The Physiology of Fear: Reconceptualizing the Role of the Central Amygdala in Fear Learning

The historically understood role of the central amygdala (CeA) in fear learning is to serve as a passive output station for processing and plasticity that occurs elsewhere in the brain. However, recent research has suggested that the CeA may play a more dynamic role in fear learning. In particular, there is growing evidence that the CeA is a site of plasticity and memory formation, and that its activity is subject to tight regulation. The following review examines the evidence for these three main roles of the CeA as they relate to fear learning. The classical role of the CeA as a routing station to fear effector brain structures like the periaqueductal gray, the lateral hypothalamus, and paraventricular nucleus of the hypothalamus will be briefly reviewed, but specific emphasis is placed on recent literature suggesting that the CeA 1) has an important role in the plasticity underlying fear learning, 2) is involved in regulation of other amygdala subnuclei, and 3) is itself regulated by intra- and extra-amygdalar input. Finally, we discuss the parallels of human and mouse CeA involvement in fear disorders and fear conditioning, respectively.

Fear can be defined as the neurophysiological processes that prepare an organism to perform innate or learned responses to cope with danger. In general, our understanding of the physiology of fear is based on models of fear learning including fear conditioning, extinction, and fear-potentiated startle. Fear conditioning involves the repeated pairing of a neutral conditioned stimulus (CS; e.g., an acoustic tone) with a noxious unconditioned stimulus (US; e.g., an electric foot shock) such that later presentation of the CS alone results in fear behaviors [conditioned response (CR); e.g., freezing]. Fear extinction is the process of repeatedly presenting the CS alone until it no longer elicits fear behaviors. Similarly, the fear-potentiated startle paradigm involves conditioning an animal to fear a light and then measuring its startle reflex in response to a loud noise in the presence or absence of the conditioning light (fear-conditioned animals will startle more than light-naive animals). Through the use of these behavioral methods, a conceptualization of the brain circuitry underlying fear learning and expression has emerged, and a preponderance of evidence suggests a pivotal role for the amygdala (15, 27, 29, 66a, 86, 87, 102).

Although often treated as a single entity, it is important to note that the amygdala is actually a collection of distinct nuclei (often subdivided further into subnuclei; FIGURE 1) that are thought to play separate but complementary roles in the acquisition, expression, and extinction of fear. Of these nuclei, the lateral/basolateral nucleus, the putative site of plasticity underlying the learned association between the CS and US (34), and the medial nucleus, which is thought to play a modulatory role in the process of predator odor-induced fear learning (146), have been the subject of extensive research and multiple reviews. However, the central amygdala (CeA), the putative output station of the fear circuit, has only recently become the focus of extensive research and currently lacks a focused and recent review on its role in fear processing (129). A review of the function of the CeA is especially timely, given emerging literature that has greatly expanded our understanding of its role in fear learning and expression. Classically, the CeA was considered to be a single homogenous structure that served as a passive relay station of fear output to fear effector brain sites, such as the lateral/paraventricular hypothalamus and the periaqueductal gray (78). However, recent research has suggested that the CeA’s structure and function are not so simple. Emerging evidence indicates that its medial and lateral subdivisions are not only anatomically distinct but also functionally separable in their contributions to fear output. Furthermore,
there is evidence that plasticity occurs within the CeA and that these small-scale functional changes contribute to the acquisition, expression, and extinction of conditioned fear. Therefore, in the following review, we will discuss the evidence for the CeA’s involvement in each of these functions, the implications of these findings for the current model of fear processing, and necessary future experiments that should further refine our understanding of the CeA’s role in the fear circuit.

Before beginning our functional discussion, we will briefly outline the major divisions of the amygdala and the connectivity of the CeA, although we note that an exhaustive anatomical discussion is beyond the scope of this review (please see Refs. 31, 80).

Divisions of the Amygdala

The amygdala is a highly conserved set of brain nuclei present in a wide range of vertebrates including mice and humans (98). It is functionally divided into four sections: the basolateral complex (BLA), the intercalated cells (ITC), the central nucleus, and the medial nucleus (MeA) (87) (FIGURE 1). The BLA is further divided into the basolateral (BL), basomedial (BM) [collectively known as the basal nucleus (BA)], and lateral (LA) nuclei (87).

Overview of the Anatomy, Histology, and Connectivity of the CeA

Anatomy and Histology

The CeA is located medial to the BLA and is bounded laterally and ventrally by the longitudinal association bundle and medially by the stria terminalis (76). It is subdivided into lateral (CEl), medial (CEm), capsular (CEc), and intermediate (CEi) divisions (124). The entire CEl, as well as the posterior part of the CEm, contains many GABAergic neurons, and while the GABAergic cells of the CEl are medium spiny neurons that show considerable dendritic branching, GABAergic CEm cells have minimally branched dendrites and few spines (19, 91, 141).

