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REVIEW

Role of the extended amygdala in short-duration versus sustained fear: a tribute to Dr. Lennart Heimer

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Abstract The concept of the "extended amygdala", developed and explored by Lennart Heimer, Jose de Olmos, George Alheid, and their collaborators, has had an enormous impact on the field of neuroscience and on our own work. Measuring fear-potentiated startle test using conditioned stimuli that vary in length we suggest that the central nucleus of the amygdala (CeA) and the lateral division of the bed nucleus of the stria terminalis ($BNST_L$) are involved in short-term versus long-term fear responses we call phasic versus sustained fear, respectively. Outputs from the basolateral amygdala (BLA) activate the medial division of the CeA (CeA_M) to very rapidly elicit phasic fear responses via CeA_M projections to the hypothalamus and brainstem. The BLA also projects to the $BNST_L$, which together with other $BNST_L$ inputs from the lateral CeA (CeA_L) initiate a slower developing, but sustained fear response, akin to anxiety. We hypothesize this occurs because the CeA_L releases the peptide corticotropin releasing hormone (CRF) into the $BNST_L$ which facilitates the release of glutamate from BLA terminals. This activates the $BNST_L$ which projects to hypothalamic and brainstem areas similar to those innervated by the CeA_M that mediate the specific signs of fear and anxiety. The generality of this idea is illustrated by selective studies looking at context conditioning, social defeat, drug withdrawal and stress induced reinstatement.

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Introduction

Based on early observations by Johnston (1923), the concept of the "extended amygdala" was developed and explored in great detail by Lennart Heimer, Jose de Olmos, George Alheid, and their many collaborators to the great benefit of behavioral researchers such as ourselves. Among their many significant findings, and of particular relevance to our research, they showed that the central (CeA) and medial (MeA) nuclei of the amygdala and the bed nucleus of the stria terminalis (BNST) were connected by columns of cells located throughout the stria terminalis, the fiber tract that connects these amygdala nuclei with the BNST, and also in a ventrally located sublenticular part of the basal forebrain (Alheid and Heimer, 1988; Alheid et al. 1998). They showed also that the CeA projected primarily to the lateral division of the BNST ($BNST_L$) and that the MeA projected primarily to the medial division of the BNST ($BNST_M$). This was followed by a series of observations that the CeA and $BNST_L$ shared many common attributes in terms of inputs, outputs, cell types, and neurochemical makeup, especially with respect to the high levels of several peptides found in both structures (Alheid et al. 1995). Based on the many similarities between these two components of the extended amygdala, and also on the known involvement of the CeA in conditioned fear (Kapp et al. 1979), our laboratory began also to evaluate the role of the $BNST_L$. This work was thoroughly reviewed in Walker et al. (2003) and will only be summarized briefly here.

CeA versus BNST involvement in fear responses: initial findings

Similar to earlier work in which freezing was used as a fear measure (Iwata et al. 1986; LeDoux et al. 1988), we found that CeA but not $BNST_L$ lesions also blocked fear-potentiated startle, in this case to a 3.2 s light that had previously been paired with footshock (Hitchcock and Davis 1986, 1991). We later found that startle also was increased after several minutes of exposure to bright light, even without prior pairings with shock (Walker and Davis 1997a). This "light-enhanced startle" effect could be reduced by anxiolytic compounds (de Jongh et al. 2002; Walker and Davis 2002; de Jongh et al. 2003) suggesting it represented an unconditioned anxiogenic effect of bright light, consistent with previous evidence that rats and mice will avoid brightly lit areas if possible and will show signs of anxiety if avoidance is not possible (DeFries et al. 1966; File and Hyde 1978; Crawley 1981). In contrast to fear-potentiated startle to short-duration conditioned fear stimuli (CSs), this light-enhanced startle effect was not blocked by local infusion of the AMPA receptor antagonist NBQX into the CeA but was blocked by local infusions into the $BNST_L$.

We also found that the $BNST_L$, but not the CeA, was involved in the startle increases produced by intraventricular infusions of the anxiogenic peptide corticotropin releasing factor (CRF). Thus, excitotoxic $BNST_L$ lesions, or local infusions of a CRF antagonist into the $BNST_L$, but not into the CeA, completely blocked CRF enhanced startle (Davis et al. 1997). In addition, CRF infusions directly into the $BNST_L$ (Lee and Davis 1997), but not into the CeA or any other part of the amygdala (Liang et al. 1992), also facilitated startle amplitude. The observed parallels between CRF- and light-enhanced startle suggested that CRF receptors in the $BNST_L$ might contribute to light-enhanced startle. Using systemic administration of a proprietary non-peptide CRF-1 receptor antagonist, we have now found that CRF-R1 blockade does indeed disrupt light-enhanced startle, but has no effect on fear-potentiated startle at the doses tested (Fig. 1, and also see de Jongh et al. 2003).

What is perhaps most valuable about these specific paradigms (i.e., fear-potentiated and light-enhanced startle) is that, procedurally, they are so very similar. Each uses increased startle as a measure of fear, and light as the stimulus that triggers that fear. Indeed, the visual stimulus used in most of our fear-potentiated startle experiments is physically identical to that used in the light-enhanced startle procedure, differing only in its duration and conditioning history. These similarities are useful in that they greatly constrain the range of possible interpretations that might account for the observed differences in terms of CeA versus $BNST_L$ involvement, and also to CRF receptor blockade.

