

REVIEW ARTICLE

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Inhibition in the amygdala anxiety circuitry

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Abstract

Inhibitory neurotransmission plays a key role in anxiety disorders, as evidenced by the anxiolytic effect of the benzodiazepine class of γ -aminobutyric acid (GABA) receptor agonists and the recent discovery of anxiety-associated variants in the molecular components of inhibitory synapses. Accordingly, substantial interest has focused on understanding how inhibitory neurons and synapses contribute to the circuitry underlying adaptive and pathological anxiety behaviors. A key element of the anxiety circuitry is the amygdala, which integrates information from cortical and thalamic sensory inputs to generate fear and anxiety-related behavioral outputs. Information processing within the amygdala is heavily dependent on inhibitory control, although the specific mechanisms by which amygdala GABAergic neurons and synapses regulate anxiety-related behaviors are only beginning to be uncovered. Here, we summarize the current state of knowledge and highlight open questions regarding the role of inhibition in the amygdala anxiety circuitry. We discuss the inhibitory neuron subtypes that contribute to the processing of anxiety information in the basolateral and central amygdala, as well as the molecular determinants, such as GABA receptors and synapse organizer proteins, that shape inhibitory synaptic transmission within the anxiety circuitry. Finally, we conclude with an overview of current and future approaches for converting this knowledge into successful treatment strategies for anxiety disorders.

Introduction

Information processing throughout the brain is critically dependent on the function of inhibitory (largely GABAergic) neurons, which provide an essential counterbalance to excitatory neurotransmission through hyperpolarization and consequent inhibition of their postsynaptic targets¹. This inhibitory control is central to all aspects of neural computation, shaping, fine-tuning and orchestrating the flow of information through neuronal networks to generate a precise neural code. Not surprisingly, therefore, alterations in inhibition have been prominently linked to psychiatric disorders, including anxiety disorders^{1–5}, and inhibitory neurons and synapses are considered to be prime targets for the development of novel anxiolytic therapies^{4,6}. A major challenge in this endeavor is the staggering complexity of the inhibitory network, which comprises a multitude of neuronal and

synaptic subtypes with highly diverse functions. Accordingly, it is increasingly appreciated that selective anxiolytic effects can only be achieved through precise knowledge of the relevant circuitry. Here we summarize what is known (and unknown) about the role of anxiety-related inhibitory neurotransmission in the amygdala, a key structure in the anxiety circuitry.

Anxiety disorders and the amygdala Adaptive vs. pathological anxiety

Anxiety is a state of increased vigilance and responsiveness that results in a range of measurable defensive behaviors. These behaviors serve to prevent or reduce harm to the organism in the face of unexpected and potentially dangerous situations, and thus, anxiety is first and foremost an adaptive, physiological mechanism that is essential for survival^{7,8}. However, dysregulation of anxiety circuits due to genetic or acquired causes (e.g., chronic stress or a traumatic brain injury) leads to pathological anxiety disorders⁸, which are among the most common neuropsychiatric diseases, with an estimated lifetime

