimuli (Libby et al., 1973). More recent studies have attempted to associate this decelerative heart rate response directly to freezing behavior in humans. Using stabilometric force platforms, these studies have confirmed that bradycardia responses are accompanied by reduced locomotion as measured by postural sway (Azevedo et al., 2005; Facchinetti et al., 2006; Roelofs et al., 2010). In the present study, we therefore hypothesized that neural activity in the amygdala–PAG pathway can be linked to freezing through an association with parasympathetically as opposed to sympathetically dominated autonomic responses.

To investigate this, we combined functional magnetic resonance imaging (fMRI) with autonomic response measures (heart rate change, pupil dilation) in a picture viewing paradigm. We expected to replicate previous findings of stronger bradycardia and pupil dilation to negatively arousing stimuli (Bradley et al., 2008), and to observe increased neural activity and connectivity within the amygdala–PAG pathway. Critically, we predicted a trial-by-trial correlation between PAG responses and parasympathetic (bradycardia) responses that is statistically independent of concomitant sympathetic (pupil dilation) responses.

Materials and methods

Participants

Eighteen (aged 19–31 years) male, healthy, right-handed volunteers were tested in a within-subject design. Exclusion criteria were history of head injury, treatment with psychotropic medications, narcotics, beta-blockers, steroids, or any other medication that affects CNS or endocrine systems, medical illness within 3 weeks before testing, self-reported mental or substance use disorder, daily tobacco use, current stressful episode or major life event, a score of 8 + on the Beck Depression Inventory (Beck et al., 1979), previous exposure to photographs used in the study, and regular exposure to violent movies or computer games. We excluded women in this study to reduce interindividual variance due to sex- and cycle-related confounds. The study was approved by the local institutional review board (CMO region Arnhem-Nijmegen, The Netherlands) in accordance with the declaration of Helsinki. All participants provided written informed consent.

General procedure and experimental paradigm

The design of this study is illustrated in Fig. 1. Participants were instructed not to use any recreational drugs for 3 days, and to refrain from drinking alcohol, exercising, and smoking for 24 h before each session. All completed Beck's Depression Inventory (Beck et al., 1979) and were invited for scan sessions between 2:00 P.M. and 6:00 P.M.

Three different sets of 160 photographs were selected from the International Affective Picture System (Lang et al., 2005) and an additional set of newly rated pictures. New pictures were selected based on their emotionality and similarity to IAPS pictures, and were rated on a 1–9 score for subjective valence and arousal using the self-assessment manikin (SAM) scales (Bradley and Lang, 1994) by a separate group of 20 healthy male participants. SAM scales are standardized for use with the IAPS and consist of small pictograms depicting valence (using positive and negative facial expressions) and arousal (illustrated by small explosions at the level of the heart). Each picture set was used in six participants, and contained 80 aversive and 80 neutral pictures. Aversive photographs had moderate to high arousal scores (mean 5.5, SD .7) and negative valence (mean 3.1, SD .7). Neutral slides had low arousal scores (mean 2.5, SD .7) and neutral valence (mean 5.3, SD .3). The three different picture sets did not differ in arousal and valence ratings, amount of newly scored photographs, and chromatic features and complexity.

During functional MRI scanning, 160 pictures were shown in pseudorandom order (no more than two consecutive pictures from the same category) in three separate blocks of 12.5 min and presented for 5 s. An interstimulus interval varying randomly between 4 and 8 s was used, during which a fixation cross was shown. To ascertain that they processed the pictures attentively and to obtain an online indication of subjective affective ratings of the stimuli, participants were instructed to categorize their content as either aversive or neutral. Responses were given with right-hand button presses. Furthermore, participants were informed that their memory for the pictures would be tested after the scan session (reported elsewhere; Henckens et al., 2009).

In the scanner, participants wore ear plugs, and foam pads were used to restrict movement. Stimuli were back-projected onto a translucent screen positioned behind the participant's head that was visible through a mirror mounted on the head coil. Scan sessions started with calibration of the eye-tracking system.

Physiological measurements

Heart rate was recorded throughout scanning using a 50 Hz finger pulse photoplethysmograph affixed to the left index finger. Data were processed using in-house software for interactive visual artifact correction and peak detection, resulting in time-series of interbeat intervals expressed in beats per minute (BPM). Data were baseline corrected using a −25 s to −1 s pre-stimulus onset average. We chose this relatively long baseline window because heart rate fluctuates with the respiratory cycle (i.e., respiratory sinus arrythmia; De Geus et al., 1995), which causes a signal fluctuation that strongly affects baselines just prior to stimulus onset. Event-related responses were quantified as averaged heart rate change between 2 and 5 s post-stimulus onset. The 0–2 s response window was discarded because heart rate decelerates nonspecifically during this period (i.e., the orienting response). The average percentage of trials (and standard deviation across participants) that were valid (i.e., contained no artifacts) was 91.0 (11.1) and 91.3 (11.0) for negatively arousing and neutral pictures, respectively. Trial-by-trial measures of heart rate change were included in (hierarchical) parametric analyses of fMRI data (see below), and were tested for effects of picture category using a paired samples *t*-test.

Pupil dilation and eye movements were monitored using a 50 Hz iView system with MR-compatible MEyeTrack-LR camera mounted on the scanner bed (SensoMotoric Instruments, Teltow, Germany). Pupil dilation data were analyzed using in-house software implemented in Matlab 7.9 (The Mathworks, Natick, MA). Signal artifacts due to eye blinks were removed using linear interpolation (Siegle, 2003). Event-related pupil dilation responses were calculated by dividing averaged pupil dilation during the 1–5 s period after picture onset by the averaged 1 s prior to onset. Note that this baseline window differs from