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Mouse models of fear related disorders: *cell-type specific manipulations in amygdala*

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Abstract

Fear conditioning is a model system used to study fear memory and its dysregulation in a variety of organisms. Newly developed tools such as optogenetics, Cre recombinase and DREADD technologies have allowed researchers to manipulate anatomically or molecularly defined cell subtypes with a high degree of temporal control and determine the effect of this manipulation on behavior. These targeted molecular techniques have opened up a new appreciation for the critical contributions different subpopulations of cells make to fear behavior and potentially to treatment of fear and anxiety disorders. Here we review progress to date across a variety of techniques to understand fear-related behavior through the manipulation of different cell subtypes within the amygdala.

Keywords

fear conditioning; fear extinction; amygdala; cell subtype manipulation

Introduction

Pavlovian Fear conditioning is a technique that has improved our understanding of the molecular mechanisms of the fear pathway. Training in Pavlovian fear conditioning involves exposing an organism to a cue conditioned stimulus (CS, usually a tone) that is temporally paired with an aversive unconditioned stimulus (US, usually a shock)(1). After training, animals demonstrate a conditioned response (CR, usually freezing- the lack of all movement except for respiration) (2-5). Within just a few trials within a single session, fear conditioning quickly creates a long lasting memory that can be manipulated with a variety of treatments at different time points around the training event (6). Fear extinction is the

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subsequent pairing of the CS without the US, resulting in the eventual diminution of the freezing response (1, 7, 8).

The amygdala is a central node for the processing of fear and threat and a variety of other emotions; it also is vital to fear acquisition and extinction. As shown in Figure 1 the major subnuclei of the amygdala include lateral (LA) including dorsal (Lad), ventral (LAv) and medial (LAm) subnuclei, basal (B), accessory basal (AB) and central nucleus (CeA) including lateral (CeL) and medial (CeM) subnuclei. Overwhelming evidence supports the LA as a structure critical for formation and storage of fear memory (6, 9). The term basolateral amygdala (BLA) has been used to refer to LA and B together (6). The BLA is morphologically and cytoarchitecturally very similar to the cortex consisting of glutamatergic projection cells expressing calmodulin kinase (CamK) and local circuit GABAergic cells that express neuropeptide Y (NPY), somatostatin (SOM) and vasoactive intestinal peptide (VIP), cholecystokinin (CCK), parvalbumin (PV) and calbindin (CB) (10).

Studies have shown that the CeA is required for the acquisition, consolidation, and expression of fear memories (11-14) potentially working in serial with the BLA (10). The CeA is largely GABAergic (15) and can be further subdivided into central lateral (CeL) and central medial (CeM) nuclei (16). The CeL receives projections from cortical and subcortical areas (17) and projects to the CeM whereas the CeM receives direct projections from the BLA (18). GABA-producing inhibitory neurons gate expression of fear via tonic inhibition of the CeM (19-23) and go on to effect downstream structures such as the periaqueductal grey (PAG), locus coeruleus (LC) and parabrachial nuclei (PB) that direct behavioral output (6). Interestingly, neurons in the CeA express a diverse population of peptides and peptide receptors including vasopressin (VP), oxytocin (OT), corticotropin releasing factor (CRF), enkephalin (ENK), neurotensin (NT), somatostatin (SOM), dynorphin (DYN) and substance P (Subs P) among others (20-22, 24-28).

Intercalated cells (ITCs) are densely packed GABAergic neurons that form islands surrounding the BLA (29-31). ITCs may regulate the interconnectivity between amygdala subnuclei and extra-amygdala structures and are named the lateral paracapsular (IITC), the dorsal (ITCd), and the ventral (ITCv) ITC (Figure 1). They receive sensory information from the BLA and thalamus (32) and project to the CeM nucleus (32-35) which gates output of conditioned fear behavior. Work has shown that ITCs express FoxP2 (36), ENK (37, 38), and the mu opioids (38, 39), dopamine 1 (38) and GABAA $\alpha 2/\alpha 3$ subunit (40) containing receptors.