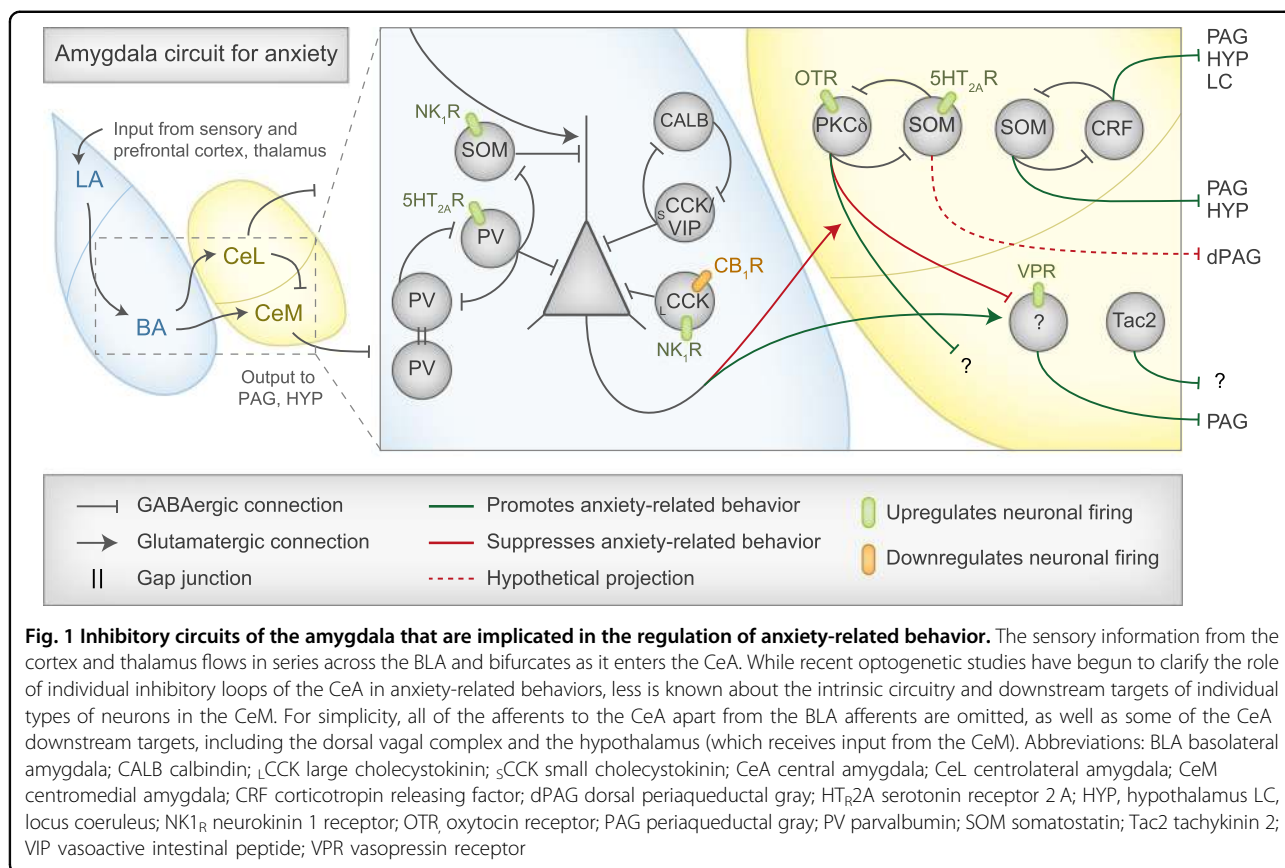
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prevalence of more than 28% in adults⁹. Moreover, pathological anxiety is still thought to be largely under-recognized and under-treated due to its broad range of symptoms and the high level of co-morbidity with other psychiatric conditions^{9,10}. Anxiety disorders can be clinically subdivided into several categories, including generalized anxiety disorder, panic disorder, agoraphobia, phobias, separation anxiety disorder, selective mutism and social anxiety disorders⁸. Apart from the emotional burden of excessive fear and apprehension, anxiety disorders represent an important source of functional impairment due to their accompanying behaviors, which include withdrawal from participating in daily activities, as well as physical symptoms, such as respiratory, gastrointestinal and cardiovascular problems^{8,10,11}. Accordingly, major research efforts aim to develop new and more effective treatments for pathological anxiety.

Studying anxiety disorders in animal models

A large variety of behavioral paradigms exist to assess anxiety-related behaviors in rodents, which aim to provide a meaningful comparison with at least one aspect of the human experience. Validated tests include the assessment of active avoidance, hyponeophagia, social interactions and conditioned emotional responses (CER), as well as

ethological tests that investigate approach-avoidance conflict, such as the elevated plus maze (EPM), open field (OF), light-dark box (LDB) and free-choice exploratory (FCE) paradigm^{9,11}. Approach-avoidance tests, which are extensively used to assess anxiety in genetic and environmental animal models due to their ethological nature, are based on the conflict between exploring a novel environment and avoiding a potentially dangerous situation (such as an environment in which the risk of being detected by a predator is high). The tests consist in letting the animals freely explore an environment that offers a 'safe' and a 'dangerous' zone (walled arms vs. open arms of the EPM, edges vs. the center of the OF, dark vs. light compartments of the LDB)^{9,11}. Mice with an anxious phenotype tend to explore less and avoid exposed, brightly lit areas, displaying an excessive avoidance of potential threats that is akin to the symptoms of anxiety in humans^{9,11}.

