Published in final edited form as:

Neurobiol Learn Mem. 2011 January ; 95(1): 86–91. doi:10.1016/j.nlm.2010.11.006.

The Central Amygdala Nucleus via Corticotropin-Releasing Factor is Necessary for Time-limited Consolidation Processing but not Storage of Contextual Fear Memory

Matthew W. Pitts¹ and Lorey K. Takahashi^{1,2}

¹Department of Cell & Molecular Biology, John A. Burns School of Medicine, University of Hawaii, 96813

²Department of Psychology, University of Hawaii, Honolulu, Hawaii, 96822

Abstract

The central nucleus of the amygdala (CeA) is traditionally portrayed in fear conditioning as the key neural output that relays conditioned information established in the basolateral amygdala complex to extra-amygdalar brain structures that generate emotional responses. However, several recent studies have questioned this serial processing view of the amygdalar fear conditioning circuit by showing an influence of the CeA on memory consolidation. We previously reported that inhibition of endogenous CeA secretion of corticotropin-releasing factor (CRF) at the time of contextual training effectively impaired fear memory consolidation. However, the time-dependent range of CeA CRF secretion in facilitating consolidation processing has not been examined. Therefore, to address this issue, we performed CeA site-specific microinjections of CRF antisense oligonucleotides (CRF ASO) at several post-training time intervals. Rats microinjected with CRF ASO at post-training intervals up to 24-h subsequently exhibited significant impairments in contextual freezing retention in contrast to animals treated 96-h after training. To further establish the validity of the results, CeA fiber-sparing lesions were made at two distinct post-training periods (24-h, 96-h), corresponding respectively to the temporal intervals when CeA CRF ASO administration disrupted or had no significant effects on memory consolidation. Similar to the CeA CRF ASO results, CeA lesions made 24-h, but not 96-h, after training induced significant freezing deficits in the retention test. In conclusion, the current results demonstrate: 1) an extended involvement of CeA CRF in contextual memory consolidation and 2) that contextual fear memory storage is not dependent on a functional CeA.

Keywords

Central amygdala; corticotropin-releasing factor; contextual fear conditioning; emotional memory consolidation; contextual memory storage; CRF antisense oligonucleotides

© 2010 Elsevier Inc. All rights reserved

Corresponding author: Dr. Matthew W. Pitts, Department of Cell & Molecular Biology, John A. Burns School of Medicine, University of Hawaii, 651 Ilalo St, Honolulu, HI, 96813, USA (mwpitts@hawaii.edu; Tel: 808-692-1516; FAX: 808-692-1968).

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1. Introduction

Memory consolidation is a process in which short-term memories are converted into long-term memories. This process has been demonstrated to be dependent upon the activation of intracellular signaling cascades and the synthesis of new proteins (Dudai, 2004; McGaugh, 2004). Much of our current understanding of memory consolidation is derived from studies using aversive conditioning procedures, such as inhibitory avoidance and pavlovian fear conditioning. In pavlovian fear conditioning, a neutral conditioned stimulus (CS) is paired with an aversive unconditioned stimulus to establish a CS-US association. Subsequent re-exposure to the CS elicits a conditioned response, which indicates a learned association.

The amygdala plays an essential role in emotional learning and memory. The basolateral amygdala complex (BLA; including the lateral, basal, and accessory basal nuclei) is a critical region involved in emotional learning (LeDoux, 2000) and a variety of neuromodulators have been shown to influence consolidation at this site, including norepinephrine, glucocorticoids, and corticotropin-releasing factor (CRF) (Paré, 2003; McGaugh, 2004; Roozendaal et al., 2008). Specifically, BLA administration of CRF receptor antagonists following both contextual fear conditioning (Hubbard et al., 2007) and inhibitory avoidance training (Roozendaal et al., 2002) has been demonstrated to disrupt memory consolidation. Yet, both of the aforementioned studies reported no impairment when CRF receptor antagonists were injected into the adjacent central nucleus (CeA), which contains the majority of CRF immunoreactive cell bodies within the amygdala (Veening et al., 1984). Furthermore, we recently reported that pre-training microinjections of CRF antisense oligonucleotides (CRF ASO) into the CeA did not disrupt freezing during training, but impaired the retention of contextual freezing when assessed 48-h later (Pitts et al., 2009). In sum, these findings suggest that CeA CRF promotes fear memory consolidation by activating BLA CRF₁ receptors.