Many studies have assessed the role of the amygdala in fear memory using lesions, pharmacological manipulation and electrophysiological techniques (6, 10, 41-43). Until recently researchers have been limited in their ability to investigate the varying contributions, at a molecular level, of different subtypes of cells within a brain region. Work by Zirlinger and colleagues (44) used microarray analysis of the amygdala followed by *in situ* hybridization to demonstrate that a majority of the genes identified by microarray show expression selective to different subnuclei of the amygdala when tested using *in situ* hybridization. This finding suggested that these cell subtypes specific to different amygdala subnuclei may each hold specific function within the emotional regulation of behavior. To

dissect the function of different cell subtypes a variety of techniques have been introduced. Along with techniques such as antisense manipulation and RNA interference, newer techniques to target and manipulate cell subtypes are being developed. One of the most revolutionary techniques, optogenetics, has offered advanced temporal precision using a combination of light presentation with genetic manipulations. Additionally, Cre/lox recombination technology for targeted deletions or inducible gene expression, DREADD (*Designer Receptors Exclusively Activated by Designer Drugs*) manipulations and targeted *in vivo* recording of amygdala cell populations have greatly increased our ability to understand the contributions of different subpopulations of neurons to fear behavior (45-48). These techniques have just begun to be utilized to give a more complete understanding of the contributions of cell specific mechanisms to fear memory. Findings from these studies highlighting fear behavior will be reviewed below (Table 1). Distribution patterns of some key cell subpopulations in the amygdala are shown in Figure 2.

Basolateral Amygdala (BLA) neuronal populations

In a study conducted to test whether there are cell responses that act in opposing directions underlying fear acquisition and fear extinction, Herry and colleagues (49) demonstrated the existence of two electrophysiologically distinct fear responsive populations of neurons in awake behaving mice. One subpopulation of neurons increased firing specifically during and after fear conditioning and decreased firing during extinction training. A second population of neurons increased firing only after fear extinction. Herry and colleagues referred to these excitatory pyramidal neurons within the BLA as 'Fear' and 'Extinction' neurons, implying that the cellular activation of these cells differentially represented the memory traces of these different functions. Importantly for the behavioral relevance of these findings, firing frequency corresponded to freezing behavior. While the molecular identity of these neurons was not investigated in this study, the data highlight the critical contribution of subsets of neurons to fear behavior.

BLA pyramidal neurons

Johansen and colleagues (50) assessed whether optogenetic activation of pyramidal neurons in the LA along with presentation of a tone is sufficient to produce fear learning. When mice were trained to a tone paired with optical activation of pyramidal cells in the LA as an US, mice demonstrated fear conditioning. Importantly, this author showed that fear memory was only formed when the tone CS preceded the optical stimulation US indicating the process is associative (51). If the tone and optical stimulation were unpaired no learning occurred. Although fear conditioning occurred with LA pyramidal neuron stimulation the resulting freezing was relatively low. The authors suggest this may be due to activation of pyramidal cells en masse rather than in a cell subtype specific manner. Specifically, LA plasticity is also involved with appetitive learning making it possible that both appetitive and aversive types of neurons were stimulated. Later work by this same group (52) showed that this effect was enhanced with the addition of a beta noradrenergic receptor agonist. Specifically, presentation of a weak training protocol (3 CS tone presentations + ChR2 optical activation) coincident with an agonist of the beta adrenergic receptor resulted in enhanced freezing behavior during testing the following day. Together, these data suggest that with limited

training a combination of Hebbian and neuromodulatory mechanisms triggers plasticity in the LA and behavioral associative learning.

Thy1 appears to mark the Fear-off / Extinction BLA Neurons

A later study by Jasnow and colleagues (53) used transgenic mice in which expression of ChR2 is driven by the Thy1-promoter to investigate the activities of Thy1-marked pyramidal neurons within the BLA during fear acquisition and extinction. The Thy1(H) line of Sanes and colleagues (54) appears to mark a specific population of neocortical and subcortical neurons, but no paleocortical populations. Jasnow and colleagues showed that the Thy1-marked cohort represented only about 1/3 of the excitatory neurons within the BLA. Pairing optogenetic activation of glutamatergic Thy1 neurons within the BLA with CS-US presentation during training inhibited fear consolidation, as indicated by attenuated freezing when tested the following day in the absence of optogenetic stimulation. To test the role of Thy1 containing neurons in fear extinction, BLA Thy1 neurons received optogenetic activation coincident with the CS alone presentation during fear extinction training. During the unstimulated extinction retention test the following day memory for extinction was enhanced (freezing was attenuated) compared to controls. These data suggest the BLA holds a subpopulation of glutamatergic neurons that are specifically tuned to inhibit the fear response possibly via an indirect connection with the CeM. Ongoing work is further examining whether the Thy1(h) subpopulation of BLA pyramidal neurons serves as a molecular mark of the Extinction neurons physiologically identified by Herry and colleagues. If so, it may provide a powerful way to drive specific fear inhibition or enhancement of extinction behavior that may help provide novel approaches to treating neuropsychiatric disease with unregulated fear processing.