Connectivity

The CeA receives input from a variety of amygdalar (102) and extra-amygdalar (90) sites (FIGURE 2A). Within the amygdala, not only do both CEl and CEm cells receive inhibitory GABAergic input from ITC cells (103, 123), but CEl cells also inhibit CEm cells (33, 53). Additionally, LA cells project directly to the CEl, and BL and BM cells project to the CEm (76, 104), possibly bypassing the ITC cells. Extra-amygdalar sources of input to the CeA include the dysgranular insula (DI) (135), which projects to the CEl, the agranular insula (AI) (135), which projects to the CEl and CEm, and the infralimbic cortex (IL) (152) and bed nucleus of the stria terminalis (BNST) (14), both of which project to the CEm. The CEl also receives input from the auditory cortex (90), the auditory thalamus (79), and the pontine parabrachial nucleus (9, 62).

The CeA sends output to areas throughout the brain that are involved in mediating a wide variety of autonomic functions (28) (FIGURE 2B). The CeA has known projections to the lateral hypothalamus (LH) (75), paraventricular nucleus of the hypothalamus (PVN) (47), dorsomedial hypothalamus (DMH) (97), medial preoptic area (mPOA) (97), and periaqueductual gray (PAG) (48). These areas are involved in the cardiovascular effects, corticosteroid release, and freezing observed during fear conditioning and extinction (28). In addition to the direct projections to these areas, there also may be an indirect projection from the CeA to the PVN via the BNST that similarly mediates the neuroendocrine response to stress (21, 28, 142).

The Role and Pathways of the CeA as an Output Station of the Fear Circuit

The CeA as the “Output Station” of the Fear Circuit

Early clues to a potential role for the CeA in fear learning came from electrical stimulation and lesioning studies showing that the CeA plays a role in generating stress responses (8, 61, 118, 148), such as those observed during open-field testing (158) and heart-rate conditioning (41-43, 65). However, the idea that the CeA is an output station for the
fear circuit was based on work by LeDoux et al., who tested the effects electrolytic and ibotenic lesions of the bed nucleus of the stria terminalis (BNST), lateral hypothalamus, and periaqueductal gray—all of which receive projections from the CeA (59, 75, 76)—on fear conditioning. Their findings suggest that lesions of the LH interfere with the autonomic responses elicited by fear conditioning (e.g., changes in blood pressure) but do not affect the behavioral response (i.e., freezing). Conversely, lesions of the PAG do not affect the autonomic responses, but do interfere with the behavioral response. Interestingly, lesions of the BNST do not affect either the autonomic or the behavioral responses (78). Later, direct lesions of the CeA in models of conditioned fear (i.e., fear conditioning and fear-potentiated startle) were shown to induce similar behavioral and autonomic deficits (17, 25, 55, 56, 94, 121, 131). Taken together, these results lead to the notion that the CeA is a critical site for the expression of fear and that it mediates these effects through its projections to downstream areas.

However, the common issue among many of these studies was that they treated the CeA as a single, homogenous entity, even though it is known that the CeA’s medial and lateral nuclei are histologically, anatomically, and functionally distinct. While many of the details of these differences are beyond the scope of this review (please see Refs. 18, 143), a few major themes relating to fear conditioning merit mention. In particular, based on tracing studies, the CeA’s medial division is thought to be the major source of the CeA’s output projections, whereas its lateral division is thought to be composed primarily of local inhibitory circuits. The latter observation indicates that, rather than being a simple output station for the fear processing that occurs in upstream areas, the CeA may play an active role in regulating fear output.