Anxiety and the amygdala

While processing of anxiety-related information involves a wide range of brain regions (reviewed in refs^{7,9}), a key structure in this network is the amygdala. Amygdala lesions in humans, monkeys, and rodents result in an inability to recognize fearful stimuli, and electrical

stimulation of the amygdala in humans generates feelings of fear and anxiety^{12,13}. Moreover, hyperexcitability of the amygdala in response to negatively valenced stimuli has been observed in patients with several types of anxiety disorders, and this is reversed following successful treatment with cognitive behavioral therapy¹³.

Anxiety-related behavioral manifestations are the end-product of a multi-stage processing of salient sensory stimuli within the amygdala circuitry (Fig. 1). The amygdala consists of multiple subdivisions, of which the basolateral amygdala (BLA) and central amygdala (CeA) are particularly important in anxiety processing¹². The BLA receives sensory information from the thalamus, cortical association areas and prefrontal cortex (PFC) through the lateral nucleus (LA), processes this information in the basal nucleus (BA), and sends it to the lateral subdivision of the CeA (centrolateral amygdala, CeL), where it may undergo additional processing (see Section 3.3). In parallel, inputs from the BLA directly excite the medial subdivision of the CeA (centromedial amygdala, CeM). In response to excitation by the BLA, projection neurons of the CeL and CeM target and regulate multiple regions implicated in anxiety, including the periaqueductal gray (PAG), bed nucleus of the stria terminalis (BNST), hypothalamus and dorsal vagal complex (DVC), to give rise to autonomic and motor responses^{7,14,15}. Thus, the excitatory output of the BLA to the CeA is translated into a behavioral reaction to aversive stimuli, including avoidance and freezing^{12,16}.

Amygdala fear vs. anxiety circuits

Much of what we know about emotional processing in the amygdala originates from studies on learned fear using the auditory fear conditioning paradigm^{7,17,18}. In this paradigm, which was originally developed to study the synaptic and circuit mechanisms that underlie memory formation, an animal is exposed to a series of auditory stimuli (known as conditioned stimuli, or CS) paired with foot shocks (known as unconditioned stimuli, or US). This exposure induces plasticity in the circuits that underlie defensive responses, such as freezing and flight, resulting in a fear response to subsequent auditory stimulus presentations even in the absence of the foot shock^{7,19}. While fear conditioning studies have contributed to elucidating the anatomical connections that underlie emotional processing in the amygdala, it is important to bear in mind that fear and anxiety are distinct emotions: fear is triggered by a real, definite threat and results in an acute and temporary response, while anxiety is activated by diffuse and less predictable threats and generates a long-lasting state of apprehension^{7,8,20–22}. Although the amygdala represents a key structure for the regulation of both sets of responses¹², it is becoming increasingly clear that the local processing of fear and anxiety information within the

amygdala likely involves entirely distinct (albeit partially overlapping) neural substrates^{7,9,12}. In the present review, we focus primarily on the circuits that mediate anxiety processing, which are substantially less well understood than those that underlie fear conditioning. For further information on the latter aspect, we refer the reader to several excellent recent reviews^{7,17,18}.

Amygdala inhibitory neurons in anxiety **Amygdala inhibitory neurons and the behavioral manifestations of anxiety**

The BLA and CeA arise from distinct cell lineages with substantially different inhibitory neuron populations: the BLA is a cortical-like structure that consists primarily of excitatory principal projection neurons with a small number of local inhibitory interneurons (10–20% of the total neuronal population in the BLA), while the CeA is a striatal-like structure that consists almost exclusively of inhibitory neurons, including both local interneurons as well as projection neurons to downstream effector regions^{12,15,23,24}. In addition to mediating the primary output of the CeA, inhibitory neurons play several roles in shaping the flow of information through the amygdala circuit.