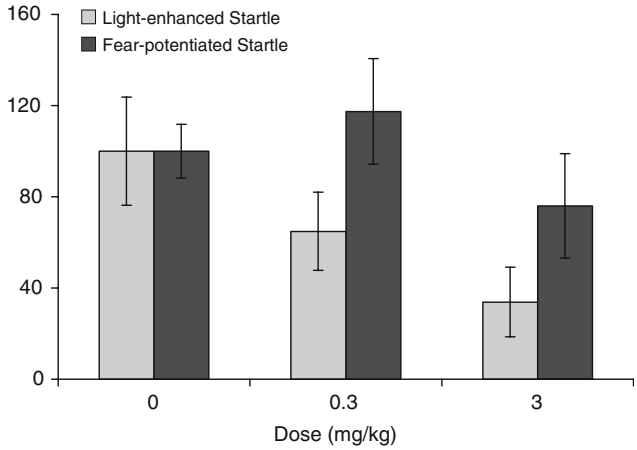

Fig. 1 A CRF1 receptor antagonist given systemically reduces light-enhanced startle, but has no effect on fear-potentiated startle at the doses tested

In our initial paper describing the differential involvement of the CeA versus $BNST_L$ in fear-potentiated and light-enhanced startle, we suggested that there were two possibilities that might account for the double dissociation between the involvement of these two structures in these two effects (Walker and Davis 1997a). One was that the CeA plays a special role in mediating *conditioned* fear responses, whereas the $BNST_L$ plays a special role in *unconditioned* responses. The other was that the CeA plays a special role in mediating *short-duration* fear responses, whereas the $BNST_L$ plays a special role in *longer-duration* responses. Based on recent data from our laboratory and elsewhere, we now believe that the second hypothesis is correct. We specifically suggest that the medial division of the CeA (CeA_M) and its projections to brainstem areas which mediate many of the behaviors that are influenced by fear, is critical for short-duration fear responses whereas the lateral division of the CeA (CeA_L) and it's CRF-containing projections to the $BNST_L$ are critical for sustained fear responses. We have also suggested that sustained fear in this model may be more akin to anxiety, as that term is commonly used, than is short-duration fear and as such may be particularly relevant clinically. These are the data we will concentrate on in this review.

Differential involvement of the CeA versus BNST in short- versus long-duration startle increases

The most direct test of these two alternatives (conditioned versus unconditioned or short- versus long-duration) would be to evaluate the effect of a $BNST_L$ manipulation on fear responses elicited by a stimulus that is both conditioned and also long in duration. If blockade of $BNST_L$ function disrupted the influence of such a stimulus, these results

would be consistent with the short- versus long-duration hypothesis and inconsistent with the conditioned versus unconditioned hypothesis. We have now developed procedures that combine these two variables and have, in fact, found that the $BNST_L$ is only involved in startle increases produced by long-duration (i.e., minutes as opposed to seconds) CSs.

In one such procedure, rats are placed into our standard startle box and presented with silence for the first 8 min followed by a continuous 8 min low-frequency-filtered noise. During this auditory stimulus, seven 0.5 s, 0.4 mA footshocks are presented using random interstimulus intervals. This procedure is done twice within a session for three total sessions spread across three consecutive days. In more recent versions we have presented seven clicker stimuli with durations ranging from 3 s to 8 min together with co-terminating footshock, also on each of three consecutive days. With both versions, the rats learn that when the CS comes on they are at risk for shock, but they do not know exactly when that shock might occur. Prior to training and also on the test day, startle amplitude is measured for 8 min before presentation of the CS and for 8 min during presentation of the CS in a context distinctively different from that used in training. For each animal, a percent change score from the pre-conditioning to the post-conditioning test is calculated. It should be emphasized that this is very different than our typical procedure in which the CS onset to shock interval is constant (typically 3.2 s) during training, and the CS onset to startle interval during testing is the same. Under those conditions, rats quickly learn to predict when the shock will occur as indicated by the fact that fear-potentiated startle is greatest when the CS-startle interval used in testing matches the CS-shock interval used in training (Davis et al. 1989).

Using this new procedure we typically see that there is relatively little change in startle amplitude from the pre- to the post-conditioning test during the pre-CS period (i.e., very little generalized context conditioning) but a large and consistent increase from the pre- to post-conditioning test when the CS is present. Using this design, we evaluated the effect of pre-test intra-$BNST_L$ and intra-CeA NBQX infusions. As indicated in Fig. 2, intra-$BNST_L$ infusions decreased the late (i.e., sustained) component of the fear-potentiated startle effect (min 5–8), but not the early component (min 1–4), whereas intra-CeA NBQX infusions had no effect using these parameters. These results are consistent with the hypothesis that the $BNST_L$ plays a selective role in long-duration fear responses. That is, once the auditory cue had been on for 4 min the $BNST_L$, but not the CeA, became critical for this sustained fear response. In fact, during the first 4 min, inactivation of the $BNST_L$ actually increased the magnitude of fear potentiated startle, consistent with recent data showing that the $BNST_L$ may

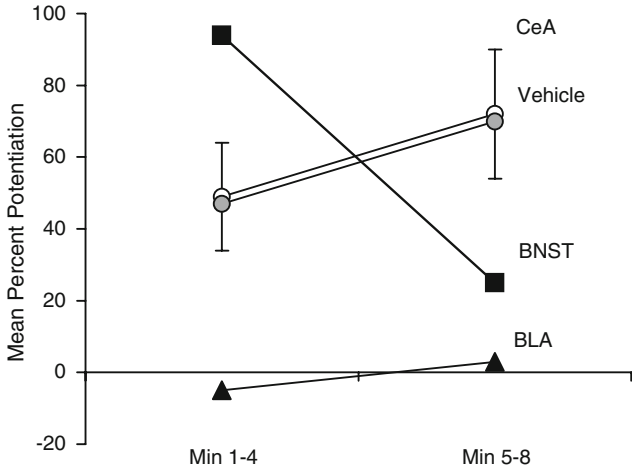

Fig. 2 Intra-$BNST_L$ infusions decreased the late (i.e., sustained) component of the fear-potentiated startle effect (min 5–8), but not the early component (min 1–n4), whereas intra-CeA NBQX infusions had no effect using these parameters. These results are consistent with the hypothesis that the $BNST_L$ plays a selective role in long-duration fear responses

tonically inhibit fear-potentiated startle (Meloni et al. 2006). In fact, in the rat, the BNST sends a very strong projection to the CeA_M (Fig. 3), which in one way or the other may tonically inhibit the expression of fear-potentiated startle.