Parvalbumin and Somatostatin Interneuron Populations

Using optogenetics and single unit recordings, a recent study (55) interrogated the role of the interneuron subtypes parvalbumin (PV) and somatostatin (SOM) in the BLA during fear learning. They found that PV and SOM neurons bidirectionally modulate fear acquisition. To show this in a cell subtype specific manner, the authors selectively expressed channelrhodopsin-2 (ChR2) in PV or SOM neurons in the BLA. PV cells received activation of ChR2 during pairing of the entire CS – US presentation or just during the US presentation, resulting in decreased freezing responses during the CS presentation the following day. Interestingly, PV neurons stimulated with ChR2 only during the CS resulted in increased freezing demonstrating opposing roles for PV neurons during CS and US processing. Additionally, Wolff and colleagues expressed the inhibitory opsin archaerhodopsin-3 (ARCH) in PV neurons and showed ARCH-mediated inhibition during the US presentation caused an increase in freezing during an unstimulated test of fear retrieval the following day. In contrast, manipulation of SOM containing neurons during the CS resulted in the opposite behavioral effects. These data suggest that within the BLA, PV and SOM expressing inhibitory neurons bidirectionally control the acquisition of fear conditioning.

While it is beyond the scope of this review to discuss all the identified inhibitory cell types within the BLA many of these neurons have been well characterized elsewhere (56-59).

Further, a recent review gives a broad overview of the diversity of GABAergic cells within the BLA (60).

Central Amygdala (CeA) neuronal populations

Vasopressin and Oxytocin

The neuropeptides VP and OT are strongly expressed in the CeA and differentially engaged in fear and anxiety behavior. A variety of studies have shown that VP enhances and OT decreases anxiety and fear behavior (61-66). Interestingly, even though VP and OT have opposing effects on fear and anxiety behavior, application of either peptide increases neuronal excitability in a variety of different brain structures (67-69). OT is expressed in the CeL and VP is expressed in the CeM (70). Huber and colleagues (70) used different receptor agonists and antagonists to determine that OT and VP activate different neuronal subpopulations. OT neurons activated GABAergic transmission into the CeM and were restricted to CeL while VP neurons were localized to CeM and sent projections outside of the CeA. The differential connectivity of these neuropeptides within amygdala and extra-amygdala circuitry may underlie their opposing effects on fear behavior.

SOM

A study by Li and colleagues (71) investigated how a specific subset of CeL neurons respond to fear conditioning and contribute to both the learning and expression of fear. First, to test whether the CeL is essential for fear memory storage, neuronal activity was suppressed in Som-IRES-cre using chemical-genetic (72) manipulation that expresses hM4Di, an engineered inhibitory G protein-coupled receptor that suppresses neuronal activity in a Cre dependent manner. SOM activity was suppressed just prior to fear conditioning and resulted in impaired fear conditioning suggesting SOM containing neurons are critical for fear memory acquisition. Further, these authors used ChR2 to activate SOM neurons in the CeL and found they could induce freezing. To test whether SOM neurons in the CeL of fear-conditioned mice are required for the expression of learned fear, the inhibitory opsin ARCH was selectively expressed in SOM neurons. Mice were then fear conditioned and SOM neurons were inhibited during fear memory recall. Freezing behavior was attenuated in mice expressing the inhibitory opsin ARCH suggesting SOM containing neurons are necessary for conditioned fear expression. As indicated by cell specific retrograde tracing, the effects on freezing behavior caused by manipulation of SOM neurons are not occurring through the CeM because SOM neurons do not project to neurons in the CeM that project to brain areas in control of freezing (discussed further below). These findings highlight the role of SOM containing neurons in the CeL as directly modulating fear behavior.