First, interneurons suppress the activity of projection neurons in the BLA²⁴, indicating that BLA interneurons may serve to constrain the excitatory output of the BLA, and hence, the magnitude of the behavioral anxiety response. In support of this notion, hyperexcitability of the BLA is associated with pathological anxiety⁵, and a subpopulation of inhibitory neurons in the BLA persistently increases its firing during the behavioral manifestations of anxiety²⁵. In the CeA, inhibitory neurons in the CeL may constrain the activation of anxiety-promoting projection neurons in the CeM, as evidenced by the fact that optogenetic inhibition of the CeL or activation of the CeM both produce strong unconditioned freezing²⁶. Together, these data indicate that inhibitory neurons in the amygdala regulate its output to prevent an excessive behavioral response to anxiogenic stimuli, which is one of the core symptoms of anxiety disorders⁹.

Second, inhibitory neurons are thought to play a key role in defining the valence of incoming sensory stimuli. Depending on whether the sensory input to the BLA is associated with a threatening or a rewarding stimulus (which can be either innate or acquired), projection neurons of the BA will specifically excite brain regions that execute threat- or reward-related behaviors^{16,27,28}. The precise mechanism that matches rewarding or threatening stimuli with target-specific projection neurons in the BLA is largely unknown, but several studies have demonstrated that (1) there are non-overlapping populations of putative projection neurons that alter their firing rates specifically during the presentation of either rewarding or threatening

Table 1 Inhibitory neurons in the basal and central amygdala that are linked to the regulation of anxiety-related behaviors

Region	Cell type	Link to anxiety
BLA	PV ⁺	The number of neurons tends to be negatively correlated with avoidance in the OF ⁴⁰ Activated by the acute delivery of anxiogenic drugs ^{38,39} Optogenetic stimulation/suppression during the acquisition phase of fear conditioning bidirectionally modulates conditioned freezing ^{a45}
	SOM ⁺	Optogenetic activation during the acquisition phase of fear conditioning reduces conditioned freezing ⁴⁵
	CALB ⁺ PV ⁻	Suppressed by exposure to innately aversive stimuli ⁴⁸
	NK ₁ R ⁺	Selective lesioning increases avoidance in the EPM ⁴⁹
	CeL	PKC δ ⁺
CeL	SOM ⁺	Chemogenetic and optogenetic suppression during fear conditioning and fear retrieval reduces conditioned freezing ⁶¹ Optogenetic stimulation induces freezing in naïve mice ^{19,61}
	Htr2a ⁺ SOM ⁺	Pharmacological/chemogenetic/optogenetic inhibition increases freezing during exposure to innately aversive smell ⁶⁶
	CRF ⁺	Optogenetic stimulation decreases freezing and promotes flight during exposure to US following fear conditioning ^{a19} Optogenetic stimulation of CRH ⁺ terminals projecting from the CeA to the Locus Coeruleus increases avoidance ⁶⁵
CeC ^b	PKC δ ⁺	Optogenetic stimulation induces freezing in naïve mice ²⁸
CeM	Tac2 ⁺	Chemogenetic suppression prior to fear conditioning reduces conditioned freezing ⁶⁸ Optogenetic stimulation induces immobility-like behavior in naïve mice ²⁸

^a This manipulation does not alter freezing in naïve animals

^b A subdivision of CeL

stimuli and (2) optogenetic activation of these valence-specific neurons correspondingly raises defensive or appetitive behavioral responses^{12,27–29}. Critically, the initiation of defensive behaviors (such as avoidance) can only occur when appetitive behaviors (such as enhanced exploration/approach) are suppressed. Accordingly, emotionally salient stimuli activate interneurons in the BLA to suppress putative neurons of opposite valence³⁰, and inhibitory neurons in the BLA are thought to be as important for encoding stimulus valence as excitatory neurons²⁹. Therefore, interneurons in the BLA regulate the excitatory circuits that underlie opposing behaviors to prevent misinterpretation of the valence of sensory stimuli—another core symptom of anxiety disorders in which negative valence is assigned to non-threatening or even rewarding stimuli⁹.

Finally, inhibitory neurons in the amygdala are involved in gating the synaptic plasticity that underlies fear learning^{7,17}. While several recent studies have begun to dissect the role of individual interneuron subtypes in the regulation of learned fear, this mechanism likely does not contribute to the processing of anxiety information and will not be discussed further here. Instead, we will focus

specifically on the different inhibitory neuron populations that are implicated in the regulation of anxiety-related processing and defensive behaviors (see also Fig 1 and Table 1).