On the other hand, it was somewhat surprising that inactivation of the CeA did not disrupt fear-potentiated startle during the first 4 min of the auditory CS, given previous findings that electrolytic (Hitchcock and Davis 1987) or chemical (Campeau and Davis 1995) CeA lesions completely block fear-potentiated startle to 3.2 s auditory CSs, and that intra-CeA NBQX infusions block fear-potentiated startle to 3.2 s visual CSs (Walker and Davis 1997b). Further experiments are underway, using a larger group of rats, to more precisely determine when short-duration fear turns into sustained fear (as defined by susceptibility to these types of manipulations), using either NBQX into the CeA or a CRF-1 receptor antagonist given systemically (see below). We predict that fear-potentiated startle at very early parts of the 8 min CS will be blocked by inactivation of the CeA and spared by systemic administration of a CRF antagonist.

It should be noted also that the two paradigms differ in that the shock is very predictable in the phasic fear paradigm, but much less so in the sustained fear paradigm. Thus, it is possible that this aspect of the procedure alone renders sustained fear CeA-independent. To a large degree, fear duration and US predictability are inexorably intertwined. One is almost invariably a confound for the other. That is, for a highly predictable US (e.g., one which always occurs 3.2 s after CS onset), fear can be very brief, lasting only for the moment in which shock is anticipated (and this

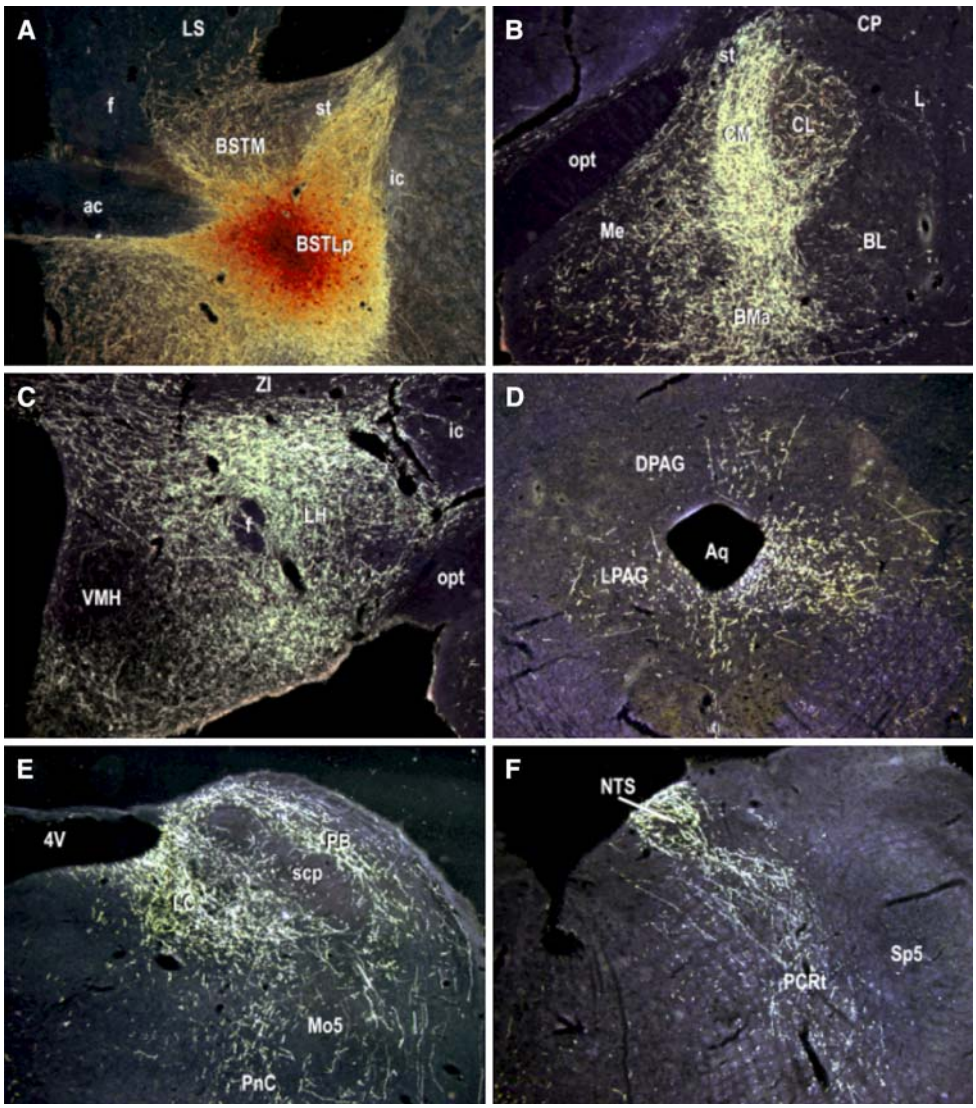

Fig. 3 Biotinylated dextran amine, an anterograde neuronal tracer, infused into the BNST (**a**) reveals projections to many of the same hypothalamic and brainstem areas involved in specific signs of fear that are also innervated by the CeA_M, (**c**–**f**), and also to the CeA_M itself (**b**), suggesting that the BNST may modulate CeA_M-mediated fear

appears to be exactly what well-trained rats do Davis et al. 1989). For a less predictable shock (e.g., one which may occur at any time during an 8 min CS), fear must be maintained for the duration of the CS. It is possible however to disentangle these variables. Very recently, we found that animals trained with the paradigm that supports good sustained fear (i.e., variable duration CSs up to 8 min with coterminating footshock) also show high levels of fear-potentiated startle to a 3.7 s CS (Walker, Miles and Davis, in preparation). Because the conditioning procedure is the same, so is US predictability (in this case, it is very unpredictable). Thus, differences in the effect of any pretest manipulation would have to be attributed to differences in response duration rather than predictability. Although we have not yet done this with BNST inactivation, we very recently found that a CRF1 receptor antagonist that completely blocks sustained fear, has no effect on short-duration fear, irrespective of the conditioning procedure (i.e., irrespective of predictability—Walker, Miles and Davis, in preparation). Results from Waddell et al. (2006a), in which BNST lesions disrupted conditioned suppression to a 10 but not 1-min min clicker CS—both with very predictable co-terminating footshocks (discussed in next section)—would also appear more consistent with the conclusion that response duration rather than predictability is the key variable.

