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REVIEW

Prefrontal cortical circuits in anxiety and fear: an overview

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Abstract **Pathological anxiety is among the most difficult neuropsychiatric diseases to treat pharmacologically, and it represents a major societal problem. Studies have implicated structural changes within the prefrontal cortex (PFC) and functional changes in the communication of the PFC with distal brain structures in anxiety disorders. Treatments that affect the activity of the PFC, including cognitive therapies and transcranial magnetic stimulation, reverse anxiety- and fear-associated circuit abnormalities through mechanisms that remain largely unclear. While the subjective experience of a rodent cannot be precisely determined, rodent models hold great promise in dissecting well-conserved circuits. Newly developed genetic and viral tools and optogenetic and chemogenetic techniques have revealed the intricacies of neural circuits underlying anxiety and fear by allowing direct examination of hypotheses drawn from existing psychological concepts. This review focuses on studies that have used these circuit-based approaches to gain a more detailed, more comprehensive, and more integrated view on how the PFC governs anxiety and fear and orchestrates adaptive defensive behaviors to hopefully provide a roadmap for the future development of therapies for pathological anxiety.**

Keywords prefrontal cortex; anxiety; fear; neural circuits; optogenetics; DREADD

Introduction

Anxiety represents a state of high arousal, negative valence, and enhanced vigilance in the absence of an immediate threat [1]. By contrast, the related emotional state of fear is induced as a response to a real or perceived imminent threat and dissipates upon removal of the eliciting stimulus [2]. Anxiety and fear elicit defensive behavioral responses that have evolved to enable an organism to avoid or reduce harm and thus ensure its survival. However, in humans, excessive fear and/or chronic anxiety can be debilitating and is considered pathological. Trauma- and stressor-related disorders (e.g., post-traumatic stress disorder, PTSD) and anxiety disorders (e.g., panic disorder, PD) impose a major burden on both affected individuals and the society in general because of their high prevalence [3–5].

The prevalence of anxiety disorders is approximately 18% among adults [6] with a lifetime prevalence of more than 28% [7]; however, a considerable portion of pathologically anxious individuals does not receive adequate treatment [8]. The medications used for anxiety disorders include benzodiazepines, selective serotonin reuptake inhibitors (SSRIs), serotonin–noradrenaline line reuptake inhibitors, pregabalin, tricyclic antidepressants and opipramol, moclobemide, phenelzine, buspirone, and hydroxyzine [9]. However, pharmacological treatments may have side effects and cause dependency. Moreover, some drugs are associated with withdrawal symptoms, abuse, and a risk of metabolic abnormalities [10], which can impede treatment adherence and the maintenance of therapeutic effects in the long run. Psychotherapy, such as cognitive behavioral therapy (CBT), is effective for PTSD and anxiety disorders [11], but issues related to availability, cost, and commitment limit its use. Even worse, a significant portion of patients show little or no response to psychotherapy. In addition, the relapse rate is high among patients who achieve remission [12,13]. Neurostimulation techniques such as repetitive transcranial magnetic stimulation (rTMS) and transcranial direct current stimulation have been studied as promising alternatives to or augmentation treatments for pharmacological and psychological therapies [14,15]. Many of these non-pharmacological therapies affect the cognitive control regions in prefrontal cortex (PFC) circuitry to achieve therapeutic efficacy [16–19]. Nevertheless, preclinical

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approaches have significant limitations, including the lack of regional and cellular specificity. Efforts have been made to identify relevant pathways from PFC with sophisticated and cutting-edge approaches in cell type- and circuit-specific manner to improve our understanding of the cognitive regulation and identify more specific pathways for intervention. This review provides an overview of recent research on local microcircuits and long-range "bottom-up" and "top-down" control of the PFC underlying anxiety and fear.

PFC in pathological anxiety

Based on the results of neuroimaging, lesion analyses, and post-mortem studies, alterations in neuronal activity, synaptic plasticity, and connectivity within the medial PFC (mPFC) are correlated with a high risk for anxiety disorders [20,21]. PFC lesions can impair the ability to inhibit cognitive interference, such as the ability to inhibit inappropriate memories [22]. Subjects with PTSD of various etiologies show reduced ventral medial PFC (vmPFC) volume and activity and increased activity in the amygdala [23–25]. Elevated activation was observed in the mPFC during perception and evaluation of emotional stimuli in patients with PTSD [26], PD [27], and other types of anxiety disorders [28–30]. As opposed to mere processing of emotional cues, which is associated with increased PFC activation in patients with anxiety disorders, the recruitment of the PFC in contexts that require emotion regulation is decreased in patients with anxiety disorders compared with healthy controls, thus increasing the anxiety. A recent meta-analysis of fMRI studies of patients with PTSD and various anxiety disorders involving an emotional reappraisal task concluded that these patients exhibit decreased activation in the bilateral dorsomedial prefrontal cortex (dmPFC), bilateral dorsal anterior cingulate cortex, left vmPFC when trying to regulate their emotions in comparison with that shown by healthy controls [31]. Correspondingly, anxiety disorders have been associated with impaired prefrontal cortex control [32,33]. Since CBT has been shown to increase prefrontal cortex control, patients with reduced prefrontal cortex control may benefit most from CBT [34].

Multiple neurotransmitter systems of the mPFC, such as the glutamatergic, GABAergic, cholinergic, and serotoninergic systems, contribute to the pathophysiology of anxiety disorders. A remarkable decrease in γ-aminobutyric acid (GABA) levels was detected in the anterior cingulate cortex (ACC) and mPFC in patients with PD [35]. In addition, abnormal reductions in $GABA_A$-benzodiazepine receptor binding were found in the dorsal anterolateral PFC via positron emission tomography [36], consistent with the abnormal inhibitory neuromodulation in PD. Treatment with rTMS that induced dorsal lateral PFC or mPFC activation remarkably reduced anxiety and avoidance ratings in patients with anxiety disorders [37,38]. These findings indicate that neurotransmission within the PFC and communication with downstream targets are dysregulated in anxiety disorders. Alterations have been observed in the structure and activity correlations between the mPFC and amygdala in patients with anxiety disorders [39,40], and CBT can normalize these alterations in functional connectivity [41,42]. Notably, prazosin, an α-adrenergic antagonist used for the treatment of PTSD, reduces flashbacks and improves concentration, suggesting that it may improve PFC function [34]. A course of treatment with the SSRI citalopram increases the functional connectivity between the dorsal lateral PFC and ventral lateral PFC during reappraisal of worry in older adults with generalized anxiety disorder [43].

These findings highlight the importance of the homeostatic function of PFC for mental health. Dysfunction of the PFC can negatively affect cognitive function and emotion by altering the local processing of afferent information and the generation of efferent activity necessary for communication with distal structures. These findings indicate the importance of further elucidating PFC network function and structure through preclinical research to allow future advances in targeted neuromodulation.

Measuring anxiety- and fear-related behaviors in rodents

In studying the pathophysiology of anxiety disorders, strategies used to measure anxiety-like behaviors in animals must meet the essential criterion of validity [44,45]. Ideally, an appropriate animal model of human anxiety should fulfil the following criteria as closely as possible: strong phenomenological similarities and pathophysiological similarities (face validity), comparable etiology (construct validity), and common treatment (predictive validity). Despite the inherent challenges of the use of model organisms to study the neural substrates of anxiety disorders, various strategies have been developed for the assessment of externally observable anxiety-related phenotypes in animals [46]. These methods can be grouped into two main subclasses, namely, ethologically based paradigms that assess animals' spontaneous or natural reactions to stress stimuli that do not explicitly involve pain or discomfort and measures of animals' conditioned responses to stressful and often painful events. The commonly used strategies for measuring anxiety-like and fear behaviors in mice are described herein (Fig. 1).

Most anxiety assays adapted for mice are ethologically based. The behavior of animals exposed to a novel situation results from competition between an exploratory tendency (motivated by curiosity or boredom) and a withdrawal tendency (motivated by fear). This behavior can be considered a form of approach-avoidance conflict. In the elevated plus-maze (EPM), a subject (typically a rat or mouse) generally makes few entries into the open