Of additional importance, the CeA is widely thought to act as the final output of the amygdala and to be essential for fear expression (Wilensky et al., 2006; Zimmerman et al., 2007; Rodrigues et al., 2009; Jimenez & Maren, 2009). The CeA receives unidirectional projections from the adjacent BLA (Pitkänen et al., 1997) and sends projections to several downstream brainstem sites that mediate conditioned fear responses (McDonald, 1998; LeDoux, 2000). While the BLA to CeA serial transmission model has been accepted by many, some studies have produced results which contradict this model (Koo et al., 2004; Pitts et al., 2009). In particular, we previously found no impairment in contextual fear retention when the CeA was inactivated immediately prior to retention testing (Pitts et al., 2009).

Thus, in our first study, we investigated the temporal role of CeA CRF in contextual fear memory consolidation by inhibiting CeA CRF with CRF ASO at several post-training intervals. Next, we evaluated the general function of the CeA in fear memory consolidation and retention by inducing fiber-sparing CeA lesions at two distinct post-training intervals (24-h and 96-h) identified in the first experiment to represent ongoing consolidation and post-consolidation periods under our training procedures. Collectively, our results demonstrate: 1) CeA CRF secretion facilitates memory consolidation for at least 24-h after exposure to contextual fear conditioning; and 2) by 96-h after conditioning, when the memory is sufficiently stabilized, CeA neurons are not required for the retrieval or expression of contextual fear memory.

2. Materials and Methods

Animals

Adult male Long–Evans rats weighing 275 – 325 g at the time of surgery were used. Rats were bred at the University of Hawaii animal facility from stock obtained from Charles River Laboratories (Raleigh, NC). Animals were singly housed in polycarbonate cages and maintained on a 12-h light–dark cycle with lights on at 06:00 A.M. Each cage was provisioned with food, water, and a layer of Sani-chips (Murphy Forest Products). All procedures were approved by the University of Hawaii Institutional Animal Care and Use Committee and in compliance with the National Institute of Health Guide for the Care and Use of Animals. Every effort was made to minimize animal use and discomfort.

Stereotaxic Surgery

Rats were anesthetized with an intraperitoneal injection of ketamine hydrochloride (100 mg/kg) and xylazine (20 mg/kg) prior to being mounted on a stereotaxic frame. Stainless steel 26-gauge guide cannulae (Plastics One), secured to the skull with stainless steel screws and dental cement, were implanted bilaterally into the CeA using the following flat-skull coordinates: CeA: –2.0 mm anteroposterior (AP) from bregma, ML $\pm$ 4.2 mm mediolateral (ML), –7.0 mm dorsoventral (DV) from skull. Dummy stylets were inserted into guide cannulae and rats were allowed a 10–12 day post-recovery period. During the recovery period, rats were handled 3 to 4 days prior to testing to adapt to the microinjection procedure.

Microinjection procedure

After removal of dummy stylets, 33-gauge stainless steel cannula injectors extending 1 mm beyond the guide cannula tip were inserted into the brain. Polyethylene tubing connected each cannula injector to a 10- μ l syringe that was driven simultaneously at a rate of 0.1 μ l/min by an infusion pump. Injectors remained in brain for an additional 3-min to confine the drug to the target site.

Drug Preparation

The CRF specific antisense oligonucleotide (CRF ASO), which we previously used (Pitts et al., 2009), was commercially obtained (Biognostik) and dissolved (2.5 nmol/1.0 μ l) in PBS. Oligonucleotides were PAGE-purified phosphorothioate end-capped sequences of 17 bases: CRF ASO: 5'- CAA GCG CAA CAT TTC AT -3' (complementary to bases 736 – 752 of the rat CRF mRNA transcript). Ibotenic acid (Tocris) was dissolved (10 μ g/ μ l) in 0.1 M phosphate buffer saline (PBS, pH 7.4).

