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Research Report

Conditioned fear inhibits c-*fos* mRNA expression in the central extended amygdala

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ABSTRACT

We have shown previously that unconditioned stressors inhibit neurons of the lateral/capsular division of the central nucleus of the amygdala (CEAl/c) and oval division of the bed nucleus of the stria terminalis (BSTov), which form part of the central extended amygdala. The current study investigated whether conditioned fear inhibits c-*fos* mRNA expression in these regions. Male rats were trained either to associate a visual stimulus (light) with footshock or were exposed to the light alone. After training, animals were replaced in the apparatus, and 2 h later injected remotely, via a catheter, with amphetamine (2 mg/kg i.p.), to induce c-*fos* mRNA and allow inhibition of expression to be measured. The rats were then presented with 15 visual stimuli over a 30 minute period. As expected, fear conditioned animals that were not injected with amphetamine, had extremely low levels of c-*fos* mRNA in the central extended amygdala. In contrast, animals that were trained with the light alone (no fear conditioning) and were injected with amphetamine had high levels of c-*fos* mRNA in the CEAl/c and BSTov. Animals that underwent fear conditioning, and were re-exposed to the conditioned stimulus after amphetamine injection had significantly reduced levels of c-*fos* mRNA in both the BSTov and CEAl/c, compared to the non-conditioned animals. These data suggest that conditioned fear can inhibit neurons of the central extended amygdala. Because these neurons are GABAergic, and project to the medial CEA (an amygdaloid output region), this may be a novel mechanism whereby conditioned fear potentiates amygdaloid output.

1. Introduction

Models of classical or Pavlovian fear conditioning in which an animal learns to predict danger based on prior experience have been the subject of intense study, and understanding the mechanisms underlying this type of fear conditioning is thought to be very relevant to understanding human anxiety disorders (Bouton et al., 2001). The central nucleus of the amygdala (CEA) is a critical structure for the expression of conditioned fear, with outputs from the medial CEA thought to control many conditioned fear responses, including potentiation of the acoustic startle reflex for example (Davis, 1992). The neural circuitry involved in classical or Pavlovian fear conditioning is relatively well understood (Pare et al., 2004; LeDoux, 2007), with activation of thalamic and cortical amygdaloid inputs by both the conditioned stimulus (such as a visual or auditory cue) and

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Abbreviations: BST, bed nucleus of the stria terminalis; CEAl/c, central nucleus of the amygdala, lateral/capsular divisions; CRH, corticotropin releasing hormone

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somatosensory unconditioned stimulus (such as pain from footshock) leading to convergence of inputs in the lateral amygdala. Current evidence suggests that synaptic plasticity occurs in both the lateral/basolateral amygdaloid complex (Malkani and Rosen, 2000; Blair et al., 2001; Maren and Quirk, 2004; LeDoux, 2007) and CEA (Pascoe and Kapp, 1985; Samson and Pare, 2005). Subsequent exposure to the conditioned stimulus alone results in information flow from the lateral amygdala, via the basal nucleus, accessory basal nucleus and/or intercalated cells of the amygdala, and ultimately to the medial subdivision of the central nucleus of the amygdala (CEA).

In addition to these pathways, the medial CEA receives input from the lateral and capsular divisions of the CEA (CEAl/c) and the oval (or dorsolateral) nucleus of the bed nucleus of the stria terminalis (BSTov) (Petrovich and Swanson, 1997; Jolkkonen and Pitkänen, 1998; Dong et al., 2001). The amygdala and BST are highly interconnected, and distinct subregions have often been associated under an "extended amygdala" concept, primarily based on afferent and efferent projections, morphological features and neurochemical phenotypes (Alheid et al., 1995). Under this system of categorization, the lateral CEA and BSTov are parts of the central extended amygdala due to their highly similar neuroanatomical features (McDonald, 1982; Cassell et al., 1986; Ju and Swanson, 1989; Ju et al., 1989; Moga et al., 1989; McDonald, 2003). The capsular CEA also receives direct input from the lateral amygdala (Pitkanen et al., 1995), and thus the BSTov and CEAl/c are potentially in a position to provide modulatory input to amygdaloid output neurons and possibly modify fear conditioned responses.