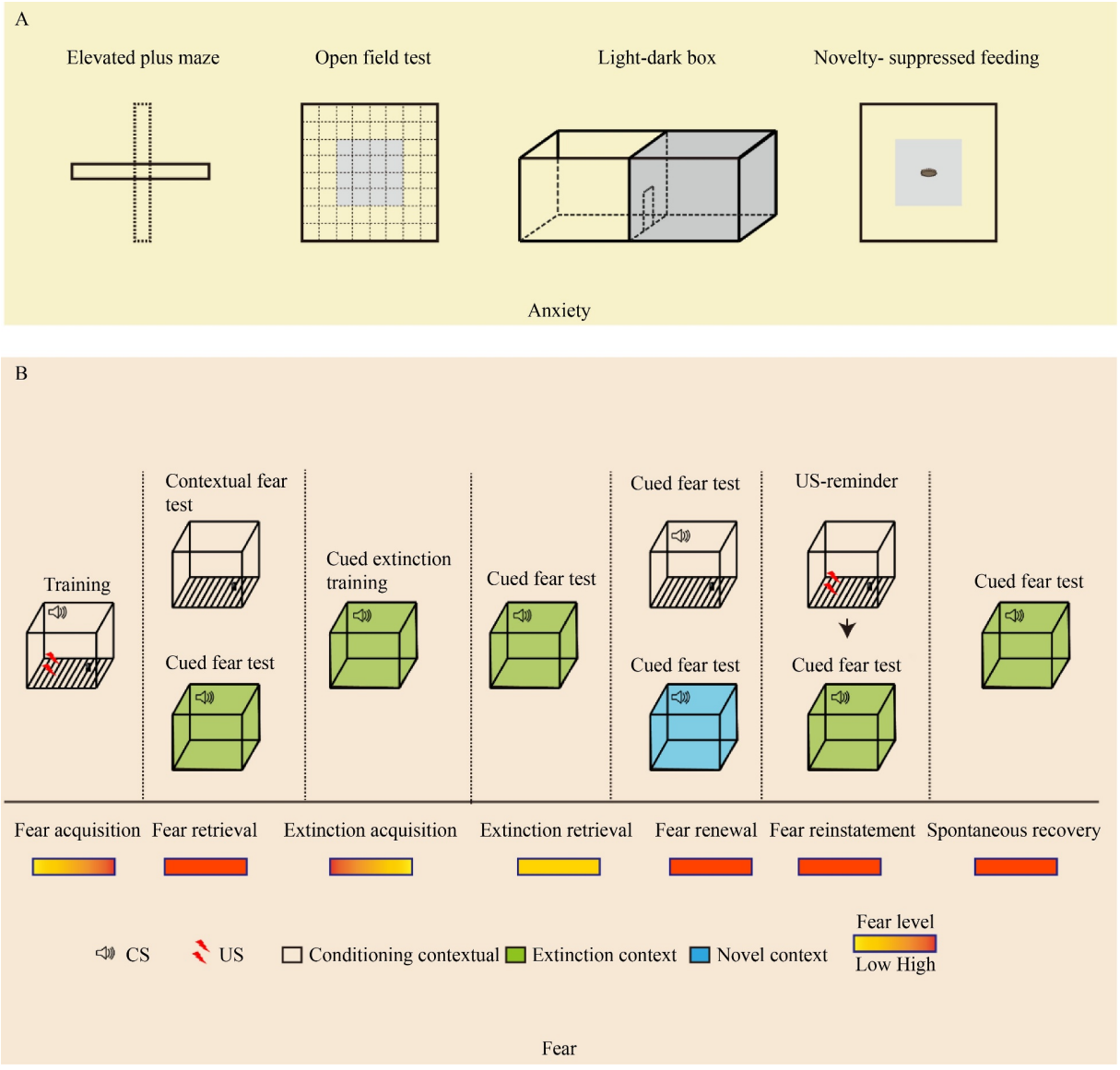

Fig. 1 Validated tests for evaluating anxiety and fear in mice. (A) Anxiety in mice is measured behaviorally through various tests, which are highlighted in yellow. (B) Examples of fear assays are highlighted in orange.

arms of the maze, and anxious mice avoid the open arms even more [47]. In the open-field test (OFT), anxious animals remain along the edges of the enclosure [48], whereas in the light-dark box assay, anxiety-related behavior is measured as the propensity to remain in the dark portion of the chamber [49]. Novelty-suppressed feeding test (NSFT) has also been used to assess anxiety-like behavior in rodents; in a novel environment, hungry animals exhibit an increased latency to feed, and this action is sensitive to benzodiazepines and SSRIs [50,51]. However, these tasks are sensitive to overall changes in locomotion; thus, the use of control tests for motor deficits is necessary when employing these measures to assess anxiety-like behavior. Complementing behavioral assays, physiologic measures of vital signs (e.g., heart rate, blood pressure, and body temperature), and circulating levels of stress hormones provide an additional indicator of anxiety-like phenotypes in mice. Although these types of measures are more commonly used to quantify stress, they can be useful for assessing anxiety in rodents considering the strong somatic components of clinical anxiety (DSM-V). In addition, simulations of a looming object that mimic an approaching aerial predator (looming stimulation test) [52] and exposure to a predator or an aggressive species (predator-based tasks) [53] can initiate defensive behaviors. These paradigms are usually used to assess innate fear response.

The most commonly used procedure for inducing a conditioned response in animals is Pavlovian fear conditioning [54,55]. In this paradigm, an initially innocuous stimulus (the to-be conditioned stimulus (CS) such as a light, tone, or particular context) is paired with an innately

aversive unconditioned stimulus (US; e.g., a foot shock). When presented alone, the CS evokes fear responses, as indicated by increased defensive behavior, stress hormone release, and activation of the sympathetic nervous system. Both short- and long-term fear-learning processes can be investigated using fear conditioning. The fear acquisition phase is typically characterized by a gradual increase in the expression of the CS when multiple CS–US pairings are presented during training. Fear memories are consolidated over time, and their retrieval can be induced and measured by presenting the CS alone in a novel context (cued fear test) or by re-exposing an animal to the conditioning context (contextual fear test). Repeated presentations of the CS alone decrease the conditioned response through a process termed extinction. Extinction learning does not completely erase conditioned fear, because fear memories can show spontaneous recovery over time when the CS is presented in the extinction context. In addition, fear of the CS can be reinstated by exposure to a single US alone or renewed by presentation of the CS in either the conditioning or novel context. Human anxiety disorders result from a dysregulation of normal fear learning mechanisms [56] and abnormal activity patterns in the cerebral networks that normally regulate fear learning [25,57]; accordingly, the fear conditioning paradigm is often used to interrogate anxiety circuits and determine how fear learning may be disrupted in anxiety disorders [58,59].

Tools for assessing PFC circuits

A mechanistic understanding of the neural underpinnings of anxiety requires the ability to manipulate specific circuit components, requiring the use of next-generation technologies in model organisms. Optogenetics is one of the leading technologies [60]. Excitatory or inhibitory effects can be elicited by the expression of different subclasses of microbial genes encoding opsins; for example, many naturally occurring channelrhodopsins (ChRs) are non-specific cation channels that depolarize (excite) neurons in response to blue light, whereas halorhodopsin-type Cl^- and bacteriorhodopsin-type proton pumps induce hyperpolarization (inhibition) in response to yellow or green light, respectively, by pumping Cl^- ions into or protons out of the cell [61]. In chemogenetics, proteins are engineered to interact with previously unrecognized small molecule chemical actuators [62]. Among these various classes of chemogenetically engineered proteins, designer receptors exclusively activated by designer drugs (DREADDs) are most widely used [63]. The DREADD hM3Dq is typically used to enhance neuronal activity, and hM4Di is the most commonly used inhibitory DREADD; both of these DREADDs can be activated by clozapine-N-oxide (CNO) [64]. Notably, clozapine, to which CNO rapidly converts *in vivo*, shows high DREADD affinity and potency, which is likely to be a major contributing factor in the activation of DREADDs after systematic administration of CNO [65].

Optogenetic and chemogenetic toolboxes can be applied with genetic specificity via viral vectors, recombinase-expressing driver animal lines, and anatomical targeting strategies (Fig. 2). Opsin/DREADD expression can be targeted to a specific cell type by injecting a virus expressing opsin/DREADD under the control of a cell type-specific promoter, such as calcium/calmodulin-dependent protein kinase type II subunit-α (CaMKIIα; biased toward excitatory cells in cortical regions). However, this strategy has not been used to target a wide range of cell types, because the sequences conferring specificity are usually too large to be packaged into viruses. Accordingly, recombinase-dependent opsin/DREADD-expressing viral vectors can be injected into transgenic animals or, along with targeted viruses that drive recombinase expression, into cells of interest. Importantly, the strategies described above can be further enhanced to allow the targeting of projections between two brain regions by delivering light/CNO to opsin/DREADD-expressing axonal terminals. Finally, the use of canine adenovirus (CAV) expressing Cre recombinase (CAV-Cre) allows for the projection-specific expression of opsin/DREADD, because CAV-Cre is preferentially retrogradely transported to neuronal somas [66]. In neuronal cell bodies, the recombination of AAV-FLEX-opsin/DREADD constructs can occur to allow the expression of opsin/DREADD in a projection-specific fashion. In addition to CAV, retrovirus rAAV2-retro, herpes simplex virus, pseudorabies virus, and rabies virus can be used as alternatives [67].

Viral infection and the expression of exogenous proteins such as opsin/DREADD at high levels can alter cellular capacitance or cellular physiology or even lead to toxicity [68]. Thus, no-light/CNO controls should be included in opsin/DREADD-expressing tissue or animals. Light used to activate opsins may also produce non-specific effects. The issue of tissue heating resulting from light deserves special consideration, because even a slight change in temperature may cause detectable tissue damage that can lead to significant physiologic [69] and behavioral [70] effects. Therefore, proper controls should be employed to account for changes that may be induced by light delivery. Notably, while light-gated proton and chloride pumps can effectively attenuate neurotransmission, sustained proton pump activity in synaptic terminals induces a pH-dependent calcium influx that increases the spontaneous release at the target circuit, and activation of light-gated chloride channels triggers neurotransmitter release upon light onset, thus potentially limiting the use of these tools for the precise manipulation of synaptic release [71]. Unlike bacterial opsins, which silence neurons via strong hyperpolarization and millisecond precision, DREADDs induce modest hyperpolarization for seconds to hours and the strong inhibition of axonal release of neurotransmitters

