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Neural correlates of pupil dilation during human fear learning

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ABSTRACT

Background: Fear conditioning and extinction are prevailing experimental and etiological models for normal and pathological anxiety. Pupil dilations in response to conditioned stimuli are increasingly used as a robust psychophysiological readout of fear learning, but their neural correlates remain unknown. We aimed at identifying the neural correlates of pupil responses to threat and safety cues during a fear learning task.

Methods: Thirty-four healthy subjects underwent a fear conditioning and extinction paradigm with simultaneous functional magnetic resonance imaging (fMRI) and pupillometry. After a stringent preprocessing and artifact rejection procedure, trial-wise pupil responses to threat and safety cues were entered as parametric modulations to the fMRI general linear models.

Results: Trial-wise magnitude of pupil responses to both conditioned and safety stimuli correlated positively with activity in dorsal anterior cingulate cortex (dACC), thalamus, supramarginal gyrus and insula for the entire fear learning task, and with activity in the dACC during the fear conditioning phase in particular. Phasic pupil responses did not show habituation, but were negatively correlated with tonic baseline pupil diameter, which decreased during the task. Correcting phasic pupil responses for the tonic baseline pupil diameter revealed thalamic activity, which was also observed in an analysis employing a linear (declining) time modulation.

Conclusion: Pupil dilations during fear conditioning and extinction provide useful readouts to track fear learning on a trial-by-trial level, particularly with simultaneous fMRI. Whereas phasic pupil responses reflect activity in brain regions involved in fear learning and threat appraisal, most prominently in dACC, tonic changes in pupil diameter may reflect changes in general arousal.

1. Introduction

Fear conditioning and extinction paradigms can be used to assess individual differences in fear learning and provide useful etiological models for anxiety disorders. Previous studies have revealed different response patterns during fear conditioning and extinction in healthy controls and psychiatric patients suffering from post-traumatic stress disorder (PTSD; Blechert et al., 2007; Glover et al., 2011; Grillon et al., 2009; Pole, 2007), panic disorder (Grillon et al., 2008; Lissek et al., 2010), social anxiety disorder (Lissek et al., 2008b) and others. A meta-analysis by Lissek et al. (2005b) and a recent update by Duits et al. (2015) reported increased physiological responding to safety cues (CS-) during fear acquisition and increased responses to fear conditioned stimuli (CS+) during extinction in patients suffering from anxiety disorders and PTSD.

To objectively assess fear learning, autonomous arousal in response to CS- as compared to CS+ is measured. This can be done by contrasting skin conductance responses (SCR), which are mainly determined by changes in sympathetic activation (Dawson et al., 2007). Another commonly applied method is startle electromyography (EMG), during which the startle reflex to sudden loud noises is quantified (for a review of startle responsivity in clinical populations see Vaidyanathan et al., 2009). The startle reflex is modulated by emotional valence (Grillon and Baas, 2003) and yields stronger responses to noises applied during CS+ presentations as compared to CS- presentations (Lindner et al., 2015; Lipp et al., 1994; Van Well et al., 2012).

However, both SCR and startle EMG have some disadvantages. SCRs can occur spontaneously and show a considerable amount of inter- and intra-subject variability (Bach et al., 2009; Benedek and Kaernbach, 2010), leading to rather high levels of noise. Furthermore, SCR shows quick habituation, leading to a high amount of zero responses (e.g., defined as responses below 0.01 mS, Dawson et al., 2007) and generally to weak-to-moderate effect sizes for the discrimination of CS- and CS+ (Pineles et al., 2009). The fear potentiated startle has been shown to yield strong effects sizes (Lissek et al., 2008a), but this measure also shows strong habituation effects (Grillon and Baas, 2003) and is more difficult to apply during fMRI measure-

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ments due to the high acoustic background noise (but see Lindner et al., 2015 for an example of combined startle EMG and fMRI). Moreover, the startle sounds themselves can elicit SCRs and can be perceived to be aversive (Lissek et al., 2005a), potentially interfering with cognitive processes of interest.

Pupillometry offers a promising, complementary method for the quantification of the conditioned response. It is non-aversive, not interfering with other measurements and can be combined with functional magnetic resonance imaging (fMRI). The pupil diameter is determined by the sympathetically innervated dilator muscle and the parasympathetically innervated sphincter muscle (Beatty and Lucero-Wagoner, 2000). It is primarily influenced by optical reflexes for light and distance, however, tonic fluctuations and phasic dilations of the pupil associated with mental processes can be detected (Beatty and Lucero-Wagoner, 2000). While tonic changes in pupil diameter have mainly been related to general alertness (Aston-Jones and Cohen, 2005; Gilzenrat et al., 2010) and wakefulness (Lowenstein et al., 1963; Wilhelm et al., 1998), phasic pupil dilations are sensitive to various cognitive and affective manipulations (for a review see Sirois and Brisson, 2014). The pupil dilates in response to aversive (Wiemer et al., 2014) and emotionally arousing stimuli (Bradley et al., 2008; Partala and Surakka, 2003) and can hence be used as a readout of fear-related processes.

Reinhard and Lachnit (2002) were the first to use pupil dilation during CS presentation as a physiological readout of human fear conditioning and reported stronger pupil dilations in response to CS + than to CS- (Reinhard and Lachnit, 2002; Reinhard et al., 2006). In the following years, pupil dilations have been used as readout of fear conditioning in several studies (De Voogd et al., 2016a; De Voogd et al., 2016b; Morriss et al., 2015; Visser et al., 2016; Visser et al., 2015; Visser et al., 2013). Furthermore, there is evidence for pupillary constriction during presentations of CS- (Pollak et al., 2010), suggesting that the pupil size may also reflect processes of fear inhibition.