Figure 2 also shows data from animals with cannula placements in the basolateral amygdala (BLA). These data show that BLA inactivation disrupts fear-potentiated startle throughout the 8 min CS, consistent with its blockade of fear-potentiated startle both to a 3.2 s tone (Campeau and Davis 1995) and sustained fear (Fig. 2) in the light-enhanced startle paradigm (Walker and Davis 1997b). We believe that the BLA's involvement in short-duration fear is mediated by projections to the CeA_M, which in turn projects to the startle pathway, and that

it's involvement in sustained fear is mediated by projections to the $BNST_L$, which also projects to the startle pathway.

In summary, these data indicate that the $BNST_L$ does indeed play an important role in the expression of conditioned fear responses, provided that those responses are maintained for a relatively long time. Based on the finding that an unconditioned visual stimulus begins to increase startle approximately 60 s after light onset (Davis et al. 1989) and the fact that inactivation of the BNST does not disrupt fear-potentiated startle to a 3.2 s visual CS (Walker and Davis 1997a), we suspect the transition from CeA_M to $BNST_L$ involvement may begin between 4 and 60 s after CS onset, although it may be several minutes before the response is fully dependent on the $BNST_L$.

Differential involvement of the CeA versus BNST in short- versus long-duration fear responses: corroborating evidence from other laboratories and paradigms

Another stimulus that is both conditioned and long in duration is context. Sullivan et al. (2004) have now reported that post-training electrolytic $BNST_L$ lesions disrupt freezing as well as corticosterone responses to a context CS but do not affect these same responses to a brief auditory CS, whereas CeA lesions disrupt freezing to both. On the basis of those findings, they suggested that the $BNST_L$ plays a special role in context-elicited fear. However, in light of our most recent findings, we wonder if a more parsimonious interpretation may be that $BNST_L$ lesions disrupt context-elicited freezing simply because context CSs are invariably long in duration, and so too are the responses they evoke. Recent work by Waddell et al. (2006b) are especially relevant in that regard. They found that pre-training $BNST_L$ lesions blocked conditioned suppression to a 10 min but not to a 1 min clicker CS. Interestingly, they also found that $BNST_L$ lesions disrupted the context-dependent reinstatement of conditioned suppression (i.e., in previously extinguished animals) by footshock. Overall, a differential involvement of the BNST in long- versus short-duration fear responses has now been demonstrated across three different response measures—startle, freezing, and conditioned suppression. As such, the pattern does not appear to be idiosyncratic to a particular response measure.

What is the role of CRF receptors?

The same hypothesis that accounts for the differential involvement of the CeA and $BNST_L$ in fear-potentiated

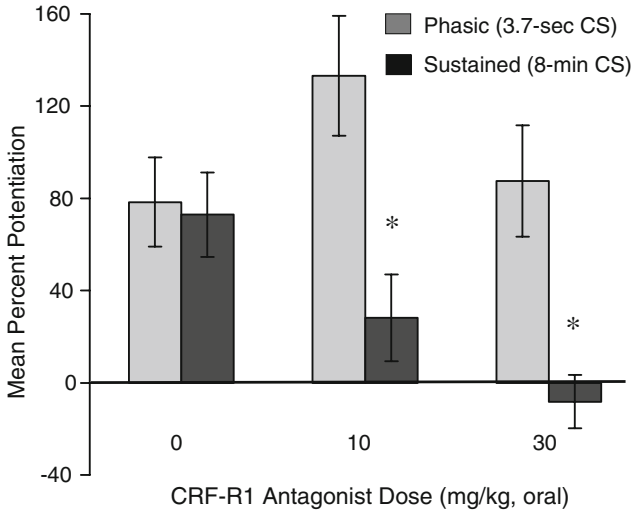

Fig. 4 Systemic administration of a CRF1 receptor antagonist dose-dependently blocked fear-potentiated startle to the long-duration auditory CS but not a 3.7 s auditory CS

versus light-enhanced startle might also account for the differential involvement of CRF receptors (as shown previously in Fig. 1). That is, CRF receptors might contribute to long- but not short-duration fear responses, irrespective of whether the fear or anxiety response is conditioned or unconditioned. In fact, prior studies have shown that the non-specific CRF antagonist, α-helical $CRF_{9–41}$, does indeed disrupt sustained freezing responses to context CSs (Kalin and Takahashi 1990; Deak et al. 1999). We recently evaluated the same CRF-1 receptor antagonist used in Fig. 1 on fear-potentiated startle to the 8 min auditory CS. As shown in Fig. 4 (black bars), systemic administration of the CRF-1 receptor antagonist dose-dependently blocked fear-potentiated startle to the long-duration CS. In contrast to the effect of intra-$BNST_L$ AMPA receptor blockade, however, the effect was apparent even during the first few minutes (the data for the entire 8 min CS period are combined in Fig. 3).