PKC δ

Previous work has found that GABAergic neurons in the CeL gate expression of fear via tonic inhibition of the CeM (19-23), the major output of the amygdala (73). Haubensak and colleagues (20) used optogenetics to investigate a GABAergic subpopulation of PKC δ containing neurons in the CeL that may be responsible for gating CeM neurons. First Haubensak and colleagues showed that optogenetic activation of PKC δ neurons inhibits

CeM output neurons that project to the PAG, a structure critical for production of freezing behavior. Further, silencing of PKC δ neurons resulted in enhanced freezing suggesting that this population of PKC δ neurons tonically inhibit CeM output neurons that gate freezing behavior.

CRF

CRF neurons are localized to the paraventricular nucleus of the hypothalamus (PVN), central nucleus of amygdala (CeA) and the bed nucleus of stria terminalis (BNST) (74-76). Dysregulation of CRF activity may underlie a number of psychiatric conditions such as PTSD (77-79). Recent studies have attempted to manipulate CRF neurons in the amygdala. Specifically, Pitts and colleagues (80, 81) used pretraining infusion of antisense against amygdala CRF neurons to test the importance of these neurons in contextual fear conditioning. No significant difference was shown during training; however when the animals were tested 48 hours later they showed significantly disrupted fear memory retention. A follow up study by the same group (81) showed post training infusion of CRF antisense into the amygdala was effective at disrupting fear memory retention when given either 5 minutes, 9 hours or 24 hours after fear conditioning, but not when given 96 hours after training. These findings highlight an extended involvement of CeA CRF neurons in contextual fear memory consolidation.

Our lab has tested the effect of inducible deletion of either the excitatory NMDA receptor, *NR1* (82) or the inhibitory, *GABAA α 1* (83) genes specifically within the CRF neuronal populations. In these inducible genetic studies, we found different effects on fear acquisition and extinction of conditioned fear. As shown in Figure 3 we found that disruption of *GABAA α 1* within CRF-containing neurons did not affect fear conditioning or retention behavior but resulted in a significant and prolonged deficit in fear extinction (83). In contrast, deletion of *NR1* within CRF-containing neurons leads to enhancement of fear conditioning and fear retention. A similar phenotype was seen within both transgenic mice and with mice using lentiviral mediated *NR1* deletion, targeting just CRF neurons within the CeA (82). These studies report, additionally, that the GABA receptor deletion was associated with enhanced Amygdala/BNST excitation, consistent with possible increased fear after extinction. Paradoxically, the *NR1* deletion mice also showed a phenotype consistent with enhanced Amygdala/BNST activation, thought to be due to compensatory increase AMPA upregulation following *NR1* deletion, resulting in increased fear acquisition and retention. Altogether, our data may suggest that different CRF neuronal subpopulations (e.g. lateral CeA (CeL) vs. BNST) selectively contribute to accelerated fear acquisition or disrupted fear extinction behavior; alternatively, these effects may primarily represent the different effects of the NR1 and GABAA α 1 neurotransmitter receptors within the CRH neuronal populations. Since disorders such as PTSD have been linked to enhanced fear conditioning as well as disrupted fear extinction (84-88), these data may highlight a potential mechanism for further investigation of this cell-type specific dissociation.

Tachykinin (Tac2) within the CeA medial nucleus (CeM)

Tac2 is highly expressed in the CeA with no expression in the basolateral amygdala (BLA) or lateral amygdala (LA). Specifically within the CeA, the highest expression of *Tac2* occurs

in the CeM, with less expression in the CeL (89, 90). Andero and colleagues (89) initially discovered that expression of *Tac2* within the CeA was dynamically altered during the consolidation of fear memory. They then showed that overexpression of *Tac2* in the CeA significantly enhanced fear memory consolidation. When the *Tac2* gene was silenced during fear learning using Designer Receptors Exclusively Activated by Designer Drugs (DREADD) technology, 30 minutes prior to fear conditioning, no effect was seen on fear behavior. However, the following day when fear memory was tested, the mice that had *Tac2* silenced during fear conditioning showed impaired fear memory consolidation. These data suggest that the *Tac2* neuronal subpopulation within CeM may represent a critical output pathway from the CeM to downstream, subcortical target nuclei representing the process of fear expression. It has long been known that the CeM nucleus of the amygdala was a final output station to the brainstem fear expression network, but specific populations of cells within CeM which could be genetically and pharmacologically targeted were previously unknown.