The method for quantifying the pupillary response varies across studies: while a subtraction of the pre-stimulus baseline pupil diameter is common (Reinhard and Lachnit, 2002; Reinhard et al., 2006; Visser et al., 2016, 2015, 2013), the response has been defined as the mean pupil diameter spanning the whole CS presentation window (Morriss et al., 2015; Pollak et al., 2010) or as the peak change in pupil diameter occurring during CS presentation (Visser et al., 2016; Visser et al., 2013). Reinhard and Lachnit (2002) demonstrated that pupil dilations discriminate most strongly between CS- and CS+ in a time window immediately preceding the unconditioned stimulus (US). Visser et al. (2015) found that the baseline-to-peak difference during CS presentation yielded identical results as the change from baseline to the second preceding US onset, indicating that the peak pupil response occurs shortly before US onset.

Besides dilating to threat stimuli, the reflexive constriction of the pupil in response to light flashes is partly attenuated during the anticipation of aversive stimuli (Bitsios et al., 1996, 2004; Hourdaki et al., 2005). Comparable to the fear potentiated startle, this 'fear inhibited light reflex' to light flashes may be modulated by emotional valence (Bitsios et al., 2004). Reinhard et al. (2006) observed such an initial inhibition of the light reflex to CS+, albeit with smaller effect sizes than pupil dilation.

Pupillary responses to CS- and CS+ have been assessed with simultaneous fMRI, mainly as indicator of conditioned responding and fear recall (De Voogd et al., 2016a, 2016b; Morriss et al., 2015; Visser et al., 2016, 2015, 2013). To our knowledge, the neural correlates of pupil dilations during fear learning have not yet been reported. In this study, we aim to identify activity associated with pupil responses to CS- and CS+ during a fear learning task comprising fear conditioning and extinction. If the magnitude of trial-wise pupil dilations correlates with brain regions involved in fear expression or threat appraisal, this would provide support for the notion that pupil dilations are a meaningful trial-by-trial readout of the conditioned response.

In fMRI experiments, the comparison CS+ > CS- typically yields a characteristic pattern of activation, referred to as the fear network (Etkin and Wager, 2007; Fullana et al., 2015). This network comprises the dorsal anterior cingulate cortex (dACC), bilateral anterior insula, thalamus and parts of the striatum, among others; amygdala activation has been specifically associated with early phases of fear conditioning in some studies (for a meta-analysis and a review see Fullana et al., 2015; Sehlmeyer et al., 2009). The reverse contrast CS- > CS+ has been associated with activity in ventromedial prefrontal cortex (vmPFC; Milad and Quirk, 2012), lateral orbitofrontal cortex (OFC), hippocampus and posterior cingulate cortex (PCC; Fullana et al., 2015).

As there are no direct cortical inputs to the pupillary dilator or constrictor muscles (Beatty and Lucero-Wagoner, 2000), cognitive influences on pupil diameter must be conveyed in an indirect way. Activity of the locus coeruleus (LC) in the brainstem has been found to strongly correlate with pupil diameter in the monkey (Aston-Jones and Cohen, 2005; Joshi et al., 2016) and also in the human brain (Gilzenrat et al., 2010; Murphy et al., 2014; Sterpenich et al., 2006). As LC receives direct inputs from anterior cingulate cortex (ACC) and OFC in the monkey brain (Aston-Jones and Cohen, 2005), it may relay activity from these cortical regions into autonomous arousal and related pupil dilations. There is also a two-way excitatory connection between LC and amygdala (Samuels and Szabadi, 2008), which have been found to co-activate in fear-related processes (Liddell et al., 2005; Sears et al., 2013). Given these anatomical connections, we may expect ACC, orbitofrontal cortex and amygdala activity to be associated with the magnitude of pupil dilations during fear learning.

In addition to these anatomical considerations, we would expect pupil dilations in a fear learning task to be associated with regions of the fear network (especially dACC and amygdala). As the pupil is known to dilate more strongly in response to CS+ than to CS-, the neural correlates of pupil dilations should resemble the CS+ > CS- contrast. Reversely, smaller pupil responses may reflect fear inhibition and the activity pattern associated with the CS- > CS+ contrast (e.g., medial prefrontal cortex).

Previous work has shown that most robust differences in pupil responses to CS- and CS+ are found in temporal proximity to the US (Reinhard et al., 2006). We therefore focused on the change in pupil diameter from CS onset until shortly before US administration as a trial-wise readout of the conditioned response, but we also aimed to evaluate the initial light reflex at stimulus onset. Furthermore we explored the dynamics of tonic changes in pupil diameter during the fear learning task and investigated its relationship to phasic pupil responses. To further assess potential effects of uncertainty and expectancy on pupil dilations during fear learning, we used two CS+ with different reinforcement rates: higher reinforcement rates may provoke larger pupil dilations during fear conditioning due to higher threat appraisal, yet partial reinforcement may provoke larger pupil dilations due to uncertainty. Finally, we evaluated whether pupil size dynamics also reflect the so-called partial reinforcement extinction effect (PREE), which has been demonstrated for SCR (Grady et al., 2016). In this context, we would expect stronger pupillary – and behavioral responses – to a partially reinforced CS+ during extinction.

2. Material and methods

2.1. Participants

Thirty-four healthy subjects (mean [M] age=25.6, standard deviation [SD]=3.0, 18 male) participated in a fear learning task with simultaneous fMRI and pupillometry recordings. All subjects were right-handed, non-smokers and had normal or (contact lens) corrected vision. Prior to participation, subjects underwent an interview and a clinical MRI screening to exclude participants with present or past psychiatric or neurological disorders, or current use of psychotropic medication. The study protocol was in accordance with the Declaration