Fear-conditioning footshock apparatus

The shock box (25.3 cm $\times$ 20.3 cm $\times$ 22.6 cm) was constructed of three white Plexiglas sides and top and a clear front wall for video recording. Scrambled electric footshock (San Diego Instruments) was delivered via the stainless grid floor. The room was illuminated using fluorescent overhead lighting. During testing, a video camera attached to a VCR recorded freezing, defined as the cessation of all body movements except those required for respiration

Contextual fear training procedure

Animals were placed in the shock box and after a 2 min pre-shock interval, five electric footshocks (1.0 mA, 1 s duration) were delivered at 2 min intervals. Contextual freezing during each 2 min postshock interval was videotaped and subsequently measured. At the

conclusion of each test, rats were returned to their homecage and the shock box was cleaned with 5% ethanol.

Contextual fear retention test

Forty-eight hours after contextual fear training, rats were returned to the shock box for a 10-min contextual fear retention test, in the absence of footshock. Contextual freezing was videotaped and the total duration in seconds of freezing was scored. At the conclusion of each test, the shock box was cleaned with 5% ethanol.

Histology and immunohistochemistry

At the completion of behavioral testing, rats were overdosed with sodium pentobarbital and transcardially perfused with ice-cold phosphate buffered saline (PBS) (pH 7.4), followed by 0.1 M phosphate buffer, pH 7.4, containing 4% paraformaldehyde (pH 7.4). Brains were post-fixed for 48-h in the same fixative (4% PFA) and then immersed daily in 0, 10, 20, and 30% sucrose in PBS. Brains were cut into 40 μ m coronal sections on a cryostat and either mounted onto precleaned Superfrost Plus slides and stained with thionin or stored as free floating sections in a cryoprotective solution (0.05 M PBS, 25% glycerol, 25% polyethylene glycol) at 4 °C. Free floating sections were stained for either myelin (fibers of passage) or NeuN (neuronal nuclei).

For myelin staining, brain sections were transferred to PBS-containing wells and rinsed for 20 min. FluoroMyelin Green (Invitrogen) was prepared immediately prior to staining by diluting the staining solution 300-fold in PBS. Tissue sections were immersed in 500 μ l staining solution and incubated for 20 min at room temperature. Sections were rinsed in PBS, mounted on Superfrost slides, and air dried before coverslipping with Vectashield mounting medium (Vector Laboratories).

NeuN immunostaining was performed using the mouse monoclonal anti-NeuN antibody (1:200 dilution; Chemicon) followed by biotinylated secondary antibody and avidin-biotin complex system (Vector ABC kit, Vector Laboratories, Burlingame, CA).

Diaminobenzidine tetrahydrochloride (DAB) (DAB Peroxidase Substrate Kit, Vector Laboratories) was used as a chromogen. Sections were mounted on slides, dehydrated, cleared, and coverslipped.

Lesion assessment

Stained sections were viewed with a Zeiss Axioimager light microscope (Carl Zeiss Microimaging). The lesion location was determined by examining both thionin-stained and NeuN-stained sections with the aid of a rat brain atlas (Paxinos and Watson, 1998).

Experiment 1: effects of post-training CeA CRF inhibition on the consolidation of contextual fear memory

We evaluated the hypothesis that CeA CRF secretion is a critical factor in a memory modulatory system that has a temporal role in consolidation processing. CeA cannulated rats were initially exposed to contextual fear conditioning and then returned to their home cage before microinjecting PBS or CRF ASO (1 nmol, 0.4 μ l/side volume) at 5-min, 9-h, 24-h, or 96-h after training. As our previous study (Pitts et al., 2009) found no behavioral differences between animals microinjected into the CeA with PBS or random sequence oligonucleotides, control animals in this study were treated only with PBS. Furthermore, in that study we demonstrated (with FITC labeled oligonucleotides) that a 0.4 μ l infusion volume was confined to the CeA and also showed that 1 nmol CRF ASO microinjected into the CeA induced a significant reduction in CRF immunoreactivity after 5 h but not after 48 h. Thus, in this study, contextual freezing was measured in the drug-free retention test 48 h

after CRF ASO microinjections (see Fig. 1A). At the conclusion of testing, rat brains were prepared for analysis. For each post training time interval, *t*-tests were conducted between groups to determine statistical significance.