Inhibitory interneuron subtypes in the BLA

Interneurons in the BLA form local circuits that provide feedforward and feedback inhibition to projection neurons and other interneurons²⁴ (Fig. 1). Like cortical interneurons¹, they can be classified into multiple groups based on the differential expression of calcium binding proteins and neuropeptides, such as parvalbumin (PV), somatostatin (SOM, in other contexts also abbreviated SST), cholecystokinin (CCK), calbindin (CALB) and calretinin (CR). These groups include: (1) PV⁺/CALB⁺ (referred to as PV⁺ interneurons in this review), (2) SOM⁺/CALB⁺ (referred to as SOM⁺ interneurons; a subset of which also express neuropeptide Y, NPY), (3) CCK⁺/CALB⁺ (referred to as CCK⁺ interneurons), and (4) CR⁺ (a subset of which also express CCK and/or vasoactive intestinal peptide (VIP, referred to as VIP⁺ interneurons below))²⁴. The different interneuron types vary in the size of their soma and the shape of their

dendritic tree, and although they all target local neurons within the BLA, they contact distinct compartments of their postsynaptic targets²⁴. While it is widely accepted that inhibition in the BLA must play a critical role in the regulation of anxiety (reviewed in ref⁵), this knowledge is largely based on the facts that hyperexcitability of the BLA is associated with pathological anxiety and that intra-BLA injections of GABA receptor agonists and antagonists modulate anxiety behaviors^{5,20}. Here, we summarize what is known about the contribution of individual interneuron populations in the BLA to the regulation of normal and pathological anxiety, a question that to date has received surprisingly little attention.

PV⁺ interneurons in the BLA

PV⁺ interneurons comprise the largest group of inhibitory neurons in the BLA, forming 50% of its interneuronal population. The majority of these cells are fast-spiking basket cells that synapse onto the soma of principal projection neurons¹⁵ (although see ref³¹), but non-basket PV⁺ interneurons that target axon initial segments and distal dendrites exist, and all three groups powerfully control and synchronize the output of BLA excitatory neurons^{32,33}. PV⁺ basket cells in the LA provide both feedforward and feedback inhibition onto the LA principal neurons to regulate the flow of information into the BLA^{34,35}. Moreover, PV⁺ interneurons in the BLA form both electrically and chemically coupled networks, indicating that, like in the cortex, they can regulate information processing by generating and maintaining oscillatory activity^{36,37}.

While there have been no studies that directly record or manipulate PV⁺ interneurons in the BLA during anxiety behaviors, several lines of indirect evidence support such a role. Acute administration of anxiogenic drugs increases the expression of the immediate early gene cFos, a marker of neuronal activity, in PV⁺ interneurons in the BLA³⁸. This response is attenuated by post-weaning social isolation, which leads to anxiety-like behavior in adult rodents³⁹. Conversely, rearing rats in an enriched environment reduces anxiety and results in an increased number of PV⁺ interneurons in the BLA, which positively correlates with decreased anxiety⁴⁰. Moreover, the inhibitory function of PV⁺ interneurons in the BLA is regulated by serotonin and possibly by corticotropin releasing factor (CRF, also known as corticotropin releasing hormone, CRH), both of which are linked to anxiety-related disorders^{41–43}. Loss of function of the serotonin 5HT_{2A} receptor reduces PV network activation in the BLA during the processing of aversive stimuli, and this mechanism may underlie the impaired oscillatory activity of the BLA that has been linked to increased fear generalization, a manifestation of anxiety^{42,44}. Together, these data indicate that PV⁺ interneurons in the BLA have an important

regulatory function in anxiety, but also that additional and more direct experiments are required to confirm and fully understand this role.