Intercalated cells (ITCs)

Work indicates that ITC populations are differentially recruited during fear acquisition (31) and fear extinction (31, 91, 92). ITC neurons receive sensory information from the BLA and thalamus (32) and BLA inputs to ITC neurons have been shown to express NMDA-dependent LTD and LTP (93, 94). ITC neurons project information to the CeM nucleus which gates output of conditioned fear behavior (32-35). Little is known about the contributions of different ITC cell subtypes to fear behavior. One study attempted to use a known cellular marker of ITCs to directly manipulate their role in fear extinction (95). The authors took advantage of the high expression level of mu opioid receptors (MORs) in ITC neurons and administered a targeted toxin directed at mu opioids receptors in ITCv neurons after fear extinction. When extinction memory was tested, ITCv lesioned rats showed impaired extinction expression suggesting ITCs were engaged in fear extinction consolidation. ITCv neurons have been shown to receive excitatory inputs from BLA after training in extinction (96). Altogether these findings suggest that a population of ITCv neurons, potentially those expressing MORs, inhibits CeM neurons during normal retention and expression of fear extinction memories.

Amygdala Projections

Recent work has also highlighted the importance of distinct subpopulations of amygdala projections to other structures and their importance in control of a variety of amygdala mediated behaviors. A recent study by (97) used optogenetic manipulation of glutamatergic fibers emanating from the BLA to the nucleus accumbens (NAc) to show that these fibers modulated rewarding behavior. Similar optogenetic stimulation of glutamatergic fibers between medial prefrontal cortex (mPFC) and the NAc did not result in changes in reward seeking behavior. Additionally, these authors showed that reward seeking behavior was attenuated when optogenetic inhibition was applied to glutamatergic fibers between BLA and NAc further supporting the critical nature of these fibers in reward related behavior. Another recent study (98) showed that a subpopulation of neurons projecting from the basal amygdala (BA) to the prelimbic (PL) area show increased expression of the immediate early gene FOS after fear conditioning compared to BA inputs into infralimbic (IL) areas that

showed increased FOS expression after fear extinction. Further, inhibition of the pathway from BA to PL increased extinction memory while inhibition of the pathway from BA to IL disrupted fear extinction suggesting the relative activity difference between these pathways contributes to extinction memory strength. A different study (99) found that optogenetically inhibiting BLA to anterodorsal BNST (adBNST) projections enhanced measures of anxiety while stimulating those projections decreased anxiety. Further testing revealed projections from adBNST to lateral hypothalamus (LH) mediated different aspects of individual measures of decreased anxiety. Specifically the adBNST projection to 1) lateral hypothalamus (LH) decreased avoidance of open spaces (2) parabrachial nucleus (PBN) contributed to decreased respiratory rate and (3) the ventral tegmental area (VTA) contributed to increased place preference (positive valence) of an environment. A final study (100) showed that optogenetic manipulation of BLA to ventral hippocampus (VH) synapses modulates anxiety. Specifically, inhibition of BLA axon terminals with NpHR in VH decreases anxiety. Activation of these same projections with ChR2 increases anxiety behaviors.

The CeL has been shown to have projections that bypass the CeM and innervate a variety of downstream structures (101-105) suggesting the CeL may directly influence fear behavior via these long range projections. Using retrograde tracing and immunolabeling a study by Penzo and colleagues (106) showed that a subset of SOM containing neurons in the CeL send long range projections directly to the midbrain periaqueductal gray (PAG) and paraventricular nucleus of the thalamus (PVN). These authors infused the CeL of SOM-Cre with a Cre-dependent virus which selectively expressed ChR2 in SOM CeL neurons. Optical stimulation of axons originating from the SOM CeL neurons reliably inhibited the ventrolateral PAG. Further, when slices from mice that were fear conditioned were tested compared to controls by recording miniature excitatory postsynaptic potentials (mEPSPs) they found that fear conditioning increased mEPSC frequency in projections between the CeL to PAG or CeL to PVN. Overall these findings implicate the projections between CeL and PAG as a secondary pathway outside of the projections from the CeM which can drive fear expression via PAG output.

A follow up study by Penzo and colleagues (107) showed that the projections from the CeL to the PVN regulate fear learning. First, the authors found fear conditioning and retrieval increased expression of c-Fos in the posterior PVN. The authors infused a retrograde tracer into the BLA and CeL and revealed dense, mostly non overlapping, projections to the CeL and BLA from posterior PVN. The authors further targeted posterior PVN projections to CeL with a DREADD virus that effectively allowed suppression of the CeL to posterior PVN pathway with infusion of a DREADD activating drug (clozapine-N-oxide) during fear conditioning or retrieval. Both fear conditioning and fear retrieval were significantly disrupted with disruption of the CeL to posterior PVN pathway. The authors further found the posterior PVN neurons preferentially innervated SOM containing neurons compared to PKC δ containing neurons and that BDNF-TrkB signaling mediates communication within this pathway and promotes SOM containing neuronal activation within the CeL.