Recent research has focused on parsing out the roles of the CEm and CEl in fear conditioning. Using optogenetics, Ciocchi et al. (26) showed that direct activation of CEm neurons leads to expression of fear behavior (freezing) and that, while injection of the GABA<sub>A</sub> receptor agonist muscimol into either the CEm alone or the entire CeA does
not change freezing behavior, injection into the CEl alone results in unconditioned freezing. Additionally, inactivation of the CEl and CeA, but not the CEm, during fear conditioning results in fear-expression deficits. Finally, inactivation of the CEm and CeA, but not the CEl, 24 h after training results in impaired fear expression. Taken together, these findings suggest that the CEl plays a role in fear learning, whereas the CEm is more involved in fear output (26). However, even this model is likely incomplete. Past neural tracing studies have shown that the CEl also sends direct projections to fear effector brain regions (20, 49, 109, 150), and Penzo et al. (106) recently used slice electrophysiology to show that these CEl projections [predominantly from somatostatin-expressing (SOM⁺) neurons] to the PAG and the paraventricular nucleus of the thalamus (PVT) show increases in the frequencies and amplitudes of their miniature excitatory postsynaptic potentials after fear conditioning. These findings establish the potential that CEl neurons are also involved in fear expression independently of CEm output (106). Thus a confluence of evidence suggests that the CeA is not a homogenous structure that simply relays information to other areas of the brain that generate fear behaviors but rather is a complex structure with multiple interconnected functional divisions that is capable of information processing. Future studies should attempt to characterize the ways in which the various CeA cell populations are distinct from each other on genetic and epigenetic levels, and the ways in which these different populations contribute to the various aspects of fear expression. Ultimately, a clear understanding of how various physiological and pathological functions map onto genetically defined cell populations could allow for more targeted interventions for treating disorders of dysregulated fear expression.

Output Pathways of the CeA and Their Roles in Fear Learning

Not only have recent studies begun to parse out the roles of the various CeA sub-nuclei, but they have also started to identify the relative contributions of the CeA’s output pathways to fear expression. It is well known that the efferents of the CeA are extensive and reach widespread brain stem structures that are essential for mediating fear expression (76, 114). As a whole, the central threat response system can be considered to have four main output targets: the periaqueductal gray in the midbrain, the lateral hypothalamus and paraventricular nucleus of the hypothalamus in the forebrain, and the rostral ventrolateral medulla (RVLM) and nucleus tractus solitarius (NTS) in the brain stem (57). The following section discusses output projections from the CeA to these regions and their roles in fear conditioning.

CeA-Periaqueductal Gray

Several groups have provided evidence that the CeA is a source of efferent projections to the PAG, the structure responsible for generating the most commonly used measure of conditioned fear: behavioral freezing (63, 69). Consistent with these prior results, Johansen et al. reported that pharmacological inactivation of the PAG before fear conditioning reduced the expression of conditioned fear and unconditioned reflex responses (63). Furthermore, these authors hypothesized that if the PAG is only involved in fear expression, then PAG inactivation should impair fear expression but not fear learning. Contrary to this idea, they found that fear acquisition was impaired by pretraining inactivation of the PAG. Therefore, these and more recent data (69) suggest that the PAG plays multiple roles during fear conditioning, contributing to both the expression and the learning of fear via reciprocal connections with the amygdala. Finally, in considering the subdivisions of the CeA, it is notable that both the CEl and CEm appear to have connections to the PAG; however, the relative contributions of CEl-PAG and CEm-PAG projections remain poorly understood.

CeA-Hypothalamus: Lateral and Paraventricular Areas

Efferent projections from the CeA to the lateral hypothalamus and brain stem are the primary neural pathways that mediate the autonomic and somatic motor components of the conditioned fear response (44, 54, 74, 78, 157). More specifically, the CEm and CEc predominantly innervate the dorso-lateral and caudolateral regions of the hypothalamus (78, 108, 144). In addition to these direct projections to the LH, the CeA has a strong projection to the BNST, which also innervates hypothalamic nuclei and plays a major role in fear conditioning (156). Although these CeA-hypothalamic circuits have been well mapped out, recent studies indicate the existence and importance of independent hypothalamic circuits involved in the expression of different types of fear (i.e., social vs. predator) (137). In addition, it has been proposed that a bias toward active or passive fear responses is mediated by a neuronal switch in the CeA (46), which could therefore lead to differential regulation of CeA-hypothalamic-brain stem interactions involved in fear expression. Viviani et al. demonstrated that the neurohormone oxytocin selectively gates output from different parts of the amygdala to downstream hypothalamic and brain stem sites that result in the differential modulation of
autonomic parameters such as blood pressure and heart rate (154).

As mentioned earlier, excitatory interactions between the CeA and downstream structures can be facilitated via indirect projections and the relay of information via brain stem pathways. The interaction between the CeA and paraventricular nucleus of the hypothalamus is thought to function in such a way (160). Although the PVN is best known for its role in the stress response and hypothalamic-pituitary-adrenal axis activation via glucocorticoid secretion, its role in the fear response remains poorly understood. Recent evidence, however, identifies the PVN as an important modulator of fear expression and extinction (83, 84, 152, 153), which may involve indirect connection between the CeA and PVN via brain stem structures.