As previously noted, the same doses did not block fear-potentiated startle to a 3.7 s visual CS. To ensure that that dissociation was not indicative of a less interesting modality-specific effect, in a different group of rats we assessed the CRF-1 receptor antagonist's ability to block fear-potentiated startle to a 3.7 s *auditory* CS. This too was unaffected (Fig. 4 gray bars). In fact, fear-potentiated startle in the intermediate dose group was actually greater, though not significantly so, than fear-potentiated startle in the vehicle group. Given the involvement of CRF in BNST functions, and evidence presented earlier that BNST inactivation actually *facilitates* fear-potentiated startle to *short-duration* threat cues (e.g., Meloni et al. 2006, and see also Fig. 2), one wonders if this nominal increase reflects random between-group variability or something more

meaningful. In any case, the results clearly suggest that CRF-1 receptors contribute to long- (minutes) but not short- (seconds) duration fear responses in these paradigms, even though the time course does not exactly mirror that of AMPA receptor blockade in the $BNST_L$. However, as mentioned above, we expect that if we used a large number of rats and sampled startle every 10 s or so during the 8 min CS, the CRF-1 receptor antagonist would not block fear-potentiated startle during the earliest part of the 8 min CS but only after the CS had been on for some time. Alternatively, it is possible that fear-potentiated startle during the first few minutes of the 8 min CS is dependent on CRF but not AMPA receptors in the $BNST_L$ or that the systemically administered CRF receptor antagonist acts elsewhere to disrupt fear-potentiated startle during the first few minutes of CS exposure. A possible site of action for the early effect is the BLA, where CRF receptors are found in even greater abundance than in the $BNST_L$ (DeSouza et al. 1985; Chen et al. 2000). Perhaps then, CRF receptors in the BLA contribute to an intermediate phase of the fear response (e.g., during the first several minutes of the long-duration CS), whereas those in the $BNST_L$ contribute to the more delayed component of this response. These ideas are currently being tested.

Circumstantial evidence for independent roles of the CeA_M versus CeA_L

The CeA can be divided into several subnuclei which include, most notably, the medial and lateral (CeA_M and CeA_L) subdivisions, respectively. Although both areas project to the $BNST_L$ (Sun et al. 1991; Petrovich and Swanson 1997; Bourgeais et al. 2001; Dong et al. 2001), they are otherwise very different. First, the CeA_M has many projections to brainstem nuclei and other areas that mediate behaviors influenced by fear (c.f., Davis 2000; Davis and Whalen 2001), including areas that mediate or modulate the acoustic startle response (Shammah-Lagnado et al. 1987; Rosen et al. 1991; Fendt et al. 1994; Meloni and Davis 1999; Shi et al. 2002). In contrast, CeA_L projections to these areas are much more limited (Schwaber et al. 1982; Veening et al. 1984; Gray and Magnusson 1987, 1992). The CeA_L projects instead to the substantia innominata, to the CeA_M, and quite prominently to the $BNST_L$ (Sun et al. 1991; Petrovich and Swanson 1997; Bourgeais et al. 2001; Dong et al. 2001).

The CeA_M and CeA_L are also very different in terms of their neurotransmitter content. Whereas CeA_L neurons stain for a variety of neuropeptide transmitters, these same peptides are largely absent from CeA_M neurons (Wray and Hoffman 1983; Veening et al. 1984; Moga and Gray 1985; Cassell et al. 1986; Gray and Magnusson 1987; Shimada et al. 1989; Otake et al. 1995; Day et al. 1999). One peptide found in great abundance in the CeA_L is CRF. In fact, CeA_L neurons are a major source of $BNST_L$ CRF. This was demonstrated by Sakanaka et al. (1986) who found that electrolytic CeA but not BLA lesions dramatically reduced $BNST_L$ CRF-immunoreactivity—nearly depleting it entirely from dorsal $BNST_L$. Many neurons within the $BNST_L$ are themselves CRF-positive (Cummings et al. 1983; Cintra et al. 1987; Gray and Magnusson 1987; Shimada et al. 1989; Phelix and Paul 1990; Gray and Magnuson 1992; Makino et al. 1994a, b; Watts and Sanchez-Watts 1995; Day et al. 1999; Veinante et al. 2003), and CRF-positive neurons in both areas invariably express GABA (Veinante et al. 1997; Day et al. 1999). As high-frequency stimulation is known to favor peptide release (e.g., Lundberg et al. 1986; Bartfai et al. 1988; Whim 1989; Bourque 1991; Ip 1994), the findings suggest that the influence of these neurons (i.e., either inhibitory or excitatory) on downstream structures may vary as a function of the pattern of afferent activity. More specifically, sustained high-frequency activation may favor the release of CRF.

It is also of some interest that CRF-positive neurons in the CeA_L and dorsal $BNST_L$ express glucocorticoid receptors (Cintra et al. 1987; Honkaniemi et al. 1992; Lechner and Valentino 1999). The regulation of these neurons by stress hormones would be compatible with the hypothesis that the CeA_L and $BNST_L$ participate in long-duration responses to sustained threats that do not need to be as temporally precise as those to more immediate or predictable threats (e.g., fixed CS-US interval).

If CeA_L neurons are involved in $BNST_L$-dependent effects, then it is necessary to account for the failure of intra-CeA NBQX infusions to disrupt light-enhanced startle in Walker and Davis (1997b) or the late stage of fear-potentiated startle to the 8 min CS in Fig. 2. One possibility is that the CeA_M neurons thought to mediate short-duration fear responses via direct projections to the brainstem are AMPA-responsive, whereas the CeA_L neurons that may mediate longer duration fear responses indirectly by way of projections to the $BNST_L$ are not. Perhaps those neurons are driven instead by activation of other receptor types such as glucocorticoid and/or calcitonin gene related peptide (CGRP) receptors. Indeed, previous studies have shown that chronic corticosterone administration upregulates CRF mRNA in CeA_L and $BNST_L$ neurons (Swanson and Simmons 1989; Makino et al. 1994a,b; Watts and Sanchez-Watts 1995; Shepard et al. 2000; Liu et al. 2004), and interacts synergistically with CRF to increase startle amplitude (Lee et al. 1994). Calcitonin gene related peptide (CGRP) is also an interesting candidate, especially given its preferential distribution (Haring et al. 1991; Honkaniemi

et al. 1992; Harrigan et al. 1994; Dobolyi et al. 2005) and that of its receptors (Kruger et al. 1988) within the lateral versus medial CeA. These receptors, when activated, produce various symptoms associated with fear and anxiety such as heart rate and blood pressure increases (Nguyen et al. 1986; Brown and Gray 1988), antinociception (Xu et al. 2003), and freezing (Kocorowski and Helmstetter 2001). CGRP-positive terminals directly innervate stress-responsive (Honkaniemi et al. 1992) CRF-containing neurons within the CeA_L (Harrigan et al. 1994). Perhaps then, glutamate selectively activates CeA_M neurons that mediate short-duration fear responses whereas CGRP, corticosterone and/or other peptide receptors selectively influence CeA_L neurons which mediate more sustained fear responses. Having shown that intra-CeA NBQX infusions selectively influence the former, it will now be interesting to evaluate the effect of some of these other ligands on short- and long-duration startle increases.