Experiment 2: effects of post-training CeA fiber-sparing lesions modulating the consolidation of contextual fear

The purpose of this study was to extend and validate the results of experiment 1 by determining whether a functional CeA is required for the consolidation and retention of contextual fear memory. CeA cannulated rats were exposed to contextual fear conditioning and returned to their home cages. At post-training intervals of 24-h or 96-h, rats were anesthetized and microinjected bilaterally with PBS or ibotenic acid (10 µg/µl, 0.2 µl/side). All rats were tested for contextual fear retention 48-h after the microinjections. At the conclusion of testing, rat brains were prepared for analysis.

Based upon the results of experiment 1, we hypothesized that CeA lesions induced 24-h, but not 96-h, after contextual fear training would impair the retention of contextual fear memory. To statistically test this hypothesis, *t*-tests were conducted between groups at each post-training interval.

3. Results

3.1. Experiment 1: CeA CRF synthesis influences contextual fear memory consolidation for at least 24 h after conditioning

Our previous study suggested that CeA CRF secretion is a critical mediator of contextual fear memory consolidation (Pitts et al., 2009) but the post-training, time-dependent period of CeA CRF secretion in facilitating the consolidation process was not assessed. Nonetheless, we expected that if CeA CRF secretion has a critical temporal involvement in facilitating the consolidation of contextual fear memory, then inhibition of CeA CRF using CRF ASO will likely impair contextual memory during the early, but not later, post-training periods.

Rats microinjected with CRF ASO into the CeA at 5-min ($t_{17} = 3.208, p < 0.01$), 9-h ($t_{13} = 2.518, p < 0.05$), and 24-h ($t_{13} = 2.513, p < 0.05$) after training exhibited significantly lower levels of contextual freezing than their respective controls. In contrast, CRF ASO administration 96-h ($t_{13} = 0.670, p > 0.05$) after contextual training induced no reliable impairments in freezing retention (Fig 1D). These novel results demonstrate that CeA CRF secretion modulates contextual fear memory consolidation for at least 24-h after exposure to our contextual fear conditioning procedures.

3.2. Experiment 2: CeA fiber-sparing lesions made 24 h, but not 96 h, after fear training impairs contextual fear memory

The results of experiment 1 demonstrate that by 96-h after contextual training, CeA CRF no longer appears to be involved in consolidation. These results raise the important issue of whether a functional CeA is necessary for freezing. To address this issue, CeA fiber-sparing lesions were performed 24-h or 96-h after fear conditioning and contextual freezing was assessed 48-h later (Fig 2A).

Fiber-sparing lesions were confined to the CeA (Fig. 2B, C) in coronal sections -2.12 to -3.14 (Paxinos and Watson, 1998). In the retention test, rats with CeA lesions 24-h after contextual training exhibited significantly lower freezing levels than their respective sham-treated controls ($t_9 = 3.60, p < 0.01$) (Fig 2D). In contrast, CeA lesions made 96-h after training produced no reliable deficits in fear retention when compared to the 96-h control group ($t_{12} = 0.15, p > 0.05$) (Fig 2D). These findings demonstrate that fear memory

consolidation is dependent on a functional CeA for at least 24-h after training. In addition, the CeA is not required for fear retention or expression during the post-consolidation period.