**CeA-Brain Stem: Nucleus Tractus Solitarius and Ventrolateral Medulla**

CeA lesioning (70) and stimulation (40) studies have shown that disrupting the fear output pathway at the level of the CeA impairs the cardiovascular and motor (freezing) responses to fear conditioning, suggesting that the CeA is an important link between upstream centers for fear processing and downstream brain stem sites involved in regulating autonomic activity. Tracing studies have established that CeA neurons project to brain regions that modulate sympathetic tone, including the nucleus tractus solitarius and rostral ventrolateral medulla (23, 127). In particular, the NTS has been shown to play a key role in modulating cardiorespiratory responses and has been implicated in cardiovascular disorders such as hypertension (6, 120). The NTS receives both direct (126, 128) and indirect input from the CeA. Afferent fibers from arterial baroreceptors and chemoreceptors also terminate in the NTS, and there is evidence to suggest that GABAergic CeA-NTS projections attenuate the baroreceptor reflex control of blood pressure (125). Future studies should add cell-type information to the anatomical and functional maps of the CeA projections using viral tract tracing and optogenetic projection targeting methods, respectively.

**The Expanding Role of the CeA in Fear Learning**

**The Background of the CeA as a Site of Plasticity with an Expanding Role in Fear Learning**

Recent research has suggested that the CeA may also be directly involved in fear learning. Early evidence suggested that the CeA exhibits synaptic plasticity in response to fear conditioning, conditioned taste aversion, appetitive taste aversion, and exposure to painful stimuli (129). Such studies showed that changes in neural activity in response to the CS occur with fear conditioning (7, 105), that CREB expression increases following fear-expression testing (51), and that infusions of NMDA receptor antagonists into the CeA block fear learning (45, 161), all of which suggest that the CeA undergoes persistent functional changes in response to fear conditioning. Further results suggested that the CeA is capable of processing fear-related information in its own right and does not rely solely on input from the BLA to generate fear responses (68).

Additionally, this work established the possibility that plasticity can occur in the CeA as well as in the BLA. These assertions began to seem even more plausible when thalamic inputs were shown to drive LTP in the CEm, even in the absence of connections between the BLA and CEm, indicating that CeA input is not limited to projections from the BLA but can also come from other subcortical structures (130).

These studies, which challenged the assumption that the CeA was a passive relay station, raised several questions. To what extent was the CeA involved in the acquisition, consolidation, expression, and extinction of fear memories? Was the plasticity of the CeA reflective of its ability to form CS-US associations? Furthermore, how did the CEm and CEl differ in their plastic properties and roles? A series of drug and optogenetic studies would shed further light on the circuit-level implications of the CeA’s potential for plasticity.

**Recent Literature on the Expanding Role of the CeA in Fear Learning**

Wilensky et al. (159) used inactivation with the GABA<sub>A</sub> receptor agonist muscimol and the protein synthesis inhibitor anisomycin to determine the extent to which the CeA plasticity is involved in fear learning and consolidation. They observed that inactivating the CeA using muscimol impairs both fear learning and expression, and blocking protein synthesis in the CeA impairs fear memory consolidation. Additional studies used muscimol inactivation to show that CeA activity is necessary for the acquisition and expression of over-trained fear (88, 117, 162). Together, these results provided evidence that the CeA is involved in multiple aspects of fear learning and consolidation and that it may even be capable of forming CS-US associations (32).

The CEm is thought to be the origin of most of the output projections to the brain stem and hypothalamus that are involved in generating fear expression behaviors (59, 78, 133, 150). The CEl, on the other hand, while having some long-distance projections to such effector sites also has GABAergic cells that inhibit the CEm (18, 92, 109, 143). Fur-
thermore, the CEI and CEc also receive input from extra-amygdalar brain regions, including sensory input from the auditory cortex and thalamus (90, 147) and nociceptive input from the pontine parabrachial nucleus (9, 62, 151). This led to the suggestion that the CEI is the site of plasticity and that the CEm acts as the output station (129), although this model does not take into account the host of projections to the CEm, such as those from the auditory thalamus (85, 147), or the evidence that LTP can be induced in the CEm following high-frequency stimulation (130). Clearly, both sub-nuclei receive input from extra-amygdalar structures and are capable of plasticity. At least two schema have been proposed for how these regions contribute to fear learning (33).

The first is that the connections between the sensory regions of the thalamus (e.g., the medial geniculate nucleus) and the CEm are excitatory and plastic, and that these synapses are strengthened during fear acquisition. This proposal is in line with the evidence showing projections from the medial geniculate to the CEm (85, 147) and evidence of LTP in the CEm as generated by high-frequency stimulation of thalamic neurons (130). However, the projections from the medial geniculate, in particular to the CEm, have not been tested for potential plasticity, and these experiments have not been conducted in vivo.

