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Norepinephrine-mediated emotional arousal facilitates subsequent pattern separation

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ABSTRACT

Pattern separation, the process by which similar experiences can be stored as distinct memories, has been ascribed to the dentate gyrus (DG) of the hippocampus. The DG is the target of noradrenergic modulation directly and indirectly via the basolateral amygdala. We tested the hypothesis that noradrenergic activation (tested using salivary alpha-amylase) potentiates DG function, enhancing pattern separation, by showing participants fearful stimuli in a pre-training task and then testing their capacity for pattern separation in a later test. Consistent with our hypothesis, we found that increased levels of salivary alpha-amylase were positively correlated with enhanced pattern separation performance even after accounting for general enhancements in recognition.

1. Introduction

Studies in rodents and humans have emphasized the role of the dentate gyrus (DG) subfield of the hippocampus in the process of pattern separation (orthogonalizing similar inputs to facilitate rapid storage; cf review by Yassa & Stark, 2011). Additionally, a large body of evidence strongly supports the notion that norepinephrine enhances the consolidation of new memories in animals and humans in a dose-dependent manner (Cahill, Prins, Weber, & McGaugh, 1994; McGaugh, 2000; McIntyre, Hatfield, & McGaugh, 2002; Segal & Cahill, 2009). The majority of this evidence stems from pharmacological experiments where post-training administration of adrenergic agonists and antagonists resulted in memory enhancement or impairment, respectively (Cahill & Alkire, 2003; Cahill et al., 1994; van Stegeren, Everaerd, Cahill, McGaugh, & Gooren, 1998; van Stegeren et al., 2005). Emotionally arousing encoding tasks result in memory enhancement, the extent of which is positively correlated with endogenous norepinephrine (NE) activation in animals and humans (McIntyre et al., 2002; Segal & Cahill, 2009). The purpose of the current experiment was to examine whether NE activation is associated with an enhanced ability to overcome interference across similar stimuli (i.e. engage in pattern separation).

The DG contains a high concentration of NE receptors and receives NE input via projections from the perforant path (PP) of the hippocampus (Young & Kuhar, 1980). The DG also receives strong modulation via direct NE projections from the locus coeruleus (LC) to the DG, and glutamatergic projections from the basolateral amygala (BLA), which is innervated by noradrenergic projections from the LC (McGaugh, 2002).

In addition to playing a role in long-term memory enhancement (McIntyre et al., 2003), long term potentiation (LTP) studies have shown that LC activation initiates a β-adrenergic and protein synthesis-dependent long-term, but not short-term increase in the synaptic strength of concurrently activated PP input to the DG (Brown, Walling, Milway, & Harley, 2005). It has been reported that NE release converts early phase frequency-induced LTP of PP input to an enduring late phase form (Harley, 2007). Based on studies showing that NE plays an integral role in memory enhancement, and synaptic plasticity of input to the PP/ DG of the hippocampus, we hypothesized that increasing endogenous NE via exposure to emotionally arousing stimuli would enhance the ability of the DG to discriminate among similar stimuli (i.e. facilitate pattern separation).

Salivary alpha amylase (sAA) is a well-established biomarker for NE (Chatterton, Vogelsong, Lu, Ellman, & Hudgens, 1996; Ehlert, Erni, Hebisch, & Nater, 2006). Secretions of sAA result primarily from NE released from sympathetic nerves that innervate acinar cells in the parotid gland (Castle & Castle, 1998; Ehlert et al., 2006; Whelton, 1996). NE binds to G-protein coupled receptors on the acinar cells of the parotid gland, activating cAMP, which results in the synthesis and secretion of alpha-amylase within

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approximately twenty seconds of NE binding to the receptors (Yoshimura, Fujita-Yoshigaki, Murakami, & Segawa, 2002).

Chatterton et al. (1996) measured sAA during various stress conditions, including aerobic exercise and written examinations, and found that sAA levels were tightly coupled to NE (and not epinephrine) levels. Pharmacological blockade of beta-adrenergic receptors using propranolol lowers sAA levels in situations where psychological stress (using emotional pictures) is used to increase arousal (van Stegeren, Rohleder, Everaerd, & Wolf, 2006). Furthermore, pharmacological enhancement of NE using yohimbine results in increased sAA levels (Ehlert et al., 2006). Salivary alpha-amylase also appears to be more accurate measurement of central NE then blood plasma measurements of NE since the latter is also subject to spillover from the adrenal medulla (Ehlert et al., 2006). Taken together, these results demonstrate that sAA is a sensitive index for endogenous NE activity. Thus, we opted to assess NE activation using sAA levels before and after exposure to emotionally arousing stimuli.

2. Materials and methods

2.1. Participants

Thirty-three females (ages 18–35, mean = 22.8 years old) from the University of California, Irvine participated in this study in exchange for course credit. We restricted participation to females because they respond more reliably to the emotional pictures used to elicit the NE response (Segal & Cahill, 2009).