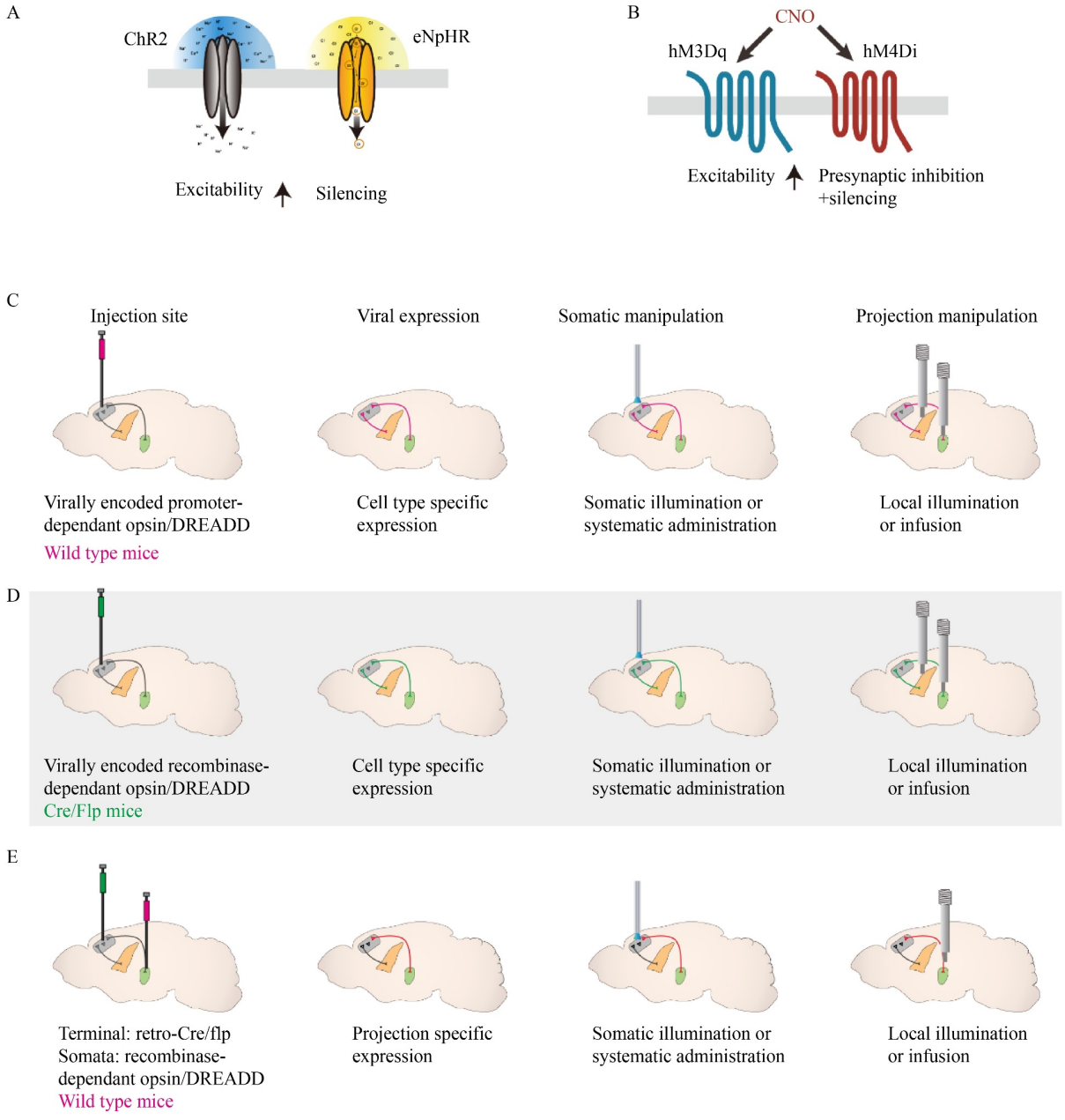

Fig. 2 Potential approaches for cell- and projection-specific modulation of neuronal activity using optogenetics and DREADDs. (A,B) Categories of single-component optogenetic (A) and chemogenetic (B) tools; transported ions, signaling pathways and potential downstream neuronal effects of activation. (C) Targeting a population by using promoter-specific AAV and somatic or terminal manipulation. (D) Additional cell-type specificity is attained by injecting a recombinase-dependent virus into a transgenic animal expressing a recombinase, such as Cre or Flp in specific cells. (E) Intersectional AAV strategy to limit AAV vector expression to a discrete projection population. The color of the syringes and the projection lines indicate different virus injected and traced.

[72,73]. Therefore, chemogenetic approaches are better choices when long-term manipulation or terminal inhibition is necessary. However, CNO may be metabolized via back-transformation to clozapine [65]. Thus, care should be taken to ensure that clozapine-like side effects (e.g., hypotension, sedation, and anticholinergic syndrome) do not occur by minimizing the dose and by always including appropriate controls (e.g., administering CNO to animals expressing GFP or a similarly irrelevant protein).

Involvement of the PFC in anxiety-like behaviors

Rodent mPFC and definition of subregions

To delineate the scope of this review, we defined the regions being considered. Although the similarity of rodent PFC to PFC in non-human primates and humans (for the discussion, see the references [74–76]) remains

disputed, the PFC of each species plays key roles in the regulation of anxiety. This review focuses on studies of the rodent PFC. Based on cytoarchitectural differences, the rodent mPFC is classified into four distinct neuroanatomical subregions along the dorsal to ventral axis: the ACC, prelimbic cortex (PL), infralimbic cortex (IL), and dorsal peduncular cortex (DP) [77]. Among these regions, the ACC and PL comprise the dorsal mPFC (dmPFC), while the IL and DP belong to the vmPFC.

Different subregions of mPFC in the regulation of anxiety

The activation of glutamatergic neurons in the mPFC by using either optogenetics or DREADDs does not affect anxiety-like behaviors [78,79]. However, the inhibition of IL mPFC increases the time in the center of OFT and on the open arms of EPM [80], while the inhibition of PL mPFC resulted in the opposite effects [81], suggesting that the mPFC is related to anxiety. Photostimulation of glutamatergic cells within the vmPFC has produced mixed results. When the CamkIIα promoter was used to target glutamatergic neurons within the vmPFC, photostimulation and photoinhibition did not affect the performance in the OFT and EPM [82]. Moreover, the optostimulation of basomedial amygdala (BMA)-projecting vmPFC neurons had no effect on anxiety-related behaviors [82]. The activation of Drd1-expressing pyramidal cells in the vmPFC of Drd1-Cre mice increased the time on the open arms of EPM and decreased the latency of NSFT. By contrast, the photostimulation of Drd2-expressing pyramidal cells had no effect on performance in tests of anxiety-like behavior [83]. Drd1 and Drd2 neurons are separate classes of excitatory cells that display different electrophysiological, morphological, and projection characteristics [84]. These results highlight the advantage of viral strategies and transgenic mice for targeting specific cellular populations and indicate the difficulty in interpreting negative results when large populations of neurons are targeted. Another large difference between the two studies above is the photostimulation protocol. In the pan-glutamatergic study, acute real-time stimulation during the behavioral test was employed [82], while the Drd1 subpopulation was photostimulated for 1 h (1 min on/1 min off) and 24 h before the behavioral tests [83]. Therefore, the manipulation of the frequency and duration of stimulation may result in strikingly different effects on behavior.

Notably, sustained photostimulation (1 min on/1 min off for 1 h) of pan-glutamatergic neurons in the IL, a subregion of the vmPFC, decreased the latency of NSFT, suggesting the anxiolytic effects [85]. By contrast, the acute stimulation of pan-glutamatergic neurons in the IL decreased the time in the center of OFT and on the open arm of EPM, suggesting anxiogenic effects, while acute inhibition resulted in the opposite (an anxiolytic) effect [80]. Consistently, the pharmacological overactivation of area 25, whose anatomical connectivity is very similar to that of roden IL cortex [86,87], reduced vagal tone and heart rate variability and altered cortisol dynamics during stress [88]. These studies further support that the sustained behavioral effects of photostimulation/DREADD-mediated inhibition are clearly different than those produced by acute manipulations and highlight the fact that the subregions of the vmPFC may have different and even opposite effects on anxiety-like behaviors. The acute stimulation of the DP, the other component of the vmPFC, increases the time in the center of OFT and on the open arms of EPM [80]. Considering that acute IL activation is anxiogenic, it may counterbalance the anxiolytic effects of DP excitation when the whole vmPFC is excited. In contrast to sustained IL photostimulation, sustained photostimulation of the PL had no effect [85]. Moreover, the acute stimulation of PL had no effect on anxiety-like behaviors, while its opto-inhibition decreased the time in the center of OFT and the open arm of EPM [81]. Finally, photostimulation of the ACC did not affect the central time in OFT but inhibited freezing in predator-based tasks [89]. Furthermore, nitric oxide-mediated defensive behavior elaborated by anterior hypothalamus neurons of mice was modulated by glutamatergic inputs from ACC [90]. The decrease in activity of glutamate connections between ACC and posterior hypothalamus by the blockade of AMPA/kainite receptors in the posterior hypothalamus resulted in decreased anxiety and panic attack-like responses [91]. Moreover, the blockade of NMDA receptors in ACC decreased the defensive behavior elicited by GABAA receptor blockade in the posterior hypothalamus [92]. Therefore, the different subregions (e.g., ACC, PL, IL, and DP) of mPFC have heterogeneous functions, and long-range inputs or outputs of these discrete subregions need to be targeted to clarify their functional heterogeneity. A summary is provided in Table 1 and Fig. 3.

Projection-specific regulation of anxiety

Alterations in structure and activity correlations between the mPFC and amygdala have been reported in patients with anxiety disorders [95,96]. Precise causal connections have been explored in animal models. Projections from the vmPFC to the amygdala are involved in anxiety; vmPFC–amygdala activation increases the time on the open arms of EMP and decreases the respiratory rate, suggesting an anxiolytic effect [82]. Interestingly, the sustained optostimulation of vmPFC Drd1 terminals in the BLA recapitulates the anxiolytic effects of somatic stimulation and acute vmPFC-amygdala activation [83]. However, neither dmPFC-amygdala activation [82] nor the control of ACC (a subregion of dmPFC) afferents to the BLA [89] results in anxiolysis or anxiolytic effects.