The second possibility is that the inhibitory control of the CEI over the CEm is plastic, and that tuning the level of inhibition of the CEm can cause corresponding shifts in the activities of the neurons projecting to the brain stem and hypothalamus. Support for this mechanism comes from the observation that the CEI receives many intra- and extra-amygdalar inputs, which could tune the activities of its inhibitory projections onto the CEm (60). Recent studies have tested the dynamics of the CEI-CEm inhibitory hypothesis. For example, Ciocchi et al. (26) were able to dissociate the functional roles of the CEI and CEm in fear learning and expression. First, they showed that optogenetic stimulation of the CEm results in unconditioned freezing. Conversely, inactivation of the entire CeA or just the CEm with muscimol does not induce freezing, but inactivation of the CEI alone does, presumably via disinhibition of the CEm. These results suggest that the CEm is independently capable of inducing unconditioned freezing and that it is normally inhibited by the CEI.

Additionally, inactivation of either the entire CeA or just the CEI, but not the CEm, during fear conditioning prevents animals from acquiring learned fear. Conversely, inactivation of either the entire CeA or just the CEm, but not the CEI, impairs fear expression. Thus the CEI may contribute to fear learning, whereas the CEm may contribute to fear expression. Using electrophysiological approaches, it was found that CEI neurons’ activity patterns in response to presentations of the CS fall into three categories: those showing increased activity (CEIon), decreased activity (CEIoff), or no change in activity. Notably, both CEIon and CEIoff neurons are able to cause inhibition of CEm neurons. Furthermore, following fear conditioning, CEm output neurons projecting to brain stem targets show a biphasic response to the CS, with the first component having a short-latency onset and brief duration (similar to the observed responses of CEIon neurons), and the second component having a delayed onset but prolonged duration (similar to the responses of CEIoff neurons). Therefore, it was proposed that an initial direct input from the thalamus excites the CEm, which is then quickly and transiently inhibited by the CEIon neurons, and then the CEIoff neurons disinhibit the CEm (by removing the CEI’s tonic inhibition on the CEm neurons), allowing for the delayed but protracted component of the response (26).

Further work revealed that most of the GABAergic CEIoff neurons express PKC-δ (PKC-δ+), and beyond their role of disinhibiting the CEm for fear expression they also make reciprocal inhibitory connections onto PKC-δ− neurons. Through the employment of optogenetic, transgenic, and neural tracing tools, these PKC-δ+ CEI neurons were shown to synapse onto CEm output neurons that project to the PAG, which is known for its role in generating freezing behavior (53). In follow-up work, it was found that fear conditioning results in an increase in the proportion of CEIoff cells, but not CEIon cells, and that extinction training results in a decrease in the proportion of CEIoff cells (32). Clearly, shifts in the activities of these CeA microcircuits are important for many aspects of fear learning and expression, yet there is only preliminary evidence on how these shifts occur.

The broader implications of these observed changes in CeA activity with fear conditioning are that activity-dependent synaptic plasticity may occur in the CEI and its afferents during fear learning, and that this plasticity is contributing to the storage of fear-related information in a manner that is at least partially independent of BLA activity. Furthermore, at least two in vitro studies (one focused on the parabrachial nucleus-to-CEI pathway and one focused on the BLA/LA-to-CEI pathway) provide evidence for LTP in these connections (37). Of these two pathways, the projections from the BLA to the CEI have been studied more extensively. Li et al. (81) studied another subset of CEI neurons, those that express the neuropeptide somatostatin (SOM−), which are largely distinct from the PKC-δ+ population. They used transgenic techniques to fluorescently label these SOM− neurons.
in mice and then used slice physiology to simultaneously record from SOM⁺ and SOM⁻ neurons while stimulating the LA (using electrical and optogenetic stimulation in independent experiments). Their results suggest that not only do shifts in the excitability of SOM⁺ and SOM⁻ neurons occur during fear conditioning but that these shifts are 1) mediated by activity-dependent strengthening of the synapses from LA cells onto the SOM⁺ cells of the CEm and 2) necessary for fear acquisition. To better characterize the connectivity of these SOM⁺ neurons, they showed that <15% of the projection neurons from the CEl to the CEm are SOM⁺. Furthermore, stimulation of these SOM⁺ CEl neurons resulted in robust IPSCs in all recorded CEm neurons but only in a few of the recorded CEm neurons and in none of the PAG-projecting SOM⁺ neurons. Finally, in an in vivo model, they showed that optogenetic stimulation of SOM⁺ CEl neurons in untrained animals results in unconditioned freezing. Furthermore, optogenetic inhibition of SOM⁺ CEl neurons during fear-expression testing abolishes conditioned freezing, indicating that the activity of these neurons is necessary for fear expression (81). Taken together, these results suggest that SOM⁺ CEl neurons inhibit the SOM⁺ CEm neurons (a majority of which are PKC-δ⁺) that are normally responsible for tonic inhibition of the CEm and suppression of fear expression.