Another difference is that the CeA_M receives input from almost all other nuclei within the amygdala whereas the rat CeA_L receives virtually no amygdala input at all (Jolkkonen and Pitkanen 1998). Instead, prominent inputs to the CeA_L include those from insular and entorhinal cortices (Yasui et al. 1991; Sun et al. 1994; McDonald et al. 1997), and also from the paraventricular nucleus of the thalamus (PVT—Berendse and Groenewegen 1991; Turner and Herkenham 1991; Moga et al. 1995; Li and Kirouac 2008; Vertes and Hoover 2008)—areas that project very lightly to CeA_M.

Projections from the PVT are especially interesting insofar as the PVT is one of the most stress-responsive areas in the brain, based on the induction of c-fos with a variety of stressors (Chastrette et al. 1991; Beck and Fibiger 1995; Cullinan et al. 1995; Duncan et al. 1996; Bhatnagar and Dallman 1998; Bhatnagar and Dallman 1999; Bubser and Deutch 1999). In fact, Bhatnagar and Dalman (1998) have suggested that the PVT to amygdala to paraventricular nucleus of the hypothalamus projection is a key regulator of the hypothalamic-pituitary-adrenal response to stress. Moreover, the PVT innervates regions in the extended amygdala, including the CeA_L that contain corticotropin-releasing factor (CRF) neurons, many of which receive apparent contacts from PVT fibers (Li and Kirouac 2008). Interestingly, the PVT also appears to be involved in circadian rhythms and the BNST shows periodicity in clock gene expression that is highly similar to, and dependent upon, the suprachiasmatic nucleus (Amir et al. 2004).

Cortical inputs to CeA_L are also interesting in that they raise the intriguing possibility that these inputs might mediate, at least in more cognitive species, the autonomic accompaniments of something more aptly described as 'worry'. In fact, the insular cortex has now been found in several fMRI studies to become active when human subjects are told to anticipate shock (Phelps et al. 2001), or learn to expect other aversive stimuli in the course of conditioning procedures (Buchel et al. 1998; Ploghaus et al. 1999), as has the BNST (and see also Kalin 2005 for threat-induced BNST activation in nonhuman primates; Straube et al. 2007). The insular cortex also projects heavily to $BNST_L$ as well as to the posterior part of the BLA (BLA_P) (e.g., Yasui et al. 1991; McDonald et al. 1999), which itself projects to the $BNST_L$.

Functional evidence for an involvement of a CRF-containing $CeA_L \rightarrow BNST_L$ projection in long-lasting anxiety and stress effects

Jasnow et al. (2004) found that social defeat behavior in Syrian Hamsters was reduced by pre-defeat *unilateral* electrolytic CeA lesions and also by pre-test *unilateral* intra-$BNST_L$ D-Phe $CRF_{12–41}$ infusions. The combined manipulation, on opposite sides of the brain, produced an even greater effect. On the basis of these results, they concluded that "stress activates CRF-containing neurons in the CeA, which then releases CRF within the BNST." Similarly, Erb et al. (2001) showed that neither *unilateral* intra-CeA TTX infusions nor *unilateral* intra-$BNST_L$ infusions of the CRF receptor antagonist D-Phe $CRF_{12–41}$ disrupted the shock-induced reinstatement of extinguished cocaine-seeking behavior, but that the combination of both treatments, again on opposite sides of the brain, did, leading them to conclude that a "CRF-containing pathway from CeA to BNST is involved in mediating the effects of CRF…on the reinstatement of cocaine seeking."

Although the results of both studies are consistent with the conclusions that were drawn, they are not definitive insofar as only a partial implementation of the crossed-lesion design was used. That is, neither study compared the effect of contralateral versus ipsilateral CRF antagonist infusions (e.g., left CeA lesion + CRF antagonist into right BNST versus CeA lesion + CRF antagonist into *left* BNST). Assuming that a serial CeA-to-BNST circuit is critical for the behavior in question, one would predict that the effect of ipsilateral CeA + BNST treatments would be less than that of contralateral CeA + BNST treatments, and equal in magnitude to that obtained by unilaterally manipulating either structure alone. Also, intra-CeA TTX infusions in the Erb et al. (2001) study and electrolytic CeA lesions in the Jasnow et al. (2004) study would have interrupted communication between the BLA and $BNST_L$ because fibers from BLA project through the CeA on their way to the $BNST_L$ (Davis and Whalen 2001; Dong et al. 2001). Thus, the observed behavioral effects in these

studies might have been attributable to an interruption of this pathway instead. Thus, it will be important to determine if fiber-sparing inactivation of the CeA will reproduce these intriguing findings.

Role of the CeA and BNST in drug withdrawal

There is now considerable evidence that some of the anxiogenic effects of drug withdrawal involve activation of the both the CeA and BNST, and may involve CRF-containing projections from the CeA_L to the BNST. This seems to be the case for the affective components of anxiety (e.g., avoidance of the place where drug withdrawal occurred) but not the somatic symptoms (e.g., classic signs of drug withdrawal such as wet dog shakes, teeth chattering, ptosis, postural abnormalities).