Table 1 Overview of studies that employed optogenetic/chemogenetic techniques to manipulate cells somas in the PFC in studies of anxiety

	Neuromodulatory approach	Target population	Effect	Test
ACC [89]	eNpHR: 5 mW, constant	Glutamatergic neurons	No effect	OFT
	ChR2: 10 ms, 10 Hz, 0.2 mW	Glutamatergic neurons	No effect	OFT
PL [81]	eNpHR: 6–9 mW, constant	Glutamatergic neurons	Anxiogenic	OFT, EPM
	ChR2: 20 Hz, 6–9 mW	Glutamatergic neurons	No effect	OFT, EPM
PL [85]	ChR2: 15 ms, 10 Hz, 5 mW, 1 min off/1 min on for 60 min	Glutamatergic neurons	No effect	NSFT
IL [85]	ChR2: 15 ms, 10 Hz, 5 mW, 1 min off/1 min on for 60 min	Glutamatergic neurons	Anxiolytic	NSFT
IL [80]	eNpHR: 6–9 mW, constant	Glutamatergic neurons	Anxiolytic	OFT, EPM
	ChR2: 5 ms, 20 Hz, 3 mW	Glutamatergic neurons	Anxiogenic	OFT, EPM
DP [80]	ChR2: 5 ms, 20 Hz, 3 mW	Glutamatergic neurons	Anxiolytic	OFT, EPM
mPFC [79]	ChR2: 40 ms, 100 Hz, 1–2 mW	Glutamatergic neurons	No effect	EPM
mPFC [78]	Gq DREADD: 2.5 mg/kg CNO	Glutamatergic neurons	No effect	OFT
mPFC [93]	Gq DREADD: 0.5 mg/kg CNO, 30 min before the test	PV neurons	No effect	OFT, NSFT
	Gq DREADD: 0.5 mg/kg CNO, 3 weeks	PV neurons	No effect	OFT, NSFT
dmPFC [94]	Gi DREADD: 5 mg/kg CNO, 30 min before the test	SST neurons	Anxiogenic	OFT, EPM, NSFT
	Gi DREADD: 0.5 mg/kg CNO, 3 weeks	SST neurons	Anxiolytic	OFT, EPM, NSFT
vmPFC [82]	eNpHR: 10 mW, constant	Glutamatergic neurons	No effect	OFT, EPM
	ChR2: 10 ms, 10 Hz, 1 mW	Glutamatergic	No effect	OFT, EPM
	ChR2: 10 ms, 10 Hz, 1 mW	$vmPFC^{BMA\text{-}projecting}$ neurons	Anxiolytic	OFT, EPM
vmPFC [83]	ChR2: 15 ms, 10 Hz, 5 mW, 1 min off/1 min on for 60 min	$Drd1^+$ glutamatergic neurons	Anxiolytic	OFT, NSFT
		$Drd2^+$ glutamatergic neurons	No effect	OFT, NSFT

ACC, anterior cingulate cortex; dmPFC, dorsal medial prefrontal cortex; DP, dorsal peduncular cortex; IL, infralimbic cortex; mPFC, medial prefrontal cortex; PL, prelimbic cortex; D1, Drd1-expressing pyramidal cells; D2, Drd2-expressing pyramidal cells; SST, somatostatin-expressing neurons; OFT, open-field test; EPM, elevated plus maze; NSFT, novelty-suppressed feeding test.

The lack of effects on anxiety-like behaviors following dmPFC-amygdala activation points to diversity in dmPFC neurons, because PL, the other component of the dmPFC, is involved in anxiety-like behaviors [81]. Notably, pharmacology studies indicated connections between ACC and both the anterior and posterior hypothalamus that play critical effects on anxiety-related behaviors [90–92].

Under physiologic conditions, the mPFC exerts inhibitory top-down control over amygdala activity, thus limiting the output of the amygdala and preventing inappropriate emotional expression [97–100]. Consistently, an inhibitory circuit—the IL-CeA pathway—was uncovered; the inhibition of this circuit decreases the time in the center of OFT and on the open arm of EPM, indicating its necessity for restraining anxiety under physiologic conditions [80]. When animals were exposed to anxiety-provoking environments such as the EPM and OFT, mPFC neurons exhibited anxiety-related features and were used by the animal to guide anxiety-related behavior [101,102]. Accordingly, an anxiogenic circuit—the IL-LS pathway —was discovered; the inhibition of this circuit increased the time in the center of OFT and on the open arms of EPM [80]. These findings provide insight into native anxiogenic and anxiolytic processes and demonstrate that anxiety is continuously regulated by balanced antagonistic pathways between the IL-LS and IL-CeA pathways. Under aversive conditions such as exposure to inescapable stress that precipitates the development of anxiety-related disorders, the prefrontal control becomes defective, resulting in aberrant amygdala activation and associated emotional disturbances [103,104]. Anxiolysis produced by IL-CeA circuit activation raised the possibility of top-down modulation of behavior elicited by pathological conditions. The activation of the IL-CeA pathway reverses stress-induced anxiety-like behaviors

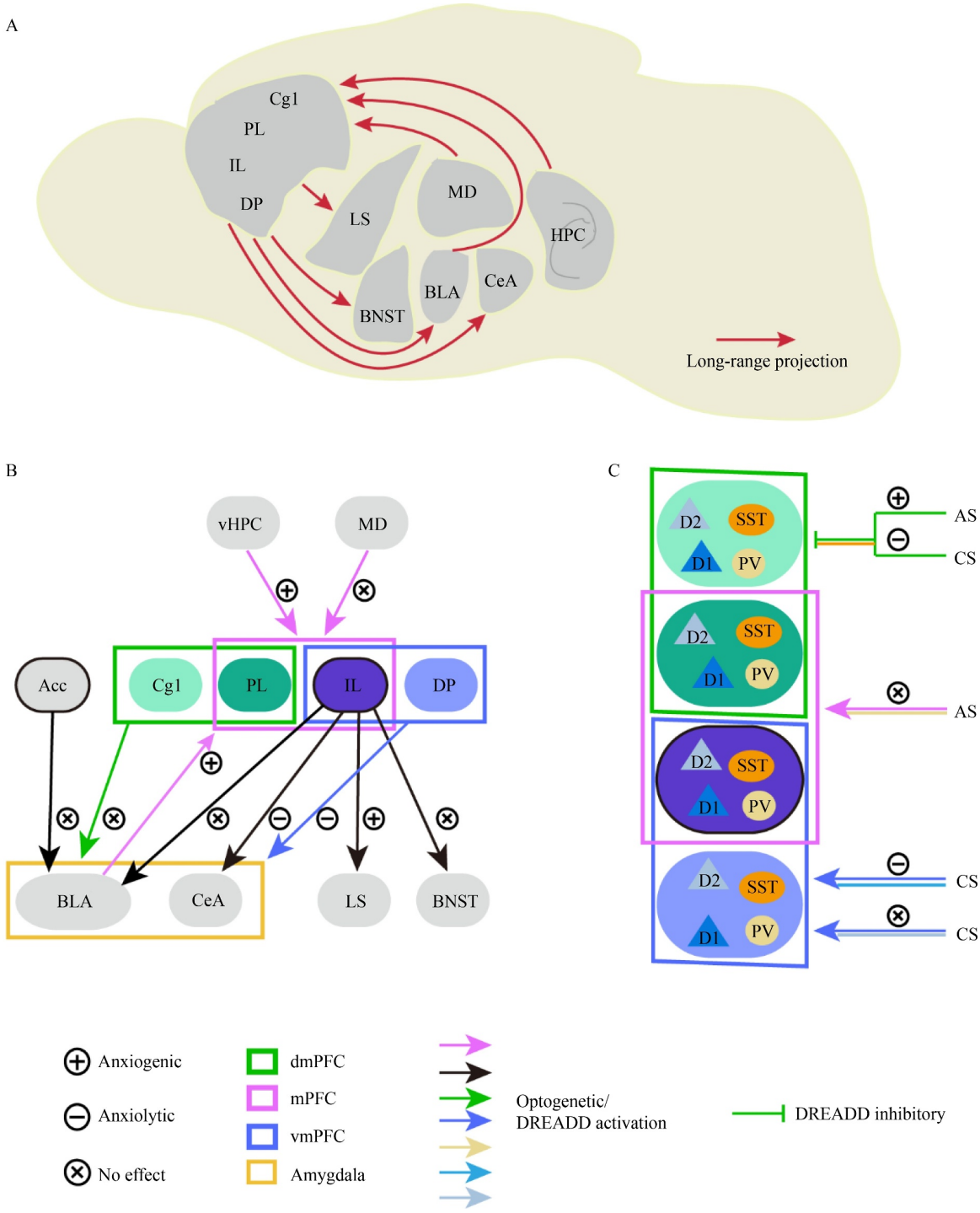

Fig. 3 Prefrontal circuits in anxiety. (A) Sagittal view of rodent brain including afferent and efferent circuits of mPFC implicated in anxiety-related behaviors. (B) Sophisticated circuitry linked to anxiety. (C) mPFC cellular populations implicated in anxiety-related behaviors. The two lines of the arrow: the color of the upper line refers to the brain region, and the color of the lower line refers to the cell type. Acc, anterior cingulate cortex; BLA, basolateral amygdala; BNST, bed nucleus of the stria terminalis; CeA, central amygdala; Cg1, cingulate cortex, area 1; dmPFC, dorsal medial prefrontal cortex; DP, dorsal peduncular cortex; IL, infralimbic cortex; LS, lateral septum; MD, medial dorsal thalamus; mPFC, medial prefrontal cortex; PL, prelimbic cortex; vHPC, ventral hippocampus; vmPFC, ventral medial prefrontal cortex; D1, Drd1-expressing pyramidal cells; D2, Drd2-expressing pyramidal cells; SST, somatostatin-expressing neurons; PV, parvalbumin-expressing neurons; AS, acute stimulation; CS, chronic stimulation.