SOM⁺ interneurons in the BLA

SOM⁺ interneurons constitute 15% of BLA interneurons and regulate excitatory transmission by forming synapses onto dendritic spines and distal dendrites of the BLA projection neurons^{17,45,46}. SOM⁺ interneurons receive inhibitory contacts from PV⁺ interneurons, which allow PV⁺ neurons to disinhibit the distal dendrites of BLA projection neurons via feedforward inhibition of SOM⁺ neurons^{45,46}. During fear conditioning, this PV-SOM microcircuit controls the freezing response to auditory stimuli, and fittingly, optogenetic excitation of SOM⁺ neurons decreases freezing in fear-conditioned animals⁴⁵. While similar studies have yet to be performed for anxiety-related processing, first evidence comes from a study showing that brain-wide disinhibition and hence activation of SOM⁺ interneurons had anxiolytic consequences in an EPM⁴⁷. The specific contribution of BLA SOM⁺ interneurons to this effect remains unknown, but in a separate study, EPM exposure resulted in the activation of putative SOM⁺ neurons in the BLA, as assessed by cFos staining⁴⁸. This indicates that under anxiogenic conditions, SOM⁺ neurons may be activated to constrain anxiety responses. Consistent with this notion, NPY⁺ (but not NPY⁻) SOM⁺ interneurons express the neurokinin 1 receptor (NK_{1r}), and selective lesioning of NK_{1r}⁺ neurons in the BLA increases anxiety-related behaviors⁴⁹. However, a subset of CCK⁺/CALB⁺ interneurons are also NK_{1r}-positive⁴⁹, and the relative contribution of these different interneuron subtypes to the anxiogenic effect of NK_{1r}-mediated lesions remains unclear.

CCK⁺ interneurons in the BLA

CCK⁺ interneurons are divided into two groups based on the size of their soma: (1) large (L)-CCK⁺ neurons that co-express CALB and (2) small (S)-CCK⁺ neurons that co-express CR and VIP⁵⁰. CCK⁺ interneurons are as effective as PV⁺ interneurons at inhibiting the output of projection neurons, and collectively, PV⁺ interneurons and (L)-CCK⁺ interneurons contribute approximately 70% of the perisomatic basket synapses onto a given projection neuron in the BLA^{32,51}. An important distinction between PV⁺ and (L)-CCK⁺ interneurons is that the latter express the cannabinoid receptor type I (CB1)^{17,52}, which predestines CCK⁺ neurons to mediate the anxiety-modulating effects of endocannabinoids (reviewed in ref⁵³). Moreover, a subset of CCK⁺ neurons were affected by the anxiogenic lesion of NK_{1r}⁺ interneurons in the BLA⁴⁹, indicating that these neurons may also contribute to the regulation of anxiety circuits.

VIP⁺ interneurons in the BLA

VIP⁺ interneurons in the BLA preferably innervate distal dendrites, but they also form perisomatic basket synapses onto both projection neurons and a subset of CALB⁺ interneurons⁵⁰. While the role of BLA VIP⁺ neurons in the regulation of anxiety-related behaviors is entirely unknown, recent studies in the cortex have identified a disinhibitory function of VIP⁺ neurons in cortical processing through inhibition of SOM⁺ neurons^{54,55}. It will be interesting to determine whether VIP⁺ neurons play a similar role in the BLA anxiety circuitry, particularly in light of recent evidence that inhibition of BLA SOM⁺ neurons by currently undetermined types of interneurons is required for the expression of the conditioned fear response⁴⁵.

Inhibitory neuron subtypes in the CeA

Inhibitory projection neurons in the CeA translate threat-related stimuli into behavioral manifestations of anxiety, including freezing, avoidance, and autonomic responses (Fig. 1). Specifically, CeL neurons form local inhibitory microcircuits (described in detail below) that receive threat-related excitatory inputs from the BLA and either inhibit or disinhibit projection neurons in the CeM^{26,56,57}. The CeM is the major output nucleus of the amygdala and plays a pivotal role in mediating anxiety-promoting behavioral responses via its inhibitory projections to downstream targets^{14,15}. The CeM receives excitatory inputs from threat-encoding projection neurons in the BLA and inhibitory inputs from the CeL, and the extent of CeM output and hence of anxiety behavior is determined by the balance between these two inputs^{16,26,27}. Accordingly, substances that increase inhibitory input to the CeM produce anxiolytic effects^{23,26,58}, and several studies have demonstrated that an increase in the general inhibitory tone within the CeM reduces responses to anxiogenic stimuli²³. For example, activation of excitatory CeM-targeting projection neurons in the BLA increases avoidance behavior¹⁶, and activation of CeM projection neurons via vasopressin receptors (VPRs) has been proposed to be a mechanism underlying the anxiogenic effects of vasopressin⁵⁹. Moreover, firing of CeM neurons increases during freezing in response to aversive stimuli, supporting a role for the CeM in the production of fear and anxiety-related behaviors⁶⁰.