In the CeA_L, naloxone-precipitated morphine withdrawal induced CRE-mediated transcription in approximately half of the neurons that are immuno-positive for CRF (Shaw-Lutchman et al. 2002), and significantly increases the number of c-Fos-positive neurons in the CeA and BNST (Gracy et al. 2001; Frenois et al. 2002; Jin et al. 2004; Frenois et al. 2005). Although c-Fos was not found in CRF-positive immunoreactive neurons in the amygdala (Hamlin et al. 2004), the number of CRF-immunoreactive neurons was significantly lower in withdrawn versus non-withdrawn rats, leading Veinante et al. (2003) to suggest that opiate withdrawal might actually have led to a massive activation and subsequent depletion of CRF from those cells. In fact, Olive et al. (2002) have shown that ethanol withdrawal does produce a large ($\sim$100%) increase in extracellular CRF in the BNST. As indicated above, naloxone-precipitated morphine withdrawal increases c-fos in the CeA as well as the BNST. Consistent with a serial circuit for withdrawal-associated effects, c-fos activation in the BNST is disrupted by excitotoxic CeA lesions, whereas c-fos activation in the CeA is not disrupted by excitotoxic BNST lesions. Moreover, lesions in either area disrupted the affective component of withdrawal, namely conditioned place aversions to the location in which withdrawal occurred (Nakagawa et al. 2005).

Although intraventricular or intra-CeA α-helical $CRF_{9–41}$ infusions, as well as systemic injections of the CRF-R1 antagonist CP-154,526 (Iredale et al. 2000; Lu et al. 2000; McNally and Akil 2002) block many of the somatic signs of morphine withdrawal (e.g., wet dog shakes, teeth chattering, ptosis, postural abnormalities), α-helical $CRF_{9–41}$ infusions directly into the BNST do not (McNally and Akil 2002). Also, the CRF antagonist D-Phe-$CRF_{12–41}$ infused into the CeA but not the BNST, has been found to reduce ethanol consumption in alcohol-dependent animals (Funk et al. 2006). Thus, CRF in the BNST does not seem to be involved in the somatic components of opiate withdrawal.

On the other hand, there is considerable evidence to show that CRF (Heinrichs et al. 1995; Contarino and Papaleo 2005; Stinus et al. 2005) and the BNST, and more specifically norepinephrine acting on β-adrenergic receptors in the BNST, are involved in the *affective* state associated with drug withdrawal. The BNST has one of the highest concentrations of norepinephrine in the brain, and opiate withdrawal activates BNST-projecting cells in the A1 and A2 noradrenergic cell groups of the caudal medulla. Lesions of the ventral noradrenergic bundle, which interrupt the projection of these cell groups to the BNST, reduce opiate-withdrawal-induced place aversion, as do injections of β-adrenergic-receptor antagonists into the BNST itself, even though these same manipulations have little effect on the *somatic* symptoms of withdrawal (Aston-Jones et al. 1999; Delfs et al. 2000; and see also Cecchi et al. 2007).

Interestingly, noradrenergic bundle lesions did not reduce conditioned place aversions when shock was used as a US (Delfs et al. 2000), suggesting the effect was specific either to drug withdrawal or to addicted animals. On the other hand, lesions of the BNST did block conditioned place aversions to somatic pain produced by intraperitoneal acetic acid injections or formalin injections in the paw, but had no effect on acetic acid-induced nociceptive writhing and actually increased formalin-induced nociceptive behaviors (Deyama et al. 2007). Taken together, these data suggest the BNST is primarily involved in the aversive affective symptoms of drug withdrawal. In fact, the reduction of the affective aspects of withdrawal using manipulations of the BNST, in the face of little or no effect on the somatic symptoms of withdrawal, may make the decrease in affective symptoms even more impressive.

BLA→$BNST_L$ projections

The preceding section focused on the possible involvement of CeA_L to $BNST_L$ projections in sustained anxiety and aversive states. It is important to note also that the $BNST_L$ receives substantial inputs from the BLA—particularly from caudal part of the BLA (BLA_p) —(Weller and Smith 1982; McDonald 1991; Dong et al. 2001). This may explain why NBQX infusions into the caudal rather than rostral BLA (Fig. 5) blocked light-enhanced startle in Walker and Davis (1997b). We have also found more recently that infusions of an NBQX/muscimol cocktail into the BLA completely block CRF-enhanced startle (Fig. 6). Although excitotoxic BLA lesions only attenuated CRF-enhanced startle in Lee and Davis (1997), the more caudal elements of the BLA were spared in that study. Moreover, electrolytic lesions of the CeA, through which BLA fibers

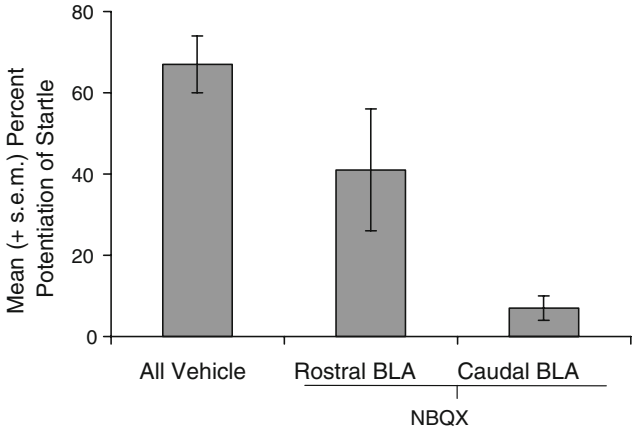

Fig. 5 NBQX infusions into the caudal rather than rostral Bla blocked light-enhanced startle in Walker and Davis (1997b)

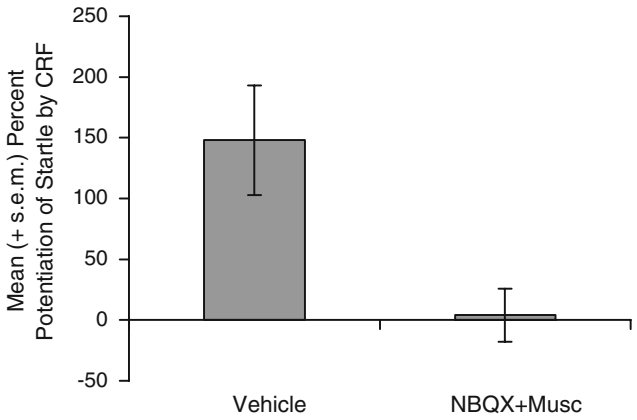

Fig. 6 NBQX/muscimol cocktail into the Bla completely blocks CRF-enhanced startle

pass on their way to the $BNST_L$ (Davis and Whalen 2001; Dong et al. 2001) also block CRF-enhanced startle (Liang et al. 1992). Together, these results suggest that BLA_P inputs to the $BNST_L$ may be involved in CRF- as well as light-enhanced startle, and perhaps in sustained fear responses more generally.