Conclusion

The amygdala complex is made up of numerous subnuclei, each of which have a variety of different excitatory, inhibitory and peptidergic cell populations (Figure 4). The selectivity of their expression within these subnuclei may suggest they underlie specific aspects of amygdala - mediated emotion regulation. Cell subpopulations within the BLA, CeA, ITC and even projections from the amygdala to other structures have shown specific roles in fear memory regulation. For example, simply activating LA pyramidal cells after presentation of a CS tone results in fear conditioning and this effect can be enhanced with LA application of an agonist of the beta adrenergic receptor. Other work targeted Thy1 pyramidal cells in the BLA and showed that optogenetic activation of glutamatergic Thy1 neurons within the BLA during training inhibited fear consolidation. The same activation given during fear extinction facilitated extinction of fear. This subpopulation of BLA neurons may be specifically tuned to inhibit the fear response. PV and SOM interneurons within the BLA have also been investigated and have been shown to play opposing roles in fear memory formation. Specifically, optogenetic activation of SOM neurons or inhibition of PV neurons impairs fear learning whereas activation of PV neurons or inhibition of SOM neurons enhances learning. The relative activity of PV and SOM neurons determines the activation level of effected principal neurons and ultimately may determine whether the effected principal neuron is recruited to the memory trace. Work in the CeA focused on neuropeptides has shown that OT, VP, SOM, Tac2 and CRF are strongly engaged during fear memory. OT, SOM and CRF are localized within the CeL which has been shown to either utilize GABAergic projections to the CeM to modulate fear behavior output or directly project to downstream structures. The GABAergic projections into the CeM have been proposed to be GABAergic PKC δ neurons in the CeL that modulate the CeM through tonic inhibition. Tac2 and VP containing neurons are mostly localized to the CeM from which they send projections to downstream structures and further modulate fear acquisition and extinction. Studies have also begun investigating projections of specific cell subtypes from the BLA or CeA. These studies have shown these projections strongly contribute to different aspects of amygdala mediated behavior and they also broaden our understanding of extra amygdala contributions to fear behavior.

Recent work showing the robust effects of manipulating neuronal subpopulations on fear and anxiety behavior highlights the importance of increased technological innovation towards understanding the contributions and changes underlying cell subpopulations in fear memory. New techniques to understand genetic changes within distinct cell populations such as *translating ribosome affinity purification* (TRAP) which combines cell type-specific expression of a transgene with purification and analysis of ribosomes that are being translated (108) will be crucial to our ability to learn about and manipulate these subpopulations of neurons.

By understanding the specific roles of individual, molecularly defined populations of neurons, the field is moving towards providing targeted, rationally designed approaches to both manipulate specific neuronal populations for research purposes, but also in the service of identifying novel cell circuit-based approaches to treating and preventing fear-related disorders in neuropsychiatry.

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Highlights

The amygdala is made of many subnuclei and cell types.

A detailed understanding of the cell-type structure is critical.

Fear disorders such as PTSD, Panic, and Phobia are related to amygdala dysfunction.

We review the CRF and other amygdala peptides in anxiety, fear and extinction.

Figure 1. Location of different subregions of the amygdala

A. Nissl stain of coronal brain section from mouse (Image Credit: Allen Brain Atlas). **B.**

Schematic diagram of amygdala complex (*Adapted from Lee et al., 2013 (94).*

Abbreviations: LAd (Lateral amygdala dorsal), LAvm (Lateral amygdala ventromedial), LAvl (Lateral amygdala ventrolateral), ITCd (Intercalated cells dorsal), ITCv (Intercalated cells ventral), IITC (Lateral Intercalated cells), BL (Basolateral amygdala), BM (Basomedial amygdala), CEI (Central lateral amygdala), CEm (Central medial amygdala).