Inhibitory neurons in the CeL

The CeL consists of two non-overlapping populations of striatal-like GABAergic medium spiny neurons, which can be distinguished by their expression of the markers SOM and protein kinase C δ (PKC δ)^{23,56,57,61}. Additionally, recent studies have identified small and partially overlapping populations of neurons that express the markers CRF/CRH, tachykinin 2 (Tac2), neurotensin

(Nts), and serotonin receptor 2a (Htr2a, encoding the 5HT_{2A} receptor)^{19,28}.

Arguably the best-studied inhibitory neurons in the amygdala anxiety circuitry are the PKC δ ⁺ neurons of the CeL, which are believed to form a monosynaptic connection with PAG-projecting neurons of the CeM^{56,61}. Optogenetic stimulation of CeL PKC δ ⁺ neurons modulates avoidance behavior during the OF, EPM and LDB tests^{62,63}, but whether this modulation is anxiogenic or anxiolytic appears to depend on the precise experimental conditions^{62,63}. PKC δ ⁺ neurons express the oxytocin receptor (OTR) and likely mediate the oxytocin-induced suppression of PAG-projecting CeM output neurons that attenuate fear responses^{56,58,59}, which is indicative of an anxiolytic effect of PKC δ ⁺ neurons. Additionally, the activity of PKC δ ⁺ neurons predicts the ability to discriminate between neutral and threat-predicting stimuli, and thus, CeL PKC δ ⁺ neurons may contribute to anxiety-related fear generalization^{26,63}.

PKC δ ⁺ neurons, in turn, are tightly regulated by local inhibitory connections with SOM⁺ neurons. Optogenetic activation of SOM⁺ neurons lifts the inhibitory control of PKC δ ⁺ neurons over the CeM and induces freezing in the absence of a threat in naïve mice^{19,56,57,61}, although this may also be partially mediated by SOM⁺ neurons that bypass the CeM and directly project to the PAG⁶⁴. In addition to inhibiting PKC δ ⁺, SOM⁺ neurons form mutually inhibitory connections with CeL CRF⁺ neurons, and during fear conditioning, this network determines the balance between conditioned flight and freezing behaviors¹⁹. Whether a similar mechanism contributes to the anxiety circuitry remains to be determined, but it was recently shown that stimulation of CeL CRF⁺ projections to the locus coeruleus produces robust anxiety-like behavior in the OF and elevated zero maze (EZM) tests⁶⁵. Finally, a subpopulation of SOM⁺ neurons also express the serotonin receptor Htr2a/5HT_{2A}^{28,66}, and inhibition of these neurons in rodents (either by systemic application of a Htr2a antagonist or by means of local manipulation using chemogenetic and optogenetic tools) enhances an innate freezing response to a fox odor, possibly by regulating dorsal PAG while simultaneously suppressing freezing to conditioned aversive stimuli via disinhibition of PKC δ ⁺ neurons⁶⁶. These data indicate that activation of Htr2a⁺ neurons by serotonin may have an anxiolytic effect by suppressing innate fear responses, consistent with the observation that reduced levels of serotonin in the amygdala are associated with anxiety in humans⁶⁷.

Inhibitory neurons in the CeM

Unlike in the CeL, where substantial progress has been made in elucidating the role of distinct neuronal populations in threat-related processing and the generation of

anxiety responses, surprisingly little remains known about similar functions in the CeM^{28,68}. A recent study identified three non-overlapping neural populations in the CeM that express either the SOM, Tac2, or Nts genes²⁸. This study demonstrated that optogenetic stimulation of Tac2⁺ neurons in the CeM elicited immobility-like behavior in naïve mice, in agreement with previous findings that showed that inhibition of Tac2⁺ neurons in the CeA impaired CS-elicited freezing in fear-conditioned mice (although importantly, this manipulation had no effect on avoidance behaviors during the OF and EPM tests)⁶⁸. The CeM contains a population of neurons that express receptors to vasopressin and orexin, which have both been hypothesized to modulate fear-related circuits^{59,69}, but how these neuromodulators might affect anxiety circuitry and behavior has not been assessed thus far. Therefore, the populations of CeM neurons that might mediate the various behavioral manifestations of anxiety remain largely unknown.