Surprisingly, given the involvement of the CEA in conditioned fear, the levels of c-*fos* mRNA or Fos protein expression, used as a tool to assess neuronal activation, are surprisingly meager in this nucleus (and in the BSTov) following conditioned fear (Pezzone et al., 1992; Beck and Fibiger, 1995; Campeau et al., 1997). A similarly low level of c-*fos* expression has been observed following exposure to unconditioned psychological or processive stressors (Cullinan et al., 1995; Campeau and Watson, 1997; Emmert and Herman, 1999; Day et al., 2001; Dayas et al., 2001). However, we have previously demonstrated that under unconditioned stress conditions such as exposure to a novel environment (Day et al., 2001), loud noise or restraint (Day et al., 2005), the levels of c-*fos* mRNA expression, induced by either amphetamine or interleukin-1β, are *reduced* in both the BSTov and CEAl/c. Similarly, exposure to a single footshock (1.5 mA, 1 s) reduced diazepam-induced EGR-1 mRNA expression in the CEA (Malkani and Rosen, 2000). This suggests that at least some neurons in these regions may be inhibited by unconditioned stressors. The present study was conducted to determine whether a similar inhibition of c-*fos* mRNA in the BSTov and CEA occurs during exposure to conditioned fear. The effect of cued fear conditioning (visual stimulus) on amphetamine-induced c-*fos* mRNA expression in the CEA and BSTov was determined by semi-quantitative in situ hybridization. The data support the hypothesis that conditioned fear can inhibit neurons of the CEAl and BSTov, which may reflect a novel and specific mechanism by which modulation of amygdaloid output neurons is achieved.

2. Results

2.1. *Experiment 1*

As previously shown, amphetamine administration (2 mg/kg i.p.) resulted in high levels of c-*fos* mRNA expression in the BSTov and CEA. Previous exposure to footshock, in a different environment, and using the same parameters as the fear conditioning procedure in Experiment 2, (15 trials at 0.6 mA for 500 ms, average ITI 2 min, on each of the 2 previous days), did not alter the amphetamine-induced c-*fos* mRNA response in these areas, compared to a control group, naïve to footshocks (BST: $p=0.805$; CEA: $p=0.945$; Fig. 1).

2.2. *Experiment 2*

Animals were divided into experimental groups on the basis of their baseline startle amplitudes, so that the mean startle amplitudes (mean of 30 baseline trials at 95, 100 and 105 dB on the second test day) were not significantly different between groups: fear conditioned+conditioned stimulus (light)=3.39±0.63 N; fear conditioned+context=3.33±0.53 N; light alone+light=3.30±0.56 N. Previous work in our laboratory has shown that the training procedure used is effective at producing fear-potentiated startle (data not shown). A highly abbreviated fear-potentiated startle test indicated that the procedure was effective in this experiment also, with the presence of the visual stimulus resulting in a significantly greater increase in acoustic startle amplitude in conditioned compared with control animals ($F(1,20)=8.40$; $p=0.009$; Fig. 2).

The levels of c-*fos* mRNA induced by the conditioned stimulus alone, or following amphetamine injection under

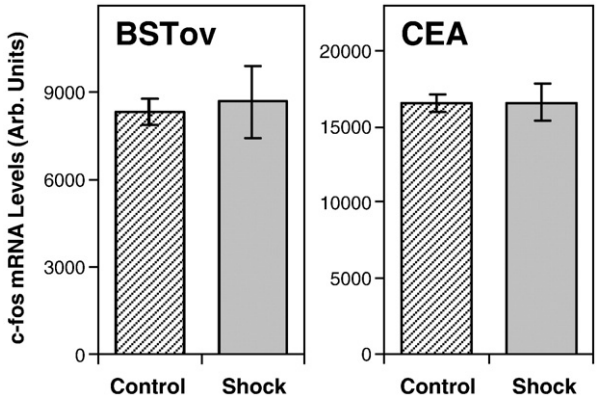

Fig. 1 – Experiment 1: Relative levels of c-*fos* mRNA in the BSTov and CEA. Animals either remained in their home cages within the colony room (Control) or were exposed to footshock, under the same paradigm used for the fear conditioning procedure (Experiment 2), on 2 consecutive days (Shock), before injection with amphetamine, 2 mg/kg i.p., in a different environment the following day. Amphetamine administration resulted in strong c-*fos* mRNA expression in both the BSTov and CEA, but previous shock experience did not diminish this expression in either brain region.