However, not all of the SOM⁺ CEl neurons project locally. There is evidence that some of these neurons project to the PAG and the paraventricular nucleus of the thalamus (PVT), both of which are involved in generating fear behaviors. Exploring these projections further with slice preparations, Penzo et al. (106) showed that optogenetic activation of these projections results in IPSCs in the neurons of the PAG but not the PVT. Furthermore, when animals underwent fear conditioning, there was a notable increase in the frequency and amplitude of EPSCs from the SOM⁺ neurons projecting to either the PAG or the PVT (106). However, the role that these projections play in fear learning has yet to be elucidated.

Very few studies have investigated the molecules that mediate this balance of excitation and inhibition in CeA microcircuits. Pitts et al. showed that corticotropin-releasing factor (CRF) in the CeA is an important mediator for contextual fear conditioning (112, 113). Similarly, Kamprath et al. showed that cannabinoid signaling in the CeA is essential for short-term plasticity and adaptation with fear conditioning (64). For fear memory consolidation, Andero et al. showed the importance of the Tac2 gene and its product neurokinin B within the CeA (5).

The Roles of Cortical and Subcortical Inputs to the CeA in Fear Learning

To convincingly argue that the CeA is capable of processing fear-related information independently of BLA input, it must first be shown that the CeA receives input from extra-amygdalar areas. Given that the CeA is both a source of fear output and a site of plasticity for fear learning, it is not surprising that it receives input from numerous cortical and subcortical structures (104, 138). While many of these projections have not been studied extensively, there have been several recent attempts to characterize the inputs to the CeA from two primary areas of the medial prefrontal cortex: the infralimbic and the prelimbic cortices (via the intercalated cells and paraventricular thalamus, respectively).

Infralimbic Cortex-ITC-CEm

The medial prefrontal cortex (mPFC), with its diffuse pattern of connectivity (50), has long been implicated in controlling fear responses (1, 96). In particular, it has been studied extensively in the framework of fear extinction, in which case it is thought to serve an inhibitory role by decreasing the activities of amygdala circuits involved in fear memory recall (115, 119). Within this context, Quirk et al. showed that lesions of the infralimbic (IL) portion of the mPFC resulted in a loss of memory of extinction (116). Furthermore, Milad and Quirk showed that response patterns of the IL were increased with tone presentations after extinction (95). Clearly, IL activity is involved in fear extinction, but the systems-level mechanisms by which the IL exerts these effects remain unclear. However, growing evidence suggests a role for IL control of the CeA in this process.

mPFC stimulation was found to robustly reduce the responsiveness of CEm neurons, indicating a functional link between the two areas (116). However, the direction of this effect was unexpected; given that most mPFC projections are excitatory, it was surprising to observe a decrease in the activity of one of its afferent targets in response to mPFC stimulation. This observation led to the hypothesis that mPFC-mediated inhibition of the CEm occurs via activation of intermediate inhibitory neurons. Currently, these neurons are thought to be the intercalated (ITC) cells of the amygdala (12), which project onto and inhibit the CEm output neurons (2, 103, 123), with increases in IL c-Fos expression correlated with increases in ITC c-Fos (12, 72). Furthermore, ITC cells respond to IL stimulation (3), and IL projections predominantly innervate medial ITC cells (although there are also direct projections from IL to lateral CeC) (111). Future
work should aim to genetically and functionally (i.e., in the context of fear learning) characterize the projections from the IL to the ITC and from the ITC to the CEm.

**Prelimbic Cortex-Paraventricular Thalamus-CEl**

Previous work has suggested that the PL is involved in fear memory consolidation (24) and expression (16), but it remains unclear by what pathways the PL mediates these effects. Given that the PL projects densely to the dorsal midline thalamus (dMT) (83) and that the dMT is necessary for fear expression 24 h after conditioning (100), it was hypothesized that the PL-dMT projection may contribute to the PL’s role in fear expression. The observation, based on c-Fos time course studies, that the PVT (a subregion of the dMT) only came online >24 h after fear conditioning suggested that its involvement in fear expression depended on plastic changes associated with memory consolidation. Additionally, PVT neurons respond more robustly to the conditioned stimulus 24 h after conditioning than at earlier time points. Furthermore, retrograde tracing and c-Fos labeling techniques showed that the PVT neurons active during fear expression were the ones that projected to the CeA.