4. Discussion

Our results provide new evidence for the prolonged involvement of CeA CRF secretion in the modulation of contextual fear memory. In experiment 1, CeA CRF ASO treatment at intervals of up to 24-h after training induced deficits in contextual fear retention, while treatment 96-h post-training produced no impairment. These results were further supported by experiment 2, which demonstrated that CeA fiber-sparing lesions administered 24-h, but not 96-h, after training significantly impaired contextual fear retention. Thus, neither CeA CRF ASO treatment nor CeA lesions produced general impairments in contextual freezing. The present work shows a specific time-dependent role of the CeA in the consolidation, but not retention, of contextual fear memory.

In the CeA, CRF cell bodies contain glucocorticoid receptors (Lechner & Valentino, 1999) and glucocorticoid administration elevates amygdalar CRF secretion (Cook, 2002) and upregulates CeA CRF mRNA (Shepard et al., 2000). Of potential relevance to our current results, a previous study reported that mice with a conditional knockout of CeA glucocorticoid receptors (CeAGRKO) failed to show an upregulation in CeA CRF mRNA following fear conditioning and also exhibited deficits in fear retention (Kolber et al., 2008). Importantly, the retention deficits in CeAGRKO mice were rescued by pre-training intracerebroventricular injections of CRF. These findings demonstrate that a functional interaction between glucocorticoids and CeA CRF plays a critical role in facilitating the consolidation of fear memory.

Prior research in this laboratory demonstrated that post-training antagonism of BLA CRF₁ receptors at 3-h, but not 9-h, impaired subsequent contextual fear retention (Hubbard et al., 2007), whereas the current results indicate that CeA CRF secretion facilitates consolidation for at least 24-h after training. These temporal differences involving BLA CRF₁ receptors and CeA CRF may reflect consolidation processing occurring not only in the BLA but also in brain sites that receive prominent CeA CRF fiber projections. CeA CRF is located predominantly in cell bodies found in the lateral part of the CeA (CeAl) (Veening et al., 1984) and the CeAl sends major CRF projections to the anterolateral regions of the bed nucleus of the stria terminalis (BST) (Sakanaka et al., 1986; Gray, 1990). Of further relevance, post-training BST microinjections of CRF has been shown to enhance retention of inhibitory avoidance (Liang et al., 2001), whereas intra-BST post-training antagonism of either NMDA receptors or β -adrenergic receptors impairs retention of this task (Liu et al., 2009). Hence, the BST, in conjunction with the BLA, may be another important site influenced by CRF during consolidation.

CeA CRF may also influence the consolidation process over the course of 24-hr by altering the activity of autonomic and endocrine systems, which are typically activated by fear conditioning and known to modulate memory consolidation. Neuroanatomical studies demonstrate that CeA CRF projects not only to the anterolateral BST region, which is well-connected to cells that mediate autonomic responses (Dong et al., 2001), but also to other autonomic centers including the dorsal vagal complex, parabrachial nuclei, and central gray (Veening et al., 1984; Sakanaka et al., 1986; Gray, 1990). In addition, the CeAl sends projections to the BST fusiform nucleus (Petrovich & Swanson, 1997), a major participant in the activation of hypothalamic-pituitary-adrenal hormone secretion (Choi et al., 2007). Thus, CeA CRF ASO treatment may have attenuated the normal rise in glucocorticoids induced by fear conditioning and this reduction, in turn, may have led to impairments in memory consolidation. Another CeA CRF projection is directed to dendrites of the locus

coeruleus-norepinephrine system (Tjoumakaris et al., 2003), which modulates arousal and processing of sensory stimuli (Valentino et al., 2008). Potential disruption in diverse brain regions that modulate autonomic, endocrine, and sensory functions made by CeA CRF inhibition and CeA lesions 24-h after contextual fear training may compromise system consolidation, a term hypothesized to reflect the reorganization over time of brain circuits involved in the encoding and storage of the memory trace throughout the brain (Dudai, 2004).