One model that could account for these results posits that the activation of CRF receptors in the $BNST_L$ potentiates either the release of glutamate from BLA terminals (i.e., pre-synaptic receptors), or the response of $BNST_L$ neurons (i.e., post-synaptic receptors) to glutamate released these same BLA_P or other neurons (Fig. 7). Findings consistent with the first possibility have been presented in abstract form by Forray et al. (2005). Using in vivo microdialysis, they found that intra-$BNST_L$ infusions of high-K^+ evoke a substantial rise in extracellular $BNST_L$ glutamate in rats that had undergone a chronic stress procedure, and that this response was completely prevented by co-infusion of the CRF-1 receptor antagonist NBI 27914. Taken as a whole, these findings are consistent with a

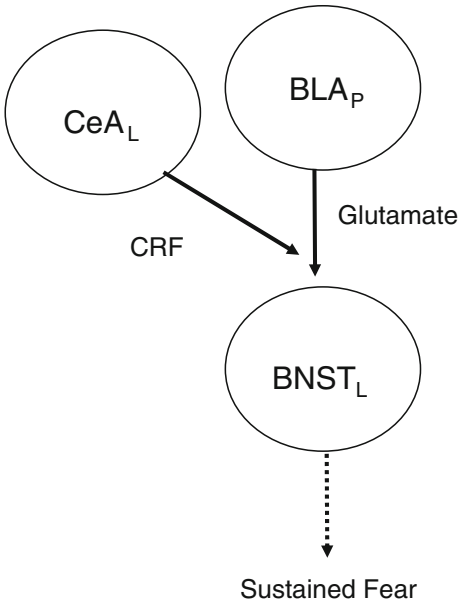

Fig. 7 Schematic model of how we believe the extended amygdala produces sustained fear. CeA_L neurons release CRF which binds to receptors located on the terminals of glutamatergic BLA_P neurons. Activation of these CRF receptors increases glutamate release and, therefore, excitatory drive onto $BNST_L$ neurons. These $BNST_L$ neurons project to other areas, mostly in the brainstem, that mediate behaviors influenced by fear

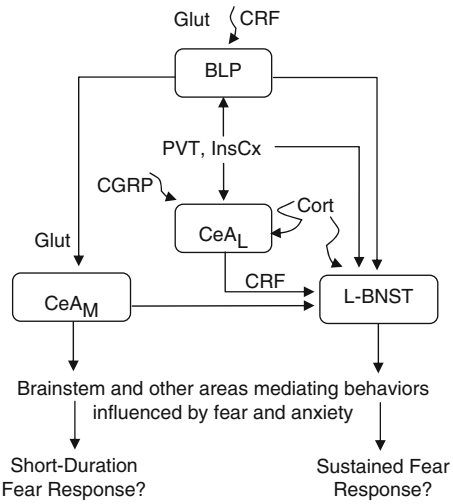

Fig. 8 Schematic diagram of the role of different parts of the extended amygdala in phasic versus sustained fear

neural model in which (a) CeA_L neurons release CRF into the $BNST_L$, (b) CRF binds to presynaptic receptors on BLA_P terminals (c) CRF receptor activation increases the release of glutamate from these terminals, and (d) glutamatergic input to $BNST_L$ neurons drives sustained threat responses (Fig. 8). We are currently testing this model using lesions that are selective for the CeA_L versus the CeA_M, microdialysis, and local drug infusions. More generally, we believe that the CeA_L and the $BNST_L$, compared

to the CeA_M play a critical role in sustained responses to long-duration threats akin to anxiety. In some cases these effects are mediated by CRF and norepinephrine in either the CeA_L, the $BNST_L$ or both.

Although we have limited this brief review to findings that we believe to be most directly relevant to the neural substrates of short- versus long-duration fear responses, many other studies have found evidence for a differential involvement of various components of the extended amygdala in a variety of other effects. Most notably these involve different patterns of morphological changes in the extended amygdala in different types of stress which have different effects on anxiety (Vyas et al. 2002, 2003), the critical role of the BNST as a relay between the hippocampus's inhibitory modulation of the paraventricular nucleus of the hypothalamus (Cullinan et al. 1993) and the importance that norepinephrine plays in this circuitry (Forray and Gysling 2004), the role of the BNST in stress-induced anorexia (Ciccocioppo et al. 2003; Ciccocioppo et al. 2004) and the "learned helplessness" model of depression (Maier et al. 1993; Hammack et al. 2004), differential modulation in the amygdala versus BNST of stress-induced anxiety by norepinephrine and galanin (Morilak et al. 2003), differential effects of repeated urocortin infusions into the amygdala versus BNST on the development of anxiety and vulnerability to lactate-induced 'panic' (Sajdyk et al. 1999; Sajdyk and Gehlert 2000; Rainnie et al. 2004; Lee et al. 2008) and the role of the BNST versus the amygdala in predator odor induced anxiety (Fendt et al. 2003). As a whole, these studies point to the widespread influence of the concept of an "extended amygdala" and, therefore, to the significance of Lennart Heimers' work—a body of research which has already proven influential in many different areas of behavioral neuroscience, including our own, and which we are certain will have an enduring impact on the field as a whole. We believe this would please Dr. Heimer very much.

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