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In vivo levels of corticotropin-releasing hormone and gastrin-releasing peptide at the basolateral amygdala and medial prefrontal cortex in response to conditioned fear in the rat

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ABSTRACT

Given the modulatory effect of exogenously administered corticotropin-releasing hormone (CRH) and gastrin-releasing peptide (GRP) on conditioned fear, the present study sought to measure the fear-induced endogenous release of CRH and GRP at the medial prefrontal cortex (mPFC) and basolateral amygdala (BLA) using *in vivo* microdialysis. Rats were divided into 2 training conditions; tone only (cue), or tone paired with shock. The day after conditioning, animals were tested for fear by scoring freezing behavior in response to the tone alone in cages different from the cages they were previously conditioned in. Freezing was scored for 10 min. Dialysates were collected over 20 min intervals from 2 h prior to testing (to establish baseline values) through to 3 h post-testing continually uninterrupted. Analyses of dialysates revealed that at the BLA, the release of both CRH and GRP was increased over time and that peptide release was significantly higher in animals that had previously received shock relative to rats that had not. Further, the release of CRH and GRP was significantly correlated with freezing levels (an indication of fear in the rat) such that animals that had higher levels of freezing also had higher interstitial peptide levels. These effects appeared site-specific, as they were not apparent at the mPFC. It appears that at the BLA, the release of CRH and GRP is related to fear.

1. Introduction

The amygdala and the prefrontal cortex have both been implicated in the development and maintenance of conditioned fear. Using classical conditioning models, where a neutral conditioned stimulus (CS) such as a tone is paired with an aversive unconditioned stimulus (US) such as a shock, the specific contributions of different cortical areas and amygdaloid regions in the induction of fear and anxiety has become clearer. In this regard, it has been suggested that the basolateral amygdala (BLA), which comprises the lateral, basolateral and basomedial nuclei, is a key complex where the interface of CS–US associations are formed (Campeau and Davis, 1995; Cousens and Otto, 1998; LeDoux et al., 1990; Maren et al., 1996). Information from the BLA is relayed to the central nucleus of the amygdala (CeA) where the expression of conditioned fear responses is initiated (Gentile et al., 1986; Hitchcock and Davis, 1986; Iwata et al., 1986; Kapp et al., 1979; Pitkanen et al., 1997; Van de Kar et al., 1991). The medial prefrontal cortex (mPFC), which encompasses the cingulate, prelimbic and infralimbic cortices, shares reciprocal connections with both the BLA and CeA and is recruited during the extinction of conditioned fear (Milad et al., 2004; Milad and Quirk, 2002; Morgan et al., 1993; Morgan and LeDoux, 1995; Quirk et al., 2003). Indeed, it is believed that the mPFC gates or inhibits amygdalar activity through the lateral amygdala and/or through intercalated cells located between the BLA and CeA (Milad et al., 2004; Milad and Quirk, 2002).

Increasing evidence has implicated amygdalar corticotropin-releasing hormone (CRH) as subserving, in part, behavioral responses indicative of stress and/or anxiety (Dunn and Berridge, 1990; Kalin et al., 1994; Swiergiel et al., 1993) and/or fear (Hubbard et al., 2007; Lee and Davis, 1997). For example, microinfusion of either the nonselective CRH_1/CRH_2 antagonist, α-helical CRH_{9-41} or the selective CRH_1 antagonist, NBI27914, into the CeA blocked footshock-induced

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freezing (Bakshi et al., 2002; Swiergiel et al., 1993). Similar results were observed in response to intra-BLA infusions of the CRH_1 antagonist DMP696 (Hubbard et al., 2007), but a corresponding role for CRH receptors in the CeA was not observed in response to this specific antagonist. Using an inhibitory avoidance task, Liang and Lee (1988) found that post-training administration of CRH to the amygdala led to enhanced task retention. In contrast, microinjections of α-helical CRH_{9-41}, into either the BLA or CeA immediately after training in the inhibitory avoidance task, produced deficits in retention in the BLA treated animals, but not in rats that had the antagonist injected into the CeA (Roozendaal et al., 2002). Further, microinjections of the CRH_1 antagonist, antalarmin, into the BLA of mice immediately preceding social defeat resulted in the reduction of defensive behaviors to a non-aggressive intruder upon testing the next day (Robison et al., 2004).

Like CRH, gastrin-releasing peptide (GRP), the mammalian equivalent of the tetradecapeptide, bombesin (BB), has also been associated with stress and/or fear-related pathology. Amygdaloid and cortical sites appear particularly relevant in this respect. The GRP gene is highly expressed in the lateral nucleus of the amygdala (LA) and enhanced memory for fear-motivated behaviors was evident in mice lacking the GRP receptor (BB_2 receptor) (Shumyatsky et al., 2002). Moreover, acute stressor exposure elicited the release of BB-like peptides from the CeA and induced a significant elevation of GRP levels at the cingulate cortex/mPFC (Adamec et al., 1998; Merali et al., 1998, 2001). As predicted, administration of GRP (intraventricularly [i.c.v.] or localized to specific amygdaloid or cortical sites), attenuated the expression of fear (as seen by reduced levels of freezing) in response to contextual cues (i.e., in a context in which animals had previously been exposed to shock), and to a tone that had previously been paired with a shock (Bédard et al., 2007; Mountney et al., 2006, 2008). Conversely, we observed that i.c.v. administration of RC-3095, a BB_2 receptor antagonist, increased freezing in this paradigm (Merali et al., 2010). Localized microinjection of the BB_2 antagonist, however, elicited a more complicated pattern of behavioral responses. Specifically, administration of the BB_2 receptor antagonist BW2258U89 into the infralimbic cortex attenuated the freezing response (Mountney et al., 2006), whereas the effects of BW2258U89 at the CeA were biphasic in that high doses reduced freezing and low doses increased freezing (Mountney et al., 2006). Administration of RC-3095 to the BLA significantly reduced the freezing response, but this outcome was observed only to contextual cues and not to the conditioned tone.