Finally, the observation that CeA lesions administered 96-h after conditioning failed to impair fear retention suggests that contextual fear expression and retention, at least in terms of freezing, is not dependent upon a functional CeA. The medial portion of the CeA (CeAm) projects to various brainstem nuclei involved in fear responses (Schwaber et al., 1982; Cassell et al., 1986) and is thought to be critically involved in fear expression (LeDoux, 2000; Rodrigues et al., 2009). Yet, the BST also sends downstream projections to many of the same target structures (Dong et al., 2004), and thus, is also well situated to coordinate fear responses. Additionally, the posterior BLA sends fibers of passage which course through the CeA to innervate the anterolateral BST (Dong et al., 2001). Our results suggest that this BLA-to-BST conduit may be vital for contextual fear expression (Fig 3). This notion is further supported by a prior report which found post-training BST lesions to attenuate freezing and corticosterone responses to contextual, but not auditory cues (Sullivan et al., 2004).

In summary, the present studies demonstrate the **temporary** role of CeA CRF in memory consolidation for at least 24-hr following exposure to contextual fear conditioning. Our CeA CRF ASO results are corroborated by the observation that CeA fiber-sparing lesions induced 24-hr, but not 96-hr, after initial conditioning subsequently impaired contextual fear retention. Together, these experiments extend our previous CeA CRF study (Pitts et al., 2009), by identifying the critical period in which CeA CRF secretion facilitates consolidation **in our conditioning paradigm**. In addition, the current studies indicate that after stabilization of contextual fear memory, a functional CeA is no longer required for the expression or retention of contextual fear.

Research Highlights

- Post-training CeA CRF inhibition impairs contextual fear memory consolidation
- The CeA has a time-dependent role in the consolidation of contextual fear memory
- CeA activity is not required for the retention of contextual freezing

Acknowledgments

The work was supported by National Institutes of Health Grant NS39406. We thank Jarrett Koo for excellent technical assistance.

References

- Cassell MD, Gray TS, Kiss JZ. Neuronal architecture in the rat central nucleus of the amygdala: a cytological, hodological, and immunocytochemical study. *J Comp Neurol* 1986;246:478–499. [PubMed: 2422231]
- Choi DC, Furay AR, Evanson NK, Ostrander MM, Ulrich-Lai YM, Herman JP. Bed nucleus of the stria terminal subregions differentially regulate hypothalamic-pituitary-adrenal axis activity:

- implications for the integration of limbic inputs. *J Neurosci* 2007;27:2025–2034. [PubMed: 17314298]
- Cook CJ. Glucocorticoid feedback increases the sensitivity of the limbic system to stress. *Physiology & Behavior* 2002;75:455–464. [PubMed: 12062310]
- Dong HW, Petrovich GD, Swanson LW. Topography of projections from amygdala to bed nuclei of the stria terminalis. *Brain Res Rev* 2001;38:192–246. [PubMed: 11750933]
- Dong HW, Swanson LW. Organization of axonal projections from the anterolateral area of the bed nucleus of the stria terminalis. *J Comp Neurol* 2004;468:277–298. [PubMed: 14648685]
- Dudai Y. The neurobiology of consolidations, or, how stable is the engram? *Ann Rev Psychol* 2004;55:51–86. [PubMed: 14744210]
- Gray, TS. The organization and possible function of amygdaloid corticotropin-releasing factor pathways. In: De Souza, EB.; Nemeroff, CB., editors. *Corticotropin-releasing factor: basic and clinical studies of a neuropeptide*. CRC press; Boca Raton: 1990. p. 53–68.
- Hubbard DT, Nakashima BR, Lee I, Takahashi LK. Activation of basolateral CRF₁ receptors modulates the consolidation of contextual fear. *Neuroscience* 2007;150:818–828. [PubMed: 17988803]
- Jimenez SA, Maren S. Nuclear disconnection within the amygdala reveals a direct pathway to fear. *Learn Mem* 2009;16:766–768. [PubMed: 19933881]
- Kolber BJ, Roberts MS, Howell MP, Wozniak DF, Sands MS, Muglia LJ. Central amygdala glucocorticoid receptor action promotes fear-associated CRH activation and conditioning. *Proc. Natl. Acad. Sci. USA* 2008;105:12004–12009. [PubMed: 18695245]
- Koo JW, Han JS, Kim JJ. Selective neurotoxic lesions of basolateral and central nuclei produce differential effects on fear conditioning. *J Neurosci* 2004;24:7654–7662. [PubMed: 15342732]
- Lechner SM, Valentino RJ. Glucocorticoid receptor-immunoreactivity in corticotrophin-releasing factor afferents to the locus coeruleus. *Brain Res* 1999;816:17–28. [PubMed: 9878680]
- LeDoux J. Emotion circuits in the brain. *Annu Rev Neurosci* 2000;23:155–184. [PubMed: 10845062]
- Liang KC, Chen HC, Chen DY. Posttraining infusion of norepinephrine and corticotropin releasing factor into the bed nucleus of the stria terminalis enhanced retention in an inhibitory avoidance task. *Chin J Physiol* 2001;44:33–43. [PubMed: 11403518]
- Liu T-L, Chen D-Y, Liang KC. Post-training infusion of glutamate into the bed nucleus of the stria terminalis enhanced inhibitory avoidance memory: an effect involving norepinephrine. *Neurobiol Learn Mem* 2009;91:456–465. [PubMed: 19186212]
- McDonald AJ. Cortical pathways to the mammalian amygdala. *Prog Neurobiol* 1998;55:257–332. [PubMed: 9643556]
- McGaugh JL. The amygdala modulates the consolidation of memories of emotionally arousing experiences. *Annu Rev Neurosci* 2004;27:1–28. [PubMed: 15217324]
- Paré D. Role of the basolateral amygdala in memory consolidation. *Prog Neurobiol* 2003;70:409–420. [PubMed: 14511699]
- Paxinos, G.; Watson, C. *The rat brain in stereotaxic coordinates*. Ed. 4. Academic; San Diego: 1998.
- Petrovich GD, Swanson LW. Projections from the lateral part of the central amygdalar nucleus to the postulated fear conditioning circuit. *Brain Res* 1997;763:247–254. [PubMed: 9296566]
- Pitkänen A, Savander V, LeDoux JE. Organization of intra-amygdaloid circuitries in the rat: An emerging framework for understanding functions of the amygdala. *Trends Neurosci* 1997;20:517–523. [PubMed: 9364666]
- Pitts MW, Todorovic C, Blank T, Takahashi LK. The central nucleus of the amygdala and corticotropin-releasing factor: insights into contextual fear memory. *J Neurosci* 2009;29:7379–7388. [PubMed: 19494159]
- Rodrigues SM, LeDoux JE, Sapolsky RM. The influence of stress hormones on fear circuitry. *Annu Rev Neurosci* 2009;32:289–313. [PubMed: 19400714]
- Roosendaal B, Brunson KT, Holloway BL, McGaugh JL, Baram TZ. Involvement of stress-released corticotropin-releasing hormone in the basolateral amygdala in regulating memory consolidation. *Proc Natl Acad Sci USA* 2002;99:13908–13913. [PubMed: 12361983]