Optogenetic inhibition of the cell bodies of PL neurons resulted in a decrease in the firing rates of some PL cells and an increase in the firing rates of others. Similarly, using the same technique to inhibit PVT-projecting PL terminals resulted in a decrease in the firing rates of some PVT cells and an increase in the firing rates of others. Furthermore, inhibiting PL projections to the PVT or PVT projections to the CeA only affected expression 7 days after conditioning. Taken together, these results suggest that the circuits recruited for fear expression change as a function of time and that projections from the PVT to the CeA are involved in the expression of distant, but not recent, fear memories. Although they generate interest in the PL-ITC to the CEm.

Next, given their finding that PVT neurons innervate SOM$^+$ cells twice as heavily as SOM$^-$ ones, they used optogenetic stimulation to explore the functional differences between PVT projections onto SOM$^+$ and SOM$^-$ CeA neurons. Surprisingly, brief optogenetic stimulation did not evoke fast synaptic responses in SOM$^+$ or SOM$^-$ cells, but high-frequency stimulation evoked slow inward currents in SOM$^+$ cells and increased the frequency of spontaneous inhibitory postsynaptic currents in SOM$^-$ cells. The observation that stimulation of PVT-CEl projections evoked slow synaptic currents in SOM$^+$ CeA cells indicated that transmission at these synapses was mediated by a neuromodulator. They then demonstrated that brain-derived neurotrophic factor (BDNF) is enriched in CeA-projecting PVT neurons and that the BDNF receptor TrkB is expressed by SOM$^+$ CeA neurons. Deleting the TrkB receptor from SOM$^+$ CeA cells abolished the PVT-driven slow inward currents, and bath-applying a BDNF scavenger abolished the PVT-driven increase in inhibition of SOM$^-$ CeA cells. Finally, using in vivo transgenic and viral techniques, they showed that both BDNF knockout from PVT neurons and TrkB receptor knockout from SOM$^+$ CeA neurons resulted in impaired fear conditioning. Furthermore, knockout of the TrkB receptor from SOM$^+$ CeA cells attenuated the presynaptic potentiation previously seen with fear conditioning, and injection of BDNF into the CeA facilitated fear conditioning (107). Together, these findings suggest that the plasticity of the SOM$^+$ neurons in the CeA caused by fear conditioning can be attributed to input from the PVT-CEl circuit and that BDNF released by projections from the PVT is necessary for this plasticity.

**The Role of CeA Neuropeptides in Fear Learning**

The CeA, via direct and indirect projections, can regulate the activity of the hypothalamic PVN and influence the synthesis and secretion of key neuropeptides involved in the physiological response to fear, such as corticotropin-releasing factor (CRF), adrenocorticotropic hormone, arginine vasopressin, and oxytocin (140). The following discussion will highlight the more extensively studied neuropeptides CRF, estrogen, vasopressin, and angiotensin II, all of which have been implicated in
fear learning and fear-related behavioral disorders such as PTSD.

CRF is distributed throughout the brain in regions such as the CeA, the PVN, and portions of the extended amygdala including the BNST (13, 132, 145). The amygdala has been shown to be a major extra-hypothalamic source of CRF-containing neurons, with high expression levels of CRF receptors (101, 149), and stress has been shown to increase CRF mRNA expression in the CeA (134). Overexpression of CRF in the CeA has profound effects on hypothalamic-pituitary-adrenal axis regulation, with associated behavioral, physiological, and reproductive consequences, all of which are involved in the development of fear disorders (66). Additionally, plasticity in CRF-expressing neurons has been implicated in fear conditioning and extinction. Mice with a GABA<sub>A</sub> receptor deletion confined to CRF-expressing neurons have increased baseline anxiety and impaired extinction of conditioned fear (39). A more recent study suggests that glutamatergic modulation of CRF neurons also contributes to changes in fear behavior (38). Although the CRF-expressing neurons of the CeA have been repeatedly implicated in fear learning and expression, the molecular mechanisms remain unclear. Future studies should investigate how changes in the excitability of CRF-expressing CeA neurons influences CRF regulation and should characterize the functional heterogeneity of the CRF-expressing neuron populations.