Figure 2. Distribution patterns of key neuronal cell populations in the amygdala

Distribution patterns of FoxP2 (Forkhead box protein P2), DRD1 (Dopamine receptor D1), PKCδ (Protein kinase C delta), Tac2 (Tachykinin 2), Nts (Neurotensin), GRP (Calcitonin Gene Related Peptide), CCK (Cholecystokinin), Cb1 (Cannabinoid receptor type 1), Sst (Somatostatin), CRF (Corticotropin Releasing Factor), Thy1 (Thy1 cell surface antigen) in the amygdala. Images from *in situ* hybridizations from Allen Brain Atlas as well as Ressler lab.

Figure 3. Disruption of *GABAAα1* or *NR1* genes within CRF neurons result in differential effects on fear acquisition and extinction

A.) CRF *GABAAα1* deficient mice (Cre+/*fGABAAα1*) show no difference from Cre-/*fGABAAα1* mice during fear conditioning. **B.)** However, Cre+/*fGABAAα1* show significant deficits in extinction memory compared to Cre-/*fGABAAα1* controls, consistent with enhanced activation of CRF neurons through disrupted GABA processing. Alternatively, disruption of CRF NR1 containing neurons (Cre+/*fNR1+*) facilitates **(C)** fear acquisition and **(D)** fear retention compared to Cre-/*fNR1+* controls, consistent with our data for compensatory increased glutamatergic AMPA signaling in CRF neurons. When CRF NR1 neurons were disrupted with virus infusion into the CeA (LV pCRF-CRE/*fNR1+*), **(E)** fear acquisition and **(F)** fear retention were similarly significantly enhanced compared to GFP infused controls (LV pCRF-GFP/*fNR1+*).

Select Neuronal Populations in Amygdala

Figure 4. Schematic diagram of Select Neuronal Populations in Amygdala
Shown are a number of different neuronal populations that have been identified via cellular markers at the mRNA or protein level, or via their function through physiological, optogenetic, and chemogenetic manipulations. Although the list is neither comprehensive or fully understood, we have attempted to outline some of the more intriguing populations discussed in this review, outlining their location within the different subdivisions of the amygdala *on the left* and their subcellular marker and function if known, based on the reviewed literature *on the right*. Abbreviations: CS-conditioned stimulus; US-unconditioned stimulus; LA- Lateral Amygdala; BL-Basolateral amygdala; BM-Basomedial amygdala; ITC-Intercalated neurons of the amygdala; CeL-Centrolateral amygdala; CeM-Centromedial amygdala; GRP-Gastrin Releasing Peptide¹⁰⁹; Parv-Parvalbumin; SST-somatostatin; MOR-mu opiate receptor; CRF-corticotropin releasing factor; PKCd – protein kinase C, delta; Tac2 – tachykinin 2; VP – vasopressin.

Table 1

A summary of recent studies targeting the amygdala that manipulate specific cell subtypes.

Region	Name of cell type targeted	Technique used	Behavioral outcome
BLA	Thy1 (Jasnow et al., 2013)	Chr2 activation during fear conditioning Chr2 activation during fear extinction.	Disrupts fear conditioning Facilitates extinction
	Parvalbumin (Wolff et al., 2014)	Chr2 activation during CS-US or US Chr2 activation during CS ARCH inhibition during US	Disrupts fear memory retention Enhances fear memory retention Enhances fear memory retention
	Somatostatin (Wolff et al., 2014)	Chr2 activation during CS	Disrupts fear learning
	GABAergic PKC δ neurons (Haubensak et al., 2010)	silencing PKC δ neurons by eliminating glutamate sensitivity during fear conditioning training and testing	Enhances freezing in high titer virus recipients
CeL	CRF GABA α 1 (Gafford et al., 2012)	Transgenic disruption	Disrupts fear extinction
	CRF NR1 (Gafford et al., 2014)	Transgenic disruption Virus mediated disruption in CeA	Disrupts fear acquisition/retention Disrupts fear acquisition/retention
	CRF (Pitts 2009, 2011)	Pretraining infusion of antisense in CeA CRF antisense 5m, 9 hours and 24 hours after fear conditioning	Disrupts fear memory retention Disrupts fear memory retention
	Somatostatin (Li et al., 2013)	DREADD impairment Chr2 activation naïve mice ARCH inhibition during fear expression	Impairs fear memory Induces freezing Suppresses conditioned freezing
	Tac2 (Andero et al., 2014)	Lentiviral mediated Tac2 overexpression	Enhances fear memory consolidation