- Roozendaal B, Schelling G, McGaugh JL. Corticotropin-releasing factor in the basolateral amygdala enhances memory consolidation via an interaction with the β -adrenoceptor-cAMP pathway: dependence on glucocorticoid receptor activation. *J Neurosci* 2008;28:6642–6651. [PubMed: 18579737]
- Sakanaka M, Shibasaki T, Lederis K. Distribution and efferent projections of corticotropin-releasing factor-like immunoreactivity in the rat amygdaloid complex. *Brain Res* 1986;382:213–38. [PubMed: 2428439]
- Schwaber JS, Kapp BS, Higgins GA, Rapp PR. Amygdaloid and basal forebrain direct connections with the nucleus of the solitary tract and the dorsal motor nucleus. *J Neurosci* 1982;2:1424–1438. [PubMed: 6181231]
- Shepard JD, Barron KW, Myers DA. Corticosterone delivery to the amygdala increases corticotropin-releasing factor mRNA in the central amygdaloid nucleus and anxiety-like behavior. *Brain Res* 2000;861:288–295. [PubMed: 10760490]
- Sullivan GM, Apergis J, Bush DE, Johnson LR, Hou M, LeDoux JE. Lesions in the bed nucleus of the stria terminalis disrupt corticosterone and freezing responses elicited by a contextual but not by a specific-cue conditioned fear stimulus. *Neuroscience* 2004;128:7–14. [PubMed: 15450349]
- Tjounmakaris SI, Rudoy C, Peoples J, Valentino RJ, Van Bockstaele EJ. Cellular interactions between axon terminals containing endogenous opioid peptides or corticotropin-releasing factor in the rat locus coeruleus and surrounding dorsal pontine tegmentum. *J Comp Neurol* 2003;466:445–456. [PubMed: 14566941]
- Valentino RJ, Van Bockstaele E. Convergent regulation of locus coeruleus activity as an adaptive response to stress. *Eur J Pharm* 2008;583:194–203.
- Veening JG, Swanson LW, Sawchenko PE. The organization of projections from the central nucleus of the amygdala to brainstem sites involved in central autonomic regulation: A combined retrograde transport-immunohistochemical study. *Brain Res* 1984;303:337–357. [PubMed: 6204716]
- Wilensky AE, Schafe GE, Kristensen MP, LeDoux JE. Rethinking the fear circuit: The central nucleus of the amygdala is required for the acquisition, consolidation, and expression of pavlovian fear conditioning. *J Neurosci* 2006;26:12387–12396. [PubMed: 17135400]
- Zimmerman JM, Rabinak CA, McLachlan IG, Maren S. The central nucleus of the amygdala is essential for acquiring and expressing conditional fear after overtraining. *Learn Mem* 2007;14:634–644. [PubMed: 17848503]

Figure 1.

Effects of post-training microinjections of CRF ASO (1 nmol/side) in the CeA on the consolidation of contextual freezing. **A**, Contextual fear conditioning testing procedure. **B**, Representative photomicrograph (magnification 2.5x) of thionin-stained brain section with cannula track terminating in the CeA. **C**, Location of bilateral cannula tip placements in the CeA for animals infused 5-min after training. The midline numbers refer to the coronal posterior distance in mm from bregma (adapted from Paxinos and Watson, 1998). Placements were similar for groups treated at other time points. **D**, Mean ($\pm$ SE) percent contextual freezing in the 10-min retention test. * $p < 0.05$, ** $p < 0.01$, significantly different from respective PBS control group (PBS: $n = 7-10$ per group; CRF ASO: $n = 7-8$ per group).

Figure 2.

Effects of post-training fiber-sparing lesions of the CeA on the consolidation of contextual freezing. **A**, Contextual fear conditioning testing procedure. **B**, Photomicrographs (magnification 5×) of thionin-stained (upper panel), NeuN immunopositive (middle panel) and FluoroMyelin Green fluorescent myelin-stained (lower middle panel) control- (left panels) and ibotenic acid CeA-lesioned (right panels) brains. Note the gliosis in thionin-stained (upper right) and neuron loss as revealed by NeuN staining (middle right) at the CeA lesion site. However, fibers of passage in the CeA were intact as shown by myelin staining in both control and lesioned brains. **C**, Schematic representations of the largest (red areas) and smallest (blue areas) ibotenic acid-induced lesions in rostral to caudal CeA coronal levels (numbers indicate mm posterior from bregma, Paxinos and Watson, 1998) for the 24-h and 96-h lesion groups. **D**, Mean ($\pm$ SE) percent contextual freezing in the 10-min retention test. *significantly different from respective sham group, $p < 0.01$ (Sham: $n = 5-7$; CeA lesion: $n = 6-7$).

Figure 3.

Model of amygdala circuitry for contextual fear expression. Contextual information regarding the conditioned stimulus is relayed from BLA to the BST via fibers of passage that course through the CeA. The BLA also projects to the CeA. The BST, in turn, projects to downstream structures which mediate fear responses, including the lateral hypothalamus (LH), paraventricular nucleus of the hypothalamus (PVN), and periaqueductal gray (PAG). These same fear response structures also receive parallel input from the CeA.