In addition to CRF, other neuropeptides also act on CeA cells to modulate the acquisition and expression of fear. Hormones like oxytocin and vasopressin have recently been shown to stimulate distinct neuronal populations in the CeA that regulate autonomic fear responses (60). In particular, work has shown that oxytocin excites CEI neurons that in turn inhibit the CEm, whereas vasopressin appears to stimulate the CEm directly (155). Following up on these findings, Knobloch et al. (73) used optogenetic stimulation of CEI-projecting oxytocinergic hypothalamic axons to show that endogenous oxytocin release causes a reduction in conditioned fear expression. Future work likely will continue to parse out the contributions of CeA neuropeptides to fear learning and expression. For example, the octapeptide angiotensin II, most notably known for its role in blood pressure regulation, and its receptors AT<sub>1</sub>, AT<sub>2</sub>, and AT<sub>4</sub> are expressed in the amygdala and other brain regions. Recently, this peptide has been shown to play a role in fear expression and extinction retention, and it is possible that these effects are mediated by its action on CeA cells (67, 89).

The CeA in Humans and Mice

Given the importance of the CeA in fear learning in rodents, it is possible that the CeA plays a role in human fear disorders as well. However, while many studies have used functional magnetic resonance imaging to examine the human amygdala (30, 58, 110), including its functional connectivity (122), responses to fearful faces (52), and even fear conditioning and extinction (77), none has yet been able to dissociate the responses of amygdala sub-nuclei, likely because existing non-invasive imaging techniques do not have adequate spatial resolution to resolve such small structures (136).

Instead, a growing number of studies have used single-nucleotide polymorphism analyses to associate genetic variants with differences in susceptibility to developing fear and anxiety disorders. For example, the Oprl1 gene and the nociceptin/orphan FQ receptor (NOP-1) have been implicated in both mouse fear conditioning and human PTSD. In particular, injection of a NOP-1 agonist into the CeA impairs consolidation of conditioned fear memory, and a single-nucleotide polymorphism within the Oprl1 gene is associated with increased severity of PTSD symptoms after a traumatic event, physiological changes in startle responses, and changes in the functional connectivity between the amygdala and insula (4). Additionally, a SNP in the promoter region of the serotonin transporter gene has been shown to convey enhanced sensitivity to fearful stimuli (52). Future studies using epigenomic and transcriptomic profiling of healthy and diseased postmortem human amygdala tissue will facilitate a better understanding of normal and pathological fear, and may inspire new avenues for treatment of fear disorders.

Conclusions and Future Directions

The CeA has had an evolving history with respect to fear learning. While initially believed to be primarily a final common output pathway of the fear circuit to the behavioral and autonomic effector regions of the brain, it is becoming increasingly clear that the CeA is involved in the acquisition, expression, and even consolidation of conditioned fear. Current evidence suggests that the role of the output station can be subscribed to the CEm, whereas the site of plasticity is the CEI. Specifically, it appears that the CEI is able to gate the activity of the CEm, which results in the expression of behavioral and autonomic responses. Furthermore, the CEI receives input from many extra-amygdalar sources, including the mPFC, pPVT, auditory thalamus and cortex, and several brainstem areas. Additionally, it appears that several of these projections release neuromodulators onto CeA...
cells, such as glucocorticoids, estrogen, CRF, and oxytocin. Together, these findings lead to the working hypothesis that local inhibitory circuits in the CeI gate fear-related information as it passes from the BLA to the CeM and that cortical and subcortical inputs directly onto CeI cells tune the activities of these local inhibitory circuits using neuromodulators. Beyond studies in rodents, recent work has suggested that the human CeA plays an important role in PTSD.

However, several unanswered questions remain. Over the past several years, our structural maps of the inputs, internal connectivity, and outputs of the CeA have grown increasingly sophisticated. Additionally, in some cases, we have been able to overlay basic functional information (i.e., electrophysiological characteristics, such as whether projections tend to be excitatory or inhibitory) onto this structural map. In even fewer cases, we have even been able to add more detailed functional information, such as which genes are expressed and which neurotransmitters and neuromodulators are released by these cells. However, as FIGURE 2B illustrates, for the majority of anatomically defined circuits, we still have little of this advanced functional information. Therefore, the most urgent priority for future studies attempting to understand the circuit- and cellular-level contributions of the CeA to fear learning is to obtain sophisticated functional data that can be superimposed onto our existing structural framework. Fortunately, an increasing number of genetically encoded fluorescent sensors (22, 35, 36, 139) and actuators (10, 11, 71, 93) (for optical recording and control of neural populations, respectively) are becoming available, and these approaches, in combination with intersectional genetic methods (99), have the power to extract this critical information.

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