[80]. The mechanism in which defined populations of IL projection neurons are integrated into local circuitry and the specific cell types that they contact in their long-range target structures have not been determined.

Single-unit recordings of mPFC neurons, which are modulated by ventral hippocampus (vHPC) θ oscillations, can be used to identify anxiogenic locations in the EPM [101]. Accordingly, DREADD-mediated activation of mPFC-projecting vHPC cells decreased the time in the center of OFT and on the open arm of EPM and increased the latency of NSFT [105], while DREADD-mediated inhibition [105] or photoinhibition [106] induced opposite effects. Similar photoinhibition of the medial dorsal thalamus (MD)-mPFC circuitry had no effect [106]. The bidirectional effects of manipulating BLA projections to the mPFC have also been observed. Photostimulation of BLA inputs to the mPFC decreased the time in the center of OFT and on the open arm of EPM, whereas photoinhibition produced opposite effects. A summary is provided in Table 2, Table 3, and Fig. 3.

GABAergic neurons of mPFC in regulation of anxiety

The mPFC contains a heterogeneous population of excitatory glutamatergic pyramidal and inhibitory GABAergic interneurons that deregulate its activity. Parvalbumin (PV)-expressing neurons and somatostatin (SST)-expressing neurons comprise most cortical interneurons and are best characterized in terms of their roles in cortical network functions [107–109]. Limited studies have directly assessed the role of GABAergic populations in anxiety. PV neurons target the soma or the axon initial segment of excitatory cells. Subsequently, this interneuron subtype limits communication with targets downstream of the mPFC. Recent efforts to understand PV-mediated regulation of anxiety-like behaviors have used DREADD-mediated activation of mPFC PV neurons after both acute and chronic activation [93]. DREADD-mediated activation of PV neurons induced by acute administration of CNO before testing has no effect on the performance of OFT and NSFT. In addition, the administration of CNO daily for 3 weeks has no effect on the performance of OFT and NSFT.

SST neurons mostly target the dendritic compartment of pyramidal cells and are thus critical for maintaining the integrity of information input. Limited studies have focused on anxiety targeting this cell population. Soumier and Sibille were the first to study SST neurons in this

Table 2 Overview of studies that employed optogenetic/chemogenetic techniques to manipulate PFC efferent targeting in studies of anxiety

	Neuromodulatory approach	Target population	Effect	Test
Jhang *et al*. [89]	eNpHR: 5 mW, constant	ACC–BLA	No effect	OFT
	ChR2: 10 ms, 10 Hz, 0.5 mW	ACC–BLA	No effect	OFT
Chen *et al*. [80]	eNpHR: 10 mW, constant and Gi	IL–LS	Anxiolytic	OFT, EPM
	DREADD: 5 μmol/L CNO	IL–CeA	Anxiogenic	OFT, EPM
	ChR2: 5 ms, 20 Hz, 3–15 mW (according	IL–LS	Anxiogenic	OFT, EPM
	to the target)	IL–CeA	Anxiolytic	OFT, EPM
		IL–BLA	No effect	OFT, EPM
		IL–BNST	No effect	OFT, EPM
Adhikari *et al.* [82]	eNpHR: 10 mW, constant	vmPFC–amygdala	Anxiogenic	OFT, EPM
	ChR2: 5 ms, 10 Hz	dmPFC–amygdala	No effect	OFT, EPM
		vmPFC–amygdala	Anxiolytic	OFT, EPM
Hare *et al*. [83]	ChR2: 15 ms, 10 Hz, 5 mW, 1 min off/1 min	$vmPFC^{Drd1+}$–BLA	Anxiolytic	NSFT
	on for 60 min			

ACC, anterior cingulate cortex; BLA, basolateral amygdala; BNST, bed nucleus of the stria terminalis; CeA, central amygdala; dmPFC, dorsal medial prefrontal cortex; IL, infralimbic cortex; LS, lateral septum; vmPFC, ventral medial prefrontal cortex; OFT, open-field test; EPM, elevated plus maze; NSFT, novelty-suppressed feeding test.

Table 3 Overview of studies that employed optogenetic/chemogenetic techniques to manipulate mPFC afferent targeting in studies of anxiety

	Neuromodulatory approach	Target population	Effect	Test
Parfitt *et al*. [105]	Gi DREADD: 1 mg/kg CNO	$vHPC^{mPFC\text{-}projecting}$	Anxiolytic	OFT, EPM, NSFT
	Gq DREADD: 3 mg/kg CNO	$vHPC^{mPFC\text{-}projecting}$	Anxiogenic	OFT, EPM, NSFT
Coreano *et al*. [106]	ArchT: 10 mW, every 2 min	vHPC−mPFC	Anxiolytic	OFT, EPM, NSFT
		MD−mPFC	No effect	EPM
Ortiz *et al*. [111]	eNpHR: 5 mW, constant	BLA−mPFC	Anxiolytic	OFT
	ChR2: 5 ms, 20 Hz, 5 mW	BLA−mPFC	Anxiogenic	OFT, EPM

BLA, basolateral amygdala; MD, medial dorsal thalamus; mPFC, medial prefrontal cortex; vHPC,ventral hippocampus; OFT, open-field test; EPM, elevated plus maze; NSFT, novelty-suppressed feeding test.

context by inducing the DREADD-mediated inhibition of dmPFC SST neurons after both acute and chronic inhibition [94]. The DREADD-mediated inhibition of SST neurons by acute administration of CNO before testing decreased the time spent in exploring the open arms in the EPM, a measure of increased anxiety-like behavior. By contrast, the administration of CNO twice per day for 3 weeks produced an anxiolytic effect. The rationale and design of the latter study was based on chronic changes in the excitation/inhibition balance, which can induce circuit-level homeostatic changes [110]. These studies highlight the need for a better understanding of how different stimulation protocols influence anxiety-like behavioral outcomes. Considering the importance of interneuron populations in regulating dmPFC function, the role of these interneuron subtypes in anxiety needs to be further studied. A summary is provided in Table 1 and Fig. 3.

Involvement of the PFC in the regulation of fear

Subregions of mPFC in the regulation of fear retrieval

Much research has been devoted into the discovery of the contribution of the mPFC to the expression of fear behavior. The role of mPFC in fear behavior is complex, because dmPFC and vmPFC may have opposing roles in fear. Subregions and subpopulations of the mPFC involved in fear expression were recently re-explored. Silencing PL glutamatergic neurons optogenetically impaired auditory fear retrieval at both 6 h and 7 days, indicating that PL is necessary for both short-term and long-term memory retrieval [112]. Notably, inactivation of area 25, whose anatomical connectivity is very similar to that of roden IL cortex [86,87], enhanced aversive Pavlovian conditioned responding in marmoset monkeys [113], which was opposite to that observed in rodents. In contrast to that of the PL, the photoinhibition of neither IL glutamatergic neurons [114] nor ACC glutamatergic neurons [89] affected auditory fear retrieval. However, the inactivation of hippocampus-projecting ACC neurons impaired contextual fear but not auditory fear retrieval [89], indicating the diversity in the ACC population and context-encoding characteristics of the hippocampus. A summary is provided in Table 4 and Fig. 4.

Top-down control of fear retrieval mediated by the mPFC

PL glutamatergic neurons are necessary for short-term and long-term memory retrieval, and researchers have

Table 4 Overview of studies that use optogenetic/chemogenetic techniques to manipulate cell somas in the PFC in studies of fear

	Neuromodulatory approach	Target population	Effect	Test
ACC [89]	eNpHR: 5 mW, constant	Glutamatergic neurons	Freezing↑	Innate fear
			Retrieval: no effect	Aud FC
	ChR2: 10 ms, 10 Hz, 0.2 mW	Glutamatergic neurons	Freezing↓	Innate fear
			Retrieval: impaired	Aud FC and Ctx FC
		$ACC^{BLA\text{-}projecting}$ neurons	Freezing↓	Innate fear
ACC [125]	eNpHR: 8–10 mW, constant	$ACC^{CA\text{-}projecting}$ neurons	Retrieval: impaired	Ctx FC
			Retrieval: no effect	Aud FC
PL [114]	eNpHR: 63.7–127.4 mW/mm^2, constant	Neurons	Extinction retrieval: no effect	Aud FC
PL [112]	eNpHR: 5 mW, constant	Glutamatergic neurons	Retrieval (6 h and 7 days): impaired	Aud FC
PL [126]	ChR2: 15 ms, 30 Hz, 159 mW/mm^2	Glutamatergic neurons	Extinction: no effect	Aud FC
PL [127]	ArchT: constant, 6–9 mW	SST neurons	Training: no effect but impaired retrieval the next day	Aud FC
			Retrieval: impaired	Aud FC
	ChR2: 10 ms, 20 Hz, 6–9 mW	SST neurons	Retrieval: no effect	Aud FC
PL [128]	PSAM DREADD: 5 mg/kg PSEM308	PV neurons	Training: no effect but impaired retrieval the next day	tFC and Ctx FC
			Retrieval: no effect	tFC and Ctx FC
			Extinction: no effect	tFC and Ctx FC
			Extinction retrieval: no effect	tFC
	Gi DREADD: 5 mg/kg CNO	$PL^{IL\text{-}projecting}$ neurons	Training: no effect	tFC
			Extinction: no effect	tFC