2.2. Experimental procedures

All experiments were conducted between the hours of 13:00 and 18:00 in order to control for the circadian rhythm of sAA. Participants were asked to fast, as well as not chew gum for two hours prior to the experiment. They were also asked to refrain from exercise and the consumption of alcohol and caffeine for 24 h prior to the experiment, so as to control for outside factors that could affect sAA levels. All experiments were presented and responses were collected using PsychToolbox running under the MATLAB 7.5 programming and runtime environment (The MathWorks, Natick, MA).

Fifteen minutes after the participant's arrival to the laboratory a baseline salivary sample was taken (timepoint 1; see Fig. 1). Participants then viewed a slideshow of 138 randomly presented, emotionally arousing images taken from the International Affective Picture Set (IAPS). During the presentation of each picture, the participants rated the intensity of their personal emotional reaction to viewing that picture on a 9-point scale, ranging from 1 (not emotionally arousing) to 9 (extremely emotionally arousing), using standard keyboard button input. Each picture appeared on the screen for 5000 ms, followed by a fixation cross for 1000 ms. Only pictures with a negative valence were used in this study, since preliminary data suggests that these stimuli are much more effective in eliciting an NE response in female participants (Segal & Cahill, 2009). Immediately following the 15 min slide show, a second salivary sample was taken (timepoint 2).

Participants then engaged in an incidental-encoding task previously used to assess pattern separation processing (Lacy, Yassa, Stark, Muftuler, & Stark, 2011), in which a set of 128 everyday items was presented in random order for 2000 ms with a 500 ms interstimulus-interval. Participants indicated whether the item was an "indoor" or an "outdoor" object by pressing keys on the keyboard ('B' for indoor, and 'V' for outdoor). Immediately following the incidental encoding task, a third salivary sample was taken (timepoint 3). Participants then experienced a 15 min delay, in which they engaged in non-arousing tasks (e.g. solving puzzles, word searches, etc.) to allow sAA levels to return to baseline and a fourth salivary sample was taken (timepoint 4).

Participants were then given a surprise subsequent memory test, in which a set of 192 pictures was randomly presented (Fig. 2). The pictures included exact repetitions of the objects previously shown during the encoding phase (64 targets), slight variations of previously shown objects (64 lures), and objects that were not previously presented (64 foils). These stimuli have been previously developed and the "mnemonic similarity" of the lures has been calibrated (see Yassa, Lacy, et al., 2010; Yassa, Stark et al., 2010; Yassa, Muftuler, Stark, 2010 and Lacy et al., 2011 for details). The task itself is based on earlier work by Koutstaal and colleagues (1997). Participants were instructed to indicate by pressing a key whether the object was old ('V' key), similar ('B' key), or new ('N' key). Pictures remained on the screen until the participant pressed a response key and then advanced to the next trial.

3. Results and discussion

3.1. Salivary alpha-amylase response

Of the 33 participants, 13 responded reliably with an increase in their sAA levels (timepoint 3–timepoint 1), which is consistent with the 36% of positive responders reported previously (Segal & Cahill, 2009). Timepoint 3 reflects sAA levels immediately after

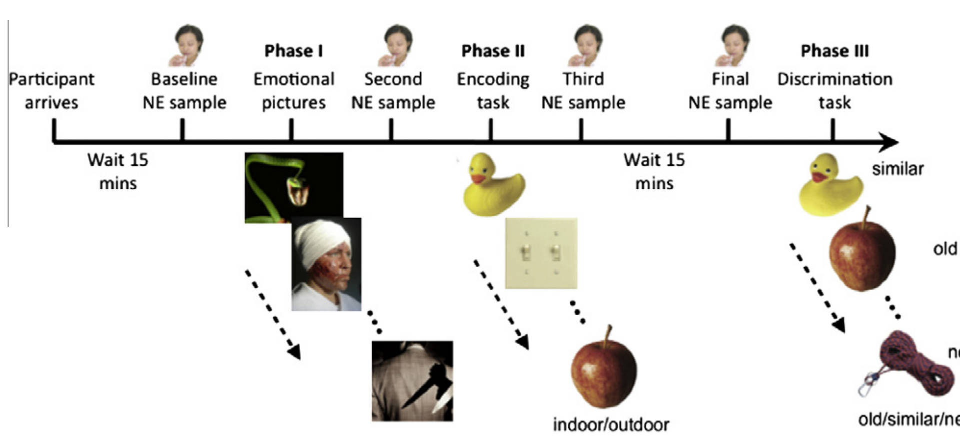

Fig. 1. Overview of experimental procedures. Participants provided salivary samples at four time points: prior to encoding emotionally-arousing pictures, prior to incidental encoding of novel pictures, immediately following the encoding phase, and immediately prior to an old/similar/new discrimination task.