Despite the complex relations between GRP alterations and fear responses, both the CRH and BB/GRP systems have been proposed as novel therapeutic targets for anxiety disorders (Holmes et al., 2003). In an attempt to clarify the role of CRH and GRP peptidergic systems in fear responses, we examined the effect of conditioned fear on *in vivo* alterations in the release of CRH and GRP at the mPFC and BLA while simultaneously assessing freezing behavior associated with conditioned fear cues.

2. Materials and methods

2.1. Subjects

Male Sprague-Dawley rats (Charles River Laboratories, St-Constant, Quebec) were maintained on a 12 h light/dark cycle (lights on at 07:00 h) in a climate-controlled environment (23 °C, relative humidity 60%). Weighing approximately 275–300 g upon arrival, animals had free access to food (Purina Lab Chow) and water throughout the experiment and were doubly housed in standard plastic cages (45 × 25 × 20 cm) until surgery. All experiments were conducted in accordance with the Canadian Council of Animal Care, and were approved by the animal care committee of the University of Ottawa. All efforts were made to minimize animal suffering and to reduce the number of animals utilized in the study.

2.2. Surgery

Animals were anesthetized with Halothane at 2.5% and were stereotaxically implanted with intracerebral guide cannula (MD-2250; Bioanalytical Systems Inc.) at the following coordinates; mPFC: A/P +2.8 mm, L +0.6 mm, D/V −3.7 mm (Fig. 1a); BLA: A/P −3.1 mm, L +5.3 mm, D/V −7.2 mm (Fig. 1b), based on (Paxinos and Watson, 1982). For pain control, rats received oral acetaminophen (Tylenol: 100–200 mg/kg) for 3 days prior to and 3 days following surgery. In addition, they received rectal

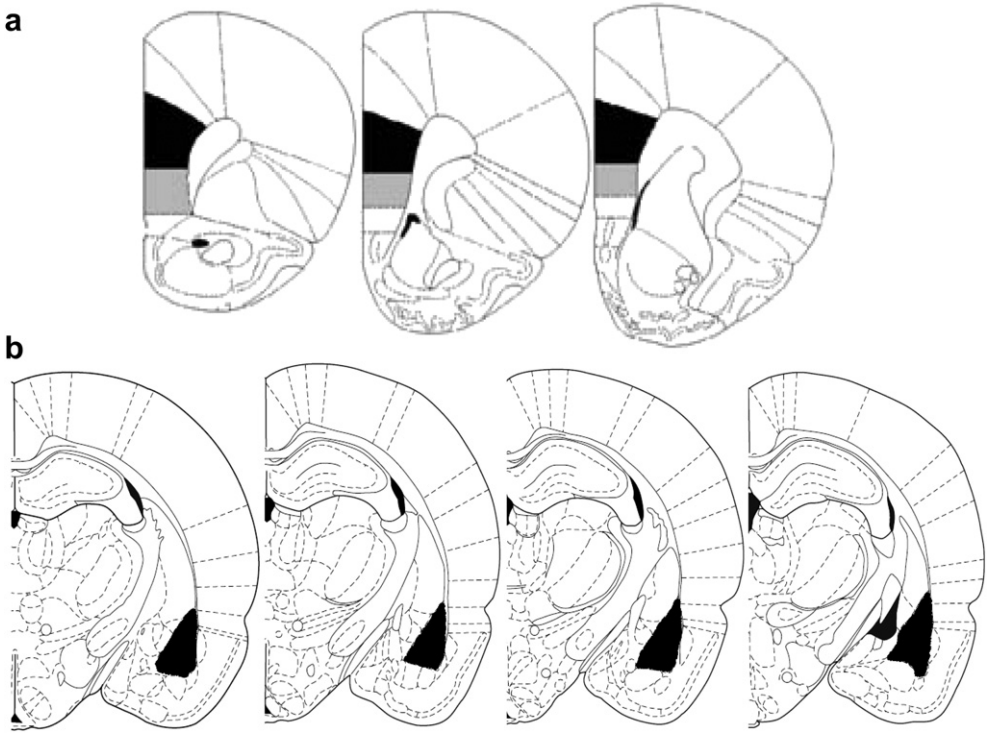

Fig. 1. Diagram of the acceptable planes (taken from Paxinos and Watson (1982)) for placements, showing a) the prelimbic cortex shaded in black and the infralimbic cortex shaded in grey of the mPFC (A/P +2.8 mm, L +0.6 mm, D/V −3.7 mm) and b) the BLA shaded black (A/P −3.1 mm, L +5.3 mm, D/V −7.2 mm).