(*Continued*)

	Neuromodulatory approach	Target population	Effect	Test
IL [80]	eNpHR: 10 mW, constant	Glutamatergic neurons	Retrieval: no effect	Aud FC
			Extinction: no effect but impaired extinction retrieval the next day	Aud FC
	ChR2: 5 ms, 20 Hz, 3 mW	Glutamatergic neurons	Retrieval: impaired	Aud FC
			Extinction: facilitated	Aud FC
IL [118]	eNpHR: 8–10 mW, constant	Glutamatergic neurons	Extinction: no effect but impaired extinction retrieval the next day	Aud FC
			Extinction retrieval: no effect	Aud FC
	ChR2: 5 ms, 20 Hz, 5 mW	Glutamatergic neurons	Retrieval: impaired	Aud FC
			Extinction: facilitated	Aud FC
			Extinction retrieval: facilitated	Aud FC
IL [114]	eNpHR: 63.7–127.4 mW/mm^2, constant	Neurons	Retrieval: no effect	Aud FC
			Extinction retrieval: impaired	Aud FC
		Glutamatergic neurons	Extinction: no effect	Aud FC
	ChR2: 20 ms, 10 Hz, 6.4 mW/mm^2	Glutamatergic neurons	Extinction retrieval: facilitated	Aud FC
IL [129]	Gi DREADD: 5 mg/kg CNO	PV neurons	Extinction: facilitated	Aud FC
	Gq DREADD: 5 mg/kg CNO	PV neurons	Extinction: impaired	Aud FC
IL [128]	PSAM DREADD: 5 mg/kg PSEM308	PV neurons	Training: no effect	tFC and Ctx FC
			Retrieval: no effect	tFC and Ctx FC
			Extinction: impaired	tFC and Ctx FC
			Extinction retrieval: no effect	tFC
	Gi DREADD: 5 mg/kg CNO	IL$^{\text{PL-projecting}}$ neurons	Training: no effect	tFC
			Extinction: impaired	tFC
mPFC [117]	KORD-DREADD: 17 mg/kg	mPFC$^{\text{BLA-projecting}}$ neurons	Extinction: no effect but impaired extinction retrieval the next day	Aud FC
dmPFC [130]	ChR2: 4 Hz, 10 mW	PV neurons	Freezing↑	Innate fear
dmPFC [131]	ArchT: 250 ms light pulse	PV neurons	Retrieval: fear promoted	Aud FC
			Extinction: impaired	Aud FC
	ChR2: 250 ms light pulse	PV neurons	Retrieval: impaired	Aud FC
vmPFC [82]	ChR2: 10 ms, 10 Hz, 1 mW	Glutamatergic neurons	Extinction: no effect but facilitated extinction retrieval the next day	Aud FC
		vmPFC$^{\text{BMA-projecting}}$ neurons	Extinction: no effect but facilitated extinction retrieval the next day	Aud FC
vmPFC [116]	Gi-DREADD: 3 mg/kg CNO	VmPFC$^{\text{RE-projecting}}$ neurons	Extinction: impaired	Aud FC
			Extinction retrieval: impaired	Aud FC

ACC, anterior cingulate cortex; BLA, basolateral amygdala; BMA, basomedial amygdala; CA, hippocampal CA3 to CA1 region; dmPFC, dorsal medial prefrontal cortex; IL, infralimbic cortex; mPFC, medial prefrontal cortex; PL, prelimbic cortex; RE, thalamic nucleus reuniens; vmPFC, ventral medial prefrontal cortex; SST, somatostatin-expressing neurons; PV, parvalbumin-expressing neurons; Aud FC: auditory fear conditioning; Cxt FC: cortical fear conditioning; tFC: trace fear conditioning.

further elucidated the neuronal circuits responsible for the respective effects [112]. Retrieval at late time points activated PL neurons projecting to the paraventricular subregion of the dorsal midline thalamus (PVT), and optogenetic silencing of this projection impaired retrieval at late but not early times. By contrast, silencing of PL

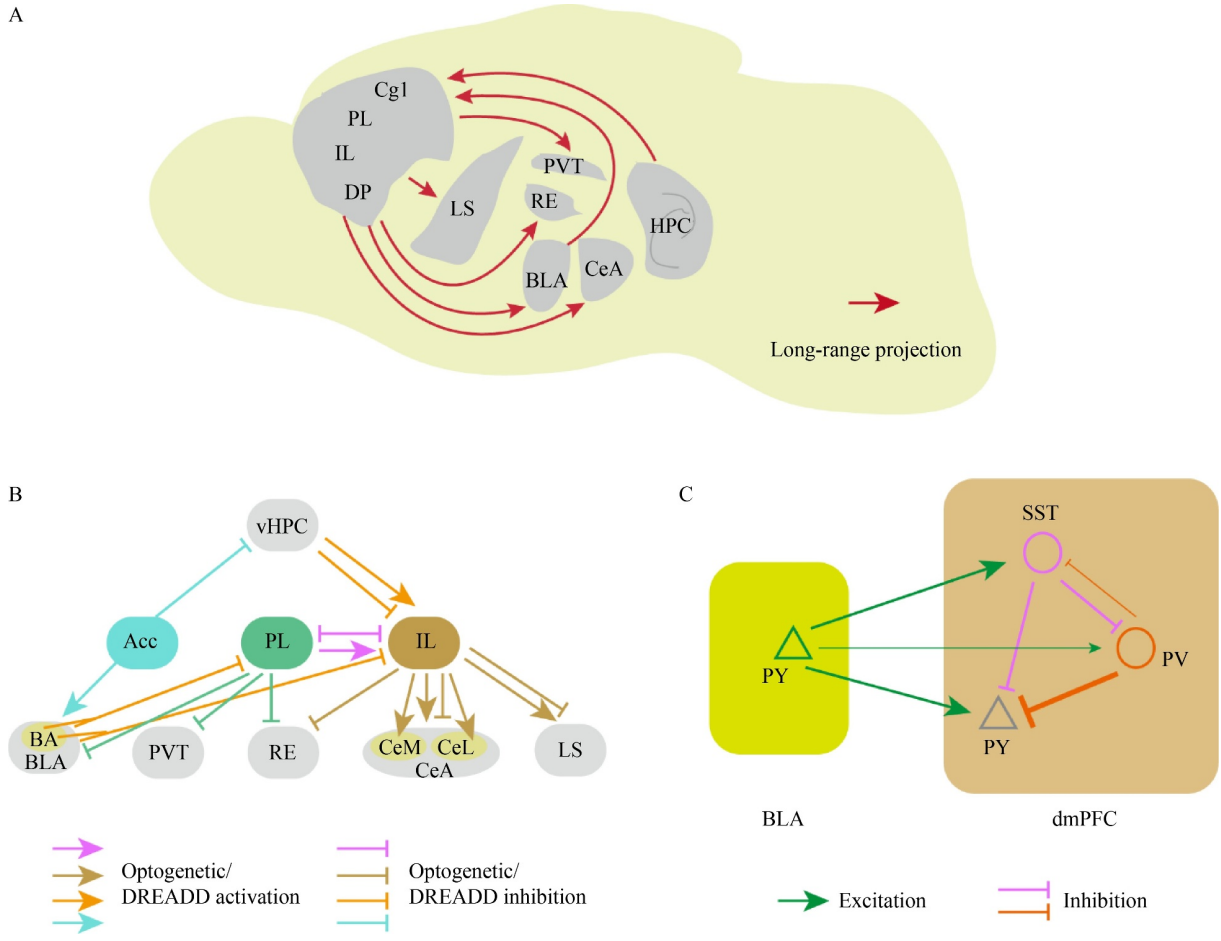

Fig. 4 Prefrontal circuits in fear. (A) Sagittal view of rodent brain, including afferent and efferent circuits of mPFC implicated in fear-related behaviors. (B) Sophisticated circuitry linked to fear. (C) dmPFC cellular response and regulation from amygdala under fear-related behaviors. The thickness of the arrow indicates the strength of regulation. ACC, anterior cingulate cortex; BA, basal amygdala; BLA, basolateral amygdala; Cg1, cingulate cortex, area 1; CeA, central amygdala; CeL, lateral subdivision of the central amygdala; CeM, centromedial subdivision of the amygdala; dmPFC, dorsal medial prefrontal cortex; DP, dorsal peduncular cortex; IL, infralimbic cortex; LS, lateral septum; PL, prelimbic cortex; PVT, paraventricular thalamus; vHPC, ventral hippocampus; RE, thalamic nucleus reuniens; PY, pyramidal neurons; SST, somatostatin-expressing neurons; PV, parvalbumin-expressing neurons.

inputs to the BLA impaired retrieval at early but not late times, indicating a time-dependent shift in retrieval circuits. Researchers have not determined the specificity of their function for the learned association and the mechanism in which PL neurons coordinate activity in their many downstream target regions to contribute to memory-guided behavior. A new knock-in mouse (TRAP2) for activity-dependent genetic labeling was used to assess PL neurons activated during fear conditioning or 1-, 7-, or 14-day memory retrieval and their contributions to 28-day remote memory [115]. Photostimulation of TRAPed neurons at later time points produced more freezing than the stimulation of TRAPed neurons at earlier time points [115]. While neurons TRAPed during earlier and later retrievals have similar broad projections throughout the brain, PL neurons TRAPed later show stronger functional recruitment of cortical targets. These studies indicate that the memory trace undergoes time-dependent reorganization, and the dynamics of interactions between the PL and identify target regions should be further studied during memory retrieval. A summary is provided in Table 5 and Fig. 4.

mPFC in the regulation of fear extinction

Much progress has been made using optogenetics and DREADDs to explore the causal role of the mPFC in the regulation of fear extinction. DREADD-mediated inhibition of thalamic nucleus reuniens (RE)-projecting vmPFC neurons during extinction training impaired within-session extinction and extinction retrieval the following day [116]. However, DREADD-mediated inhibition of BLA-projecting mPFC neurons during extinction training did not affect within-session extinction but impaired the retrieval of extinction the following day [117]. Moreover, the optogenetic silencing of IL glutamatergic neurons during extinction training resulted in the same effects as

Table 5 Overview of studies that used optogenetic/chemogenetic techniques for the manipulation of PFC efferent targeting in studies of fear

	Neuromodulatory approach	Target population	Effect	Test
Jhang *et al*. [89]	eNpHR: 5 mW, constant	ACC–BLA	Freezing↑	Innate fear
		PL–BLA	No effect	Innate fear
	ChR2: 10 ms, 10 Hz, 0.5 mW	ACC–BLA	Freezing↓	Innate fear
Do-Monte *et al*. [112]	eNpHR: 10 mW, constant	PL–BLA	Retrieval (6 h): impaired	Aud FC
			Retrieval (7 days): no effect	Aud FC
		PL –PVT	Retrieval (6 h): no effect	Aud FC
			Retrieval (7 days): impaired	Aud FC
Marek *et al*. [126]	eNpHR: constant	PL–IL	Extinction: impaired	Aud FC
	ChR2: 15 ms, 30 Hz, 159 mW/mm^2	PL–IL	Extinction: facilitated	Aud FC
Mukherjee *et al*. [128]	Gi-DREADD: 5 mg/kg CNO	PL–IL	Extinction: no effect	tFC
		IL–PL	Extinction: impaired	tFC
Ramanathan *et al*. [116]	Gi-DREADD: 1 mmol/L CNO	PL–RE	Extinction retrieval: impaired	Aud FC
		IL–RE	Extinction retrieval: impaired	Aud FC
		mPFC–RE	Extinction retrieval: impaired	Aud FC
	Gi-DREADD: 5 µmol/L CNO	IL–LS	Retrieval: no effect	Aud FC
			Extinction: facilitated	Aud FC
		IL–CeA	Retrieval: no effect	Aud FC
			Extinction: impaired	Aud FC
	ChR2: 5 ms, 20 Hz, 7–15 mW	IL–LS	Retrieval: no effect	Aud FC
			Extinction: impaired	Aud FC
		IL–CeA	Retrieval: no effect	Aud FC
			Extinction: facilitated	Aud FC
		IL–CeL	Retrieval: no effect	Aud FC
			Extinction: no effect	Aud FC
		IL–CeM	Retrieval: no effect	Aud FC
			Extinction: facilitated	Aud FC
Adhikari *et al*. [82]	ChR2: 10 ms, 10 Hz, 10 mW	dmPFC–amygdala	Acquisition: no effect	Aud FC
			Extinction: no effect but impaired extinction retrieval the next day	Aud FC
			Retrieval: no effect	Ctx FC
		vmPFC–amygdala	Acquisition: no effect	Aud FC
			Extinction: facilitated	Aud FC
			Retrieval: impaired	Ctx FC
Bukalo *et al*. [132]	eNpHR: 10 mW, constant	vmPFC–amygdala	Extinction: no effect but impaired extinction retrieval the next day	Aud FC
			Extinction retrieval: no effect	Aud FC
	ChR2: 10 ms, 10 Hz, 10 mW	vmPFC–amygdala	Extinction: no effect but facilitated extinction retrieval the next day	Aud FC
			Extinction retrieval: no effect	Aud FC

ACC, anterior cingulate cortex; BLA, basolateral amygdala; CeA, central amygdala; CeL, lateral subdivision of the central amygdala; CeM, centromedial subdivision of the amygdala; dmPFC, dorsal medial prefrontal cortex; DP, dorsal peduncular cortex; IL, infralimbic cortex; LS, lateral septum; PL, prelimbic cortex; PVT, paraventricular thalamus; RE, thalamic nucleus reuniens; Aud FC, auditory fear conditioning; Cxt FC, cortical fear conditioning.

the inhibition of BLA-projecting mPFC neurons [80,118]. Notably, the photostimulation of vmPFC glutamatergic neurons or BMA-projecting vmPFC neurons during extinction training had no effect on within-session extinction but facilitated extinction memory the following day [82], while the activation of IL neurons during extinction training reduced fear expression and strengthened extinction memory the following day [118]. In addition, the DREADD-mediated inhibition of RE-projecting vmPFC neurons impaired the expression of extinction memory [116], while the optogenetic silencing of IL glutamatergic neurons did not affect the retrieval of extinction memory [118]. IL activity is not necessary for the retrieval of extinction memory [118], which is contrary to current models, because several studies that employed various techniques have specifically implicated the IL in rodents and vmPFC in humans in controlling fear expression after extinction [119–123]. This study only targeted IL glutamatergic pyramidal cells. Consistent with this, another study found that the inactivation of both glutamatergic neurons and GABAergic neurons in the IL produced extinction retrieval deficits, while the inhibition of pyramidal neurons alone did not produce a deficit [114]. Notably, rat/mouse strain is an important factor for fear extinction concerning the IL cortex, because focal electrolytic lesions of IL impaired the retrieval of extinction Sprague-Dawley (SD) rats but not Long-Evans rats [124]. The use of optogenetics demonstrated IL involvement in extinction apart from SD rats/C57 mice [80,118]. Notably, the inactivation of area 25, whose anatomical connectivity is very similar to that of roden IL cortex [86,87], enhanced fear extinction in marmoset monkeys [113], which was opposite to that seen in rodents. Therefore, fear extinction learning recruited IL neurons and RE-projecting vmPFC neurons, while the retrieval of extinction memory recruited not only IL neurons and RE-projecting vmPFC neurons but also BLA-projecting mPFC neurons and BMA-projecting vmPFC neurons. A summary is provided in Table 4 and Fig. 4.

Projection-specific regulation of fear extinction

Studies on vmPFC efferent support the role of vmPFC circuitry in altered fear extinction. The use of optogenetic constructs to bidirectionally control vmPFC targets in the amygdala demonstrates that photoinhibition impairs fear extinction [132], while photostimulation has the opposite effect [82,132]. Moreover, precise causal targets of subregions of the vmPFC in modulating fear were established. Two projections exert opposite effects on fear extinction, in which the IL-LS pathway promoted fear-related freezing, whereas the IL-CeA pathway exerted a fear-releasing influence on the same features [80]. Therefore, the mPFC has two antagonized top-down control circuits for the modulation of fear extinction with high efficiency and precision. The fact that LS- and CeA-projecting IL neurons are intermingled in a salt-and-pepper-like manner rather than being anatomically segregated may help to establish local interactions important for rapid behavioral switching. Although the vmPFC-amygdala pathway is not necessary for extinction retrieval [132], DREADD-mediated inhibition of IL-RE projection was shown to impair extinction retrieval [116]. RE is critically involved in extinction recall, thus supporting the importance of the midline thalamus in both memory and emotion [133–135]. RE has been suggested as a critical hub that interconnects the mPFC and hippocampus [136,137], suggesting its broader role in adaptive emotional regulation.

Interestingly, the optostimulation of dmPFC- and vmPFC-amygdala projections exhibited opposite effects on fear extinction [82], indicating the critical role of PFC in mediating the flexible adaptive control of behavior and raising the question of how subregions within it might interact to influence learning. Anatomical [138] and electrophysiological studies [139,140] suggest the presence of significant connections between IL and PL. Later studies detected prominent reciprocal layer 5/6 connectivity between the PL and IL via neural tracing [126,128]. In addition, PL-to-IL connectivity is active during extinction [126]. Consistently, the stimulation of PL afferents in the IL enhances extinction, whereas the stimulation of cell soma in the PL did not have an effect [126]. By contrast, silencing PL afferents to the IL during extinction impairs extinction [126]. However, by using DREADD-mediated inhibition, another study showed that inhibition of PL-IL projection had no effect on fear extinction, whereas the inhibition of IL-PL projections impaired fear extinction in a trace fear condition model [128]. These studies uncover the interactions between mPFC subregions in regulating extinction memory in different behavioral models. Notably, the DREADD-mediated inhibition of the PL terminal in RE impaired extinction retrieval [116], together with the PL-IL circuit in the regulation of fear extinction [126], redefining the role of the PL in extinction-related behaviors. A summary is provided in Table 5 and Fig. 4.

Afferents-specific regulation of fear extinction

Several studies have used optogenetics or DREADDs to target mPFC afferents. The amygdala and the mPFC are interconnected brain structures that are involved in the extinction of conditioned fear. A study targeted subpopulations of basal amygdala (BA) neurons based on their projection targets in the mPFC [141]. Results show that fear neurons projected specifically to the PL, whereas extinction neurons projected to the IL. Pathway-specific optogenetic manipulations demonstrated that the activity balance between pathways was causally involved in fear

extinction, and the eNpHR-mediated inhibition of IL-projecting BA neurons during extinction training did not affect within-session extinction but impaired strengthened extinction memory the following day, whereas the inhibition of PL-projecting BA neurons produced the opposite effects. However, the high-frequency optogenetic stimulation of the ChR variant $ChETA_{TC}$ induced robust synaptic depression, and synaptic depression in both the BLA-IL and BLA-PL circuits before extinction training facilitated within-session extinction [142]. Differences in stimulation parameters or variations in the subpopulations of neurons targeted may have caused the contradictory outcomes.

Hippocampal projections to the mPFC have been identified [143]. The precise projection patterns, the local circuits driven by these inputs, and the functional role of these projections have been explored [144], and the results show that the optogenetic activation of the vHPC generated significant feed-forward inhibition of IL pyramidal neurons through the recruitment of PV interneurons. The DREADD-mediated activation of vHPC-IL projections impaired extinction renewal, whereas the silencing of IL projections diminished fear renewal, suggesting a necessary role for the vHPC-IL circuit in fear relapse. Notably, vHPC-mediated inhibition was most pronounced in amygdala-projecting pyramidal neurons located in layers 2 and 3 of the IL, suggesting that the vHPC-mPFC-amygdala might orchestrate the relapse of extinguished fear. A summary is provided in Table 6 and Fig. 4.

Inhibitory circuits of mPFC in the regulation of fear

For the identification of cellular plasticity mechanisms underlying fear conditioning *in vivo*, the constraints imposed by control elements in the circuit should be addressed. Local circuit inhibitory interneurons represent one type of regulatory control element. Single-unit recordings revealed two main subclasses of interneurons in the dmPFC with opposite CS-evoked responses during fear expression, including type 1 interneurons, which display CS-evoked activity correlated with high fear states, and type 2 interneurons, which are strongly inhibited during a high-fear state [131]. By combining data with optogenetic manipulations, the authors demonstrated that the type 2 interneurons were PV neurons. The optogenetic inhibition of PV interneuron activity disinhibited prefrontal cortex projection neurons and synchronized their firing by resetting local θ oscillations, leading to fear expression. In another study, *in vitro* patch recording and fiber photometry revealed that the type 1 interneurons were SST neurons [127]. Moreover, the activation of SST neurons played a causal role in both the expression and initial acquisition of cued fear responses. In network disinhibition, PL circuits formed by SST and PV interneurons exhibited functional differences that may facilitate their complementary roles. Output from PV neurons is heavily biased toward pyramidal neurons (PNs) over SST neurons, implying that they were specialized for the suppression of PN firing [127]. By contrast, the potential for PN disinhibition during SST neuron activity is high because of the ability of SST neurons to inhibit PV interneurons relatively strongly [127]. These provide more information for the characterization of the intricate organization of interneuron ensembles in cortical and subcortical areas [145–147] and the identification of disinhibitory microcircuits as key features of the neuronal networks that mediate learning and memory.

A recent study elucidates the local circuit mechanism by which IL plasticity regulates extinction [129], in which fear extinction induced the reconstruction of the PV network configuration that was dependent on neuregulin 1-ErbB4 signaling. Interference with PV configuration shifts following extinction blocked both extinction-induced PV network plasticity and previous extinction learning, indicating that extinction-induced PV network plasticity is required for extinction. Moreover, the regulation of fear extinction by basal medial amygdala

Table 6 Overview of studies that used optogenetic/chemogenetic techniques for the manipulation of mPFC afferent targeting in studies of fear

	Neuromodulatory approach	Target population	Effect	Test
Marek *et al*. [144]	Gi DREADD: 1–3 mg/kg	$vHPC^{IL\text{-}projecting}$ neurons	Renewal: impaired	Aud FC
	Gq DREADD: 1–3 mg/kg	$vHPC^{IL\text{-}projecting}$ neurons	Renewal: promoted	Aud FC
Senn *et al*. [141]	eNpHR: 10 mW, constant	$BA^{IL\text{-}projecting}$ neurons	Extinction: no effect but impaired extinction retrieval the next day	Aud FC
		$BA^{PL\text{-}projecting}$ neurons	Extinction: no effect but facilitated extinction retrieval the next day	Aud FC
Klavir *et al*. [142]	ChETA: 9 s burst of 100 Hz, 3 ms pulse every 60 s for 15 min	BLA–PL	Extinction: facilitated	Aud FC
		BLA–IL	Extinction: facilitated	Aud FC

BA, basal amygdala; BLA, basolateral amygdala; IL, infralimbic cortex; PL, prelimbic cortex; vHPC, ventral hippocampus; Aud FC, auditory fear conditioning.

(BMA)-projecting IL neurons is dependent on PV network configuration. In conclusion, PV network plasticity mediated by neuregulin 1-ErbB4 signaling in IL mediates fear extinction through connectivity to BMA. However, the DREADD-mediated excitation of PV neurons in the IL does not affect extinction retrieval [128]. This finding does not exclude the involvement of PV neurons in the IL in the expression of extinction memory, again highlighting the importance of loss-of-function studies. Alternatively, other subtypes of GABAergic interneurons in the IL might contribute to extinction retrieval. A summary is provided in Table 4 and Fig. 4.

Considerations for future studies

The studies detailed herein support that mPFC neurons, projection target regions, and afferent innervations regulate anxiety- and fear-related behaviors in preclinical models. The best available intervention for the treatment of anxiety disorders is CBT [148], which aims to replace maladaptive interpretations of events with more helpful interpretations, and this process almost certainly occurs through plastic changes in the interpretation circuits. These circuits are expected to be complex. While the indiscriminate acute stimulation of mPFC glutamatergic neurons during testing does not affect anxiety-like behavior [78,79,82], targeting of subregions or subpopulations of mPFC neurons has significant effects [81,85,89,93,94]. Considering the inherent challenges of human studies, preclinical studies should clearly delineate the neuronal population, subregion, and stimulation paradigm to provide information that can be used for the design and refine clinical interventions to produce more selective circuit effects in patients.

In addition, optogenetic and chemogenetic manipulations can produce persistent neuroplastic effects that may affect behavior. For example, long high-frequency stimulation (9-s bursts of 100 Hz, 3-ms blue light pulses every 60 s for 15 min) of BLA-mPFC terminals induces persistent synaptic depression and facilitated fear extinction [142]. The ability to produce sustained synaptic and behavioral responses indicates that clinical interventions could be designed to produce similar effects by DBS, and these circuits are potential therapeutic targets. In addition, the sustained behavioral effects of DREADD-mediated inhibition are clearly different than those achieved by acute manipulations. For instance, the acute inhibition of SST neurons in the dmPFC increases behavioral emotionality, whereas chronic inhibition decreases behavioral emotionality [94]. Understanding the mechanistic drivers that produce these sustained effects could also inform the refinement of clinical targets and pharmacological treatments in the future.

An innovative strategy requires a comprehensive understanding of the dynamic interactions occurring within the distributed networks underlying anxiety and fear. By combining whole-brain precise imaging with state-of-the-art viral genetic tools, recent studies have provided a comprehensive atlas of the long-range inputs to pyramidal, PV, SST and VIP neurons in the mPFC [149,150]. Together with previous anterograde tracing studies [82,112,132,151–153], these studies have clearly elucidated the anatomical organization in terms of inputs and outputs. In the future, even more refined intersectional optogenetic approaches that allow projection-specific and cell-type-specific targeting of circuit elements will be conducted to reveal additional details about circuit function in fear and anxiety. The mechanism in which PL cortex is involved in fear extinction needs to be determined. Conventional models for fear learning suggest that PL is not required for fear extinction, because the activation of pyramidal neurons or PV neurons and the inhibition of neurons in PL cortex have no effect on fear extinction [114,126,128]. However, opto- or chemo-manipulated PL efferents and afferents do not participate in fear extinction [116,126,141,142]. Further studies need to resolve the conflicts.

Future studies will investigate how local microcircuits interact with long-range projections in regulating anxiety and fear. One key issue among them is the inhibitory mechanism underlying projection-specific control of fear extinction. IL-BMA and IL-CeA circuit facilitated fear extinction [80,82], but the regulation of fear extinction by BMA-projecting but not CeA-projecting IL neurons was dependent on PV network plasticity [129]. The mechanism in which CeA-projecting IL neurons are integrated into the local inhibitory circuits has not been determined. This information can be obtained if different levels of analysis are integrated. Hence, studies should characterize cellular and molecular mechanisms within defined functional networks. Future studies should go beyond functional and anatomical analysis of these circuits and address the computations they carry out. For example, researchers found that two different IL mPFC outputs exerted opposite effects on fear and anxiety [80], but it remains unclear when and how this projection-specificity could be functionally recruited in control of anxiety states. Accordingly, future research needs to address how stimulus representations, associations, and behavioral output programs are encoded at the level of larger-scale neuronal populations that are organized in defined circuits. This will require implementation of *in vivo* imaging by using two-photon microscopy or fiber photometry and multielectrode electrophysiology to further our understanding of cortical function.

Lastly, new behavioral approaches or behavioral biomarkers are needed to increase the diversity of fear and anxiety parameters and may help provide insight into the current mixed set of results. Non-uniform measurement

paradigms, variability in apparatus use, and individual differences in animals confound study results [154,155]. Recently, artificial intelligence approaches have been used to screen for more behavioral biomarkers [156,157] and began to be applied to the anxiety model [158].

The combination of optogenetic and chemogenetic tools with viral tools that allow cell population-specific control allows powerful insight into the diverse role of mPFC neuronal populations in preclinical studies of anxiety and fear-related behaviors. Characterizing the distributed and highly organized neuronal circuits underlying the acquisition and expression of defensive behaviors not only will lead to a better understanding of fear, fear extinction, and anxiety processes, but also has the potential to reveal the general principles of brain organization and function.

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Compliance with ethics guidelines

Yihua Chen, Nengyuan Hu, Jianming Yang, and Tianming Gao declare that they have no conflicts of interest. This manuscript is a review article and does not involved a research protocol requiring approval from relevant institutional review board or ethics committee.

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