Overlapping circuits for anxiety, fear, and appetitive behaviors

An interesting additional finding arising from the above studies is that the role of inhibitory neurons in amygdala anxiety circuits overlaps substantially not only with fear circuits, but also with circuits that mediate appetitive behaviors. For example, optogenetic activation of the neurons in the CeM elicits strong unconditioned freezing²⁶, but, surprisingly, also promotes appetitive behaviors²⁸. Similarly, PKC δ ⁺ and SOM⁺ neurons in the CeL are not only implicated in anxiety behaviors, but also in the regulation of feeding and reward-triggered approach^{28,62}. While the exact mechanism of how the same population of neurons may mediate behaviors of opposite valence has yet to be determined, it is possible that individual members of the same population project to distinct regions, and, thus, regulate different behaviors; that various degrees of engagement of mutually inhibitory connections during experimental activity manipulations may result in indirect effects; or that the same neurons indeed mediate distinct behaviors of opposite valence^{14,56–58,70}. In either scenario, these multifaceted roles highlight the difficulty in identifying and targeting neuronal populations that may specifically regulate anxiety behaviors and underline the need to fully understand the circuitry to establish selective therapeutic approaches. This includes not only the cellular components of the circuitry, but also the molecular machinery that regulates synaptic transmission within the amygdala inhibitory network.

Molecular determinants of anxiety in the amygdala

All neurons communicate with each other through synaptic connections, and accordingly, the molecular composition and function of these synapses play key roles in regulating the flow of information through neuronal networks. The efficacy of synaptic transmission can be modified by genetic or pharmacological influences, and several lines of evidence indicate that alterations in the function of inhibitory synapses can substantially influence anxiety processing and regulate anxiety-related behavioral output. First, it has long been known that the benzodiazepine class of anxiolytic drugs, still widely used in the treatment of anxiety disorders, act as GABA_A receptor (GABA_AR) agonists^{4,6}. Second, an increasing list of genetic variants in the molecular components of inhibitory synapses have been linked to pathological anxiety in humans^{71–73} and/or anxiety behaviors in mice^{73–82}. Together, these findings indicate that a detailed understanding of the synaptic and circuitry mechanisms that link alterations in inhibitory synapse components to pathological anxiety is essential, and that studies using genetic animal models of anxiety disorders will provide critical complementary insights to studies on the circuitry underlying normal, adaptive anxiety in wild-type mice using the modern circuitry approaches described above. This is particularly true given the notion that anxiety disorders have a strong developmental component²¹ and that genetic and environmental influences may induce alterations in brain wiring, such that the circuits underlying pathological anxiety may be substantially different from those that mediate adaptive anxiety. To date, however, surprisingly little is known about the specific functions of the known inhibitory synapse components in the amygdala anxiety circuitry. Here, we summarize the current state of knowledge on amygdala GABA_ARs, GABA_BRs, glycine receptors, and inhibitory synapse organizers in anxiety processing (Fig. 2, Table 2, Table 3).

GABA_A Receptors

Fast inhibitory synaptic transmission is primarily mediated by ionotropic GABA_ARs, which are pentameric chloride channels that are composed of various combinations of 19 different subunits (α 1-6, β 1-3, γ 1-3, δ , ϵ , θ , π , ρ 1-3)^{4,5,20,83}. While many different combinations of these subunits exist, the most common ones contain two α -subunits, two β -subunits, and one γ -subunit. Different GABA_AR subunits are differentially distributed with respect to their regional expression, as well as their subcellular targeting to different synapse types (perisomatic, dendritic, axo-axonal etc.), and each subunit confers distinct electrophysiological and pharmacological properties on the receptor. Importantly in the context of the anxiety circuitry, only specific GABA_AR subunits act as targets for

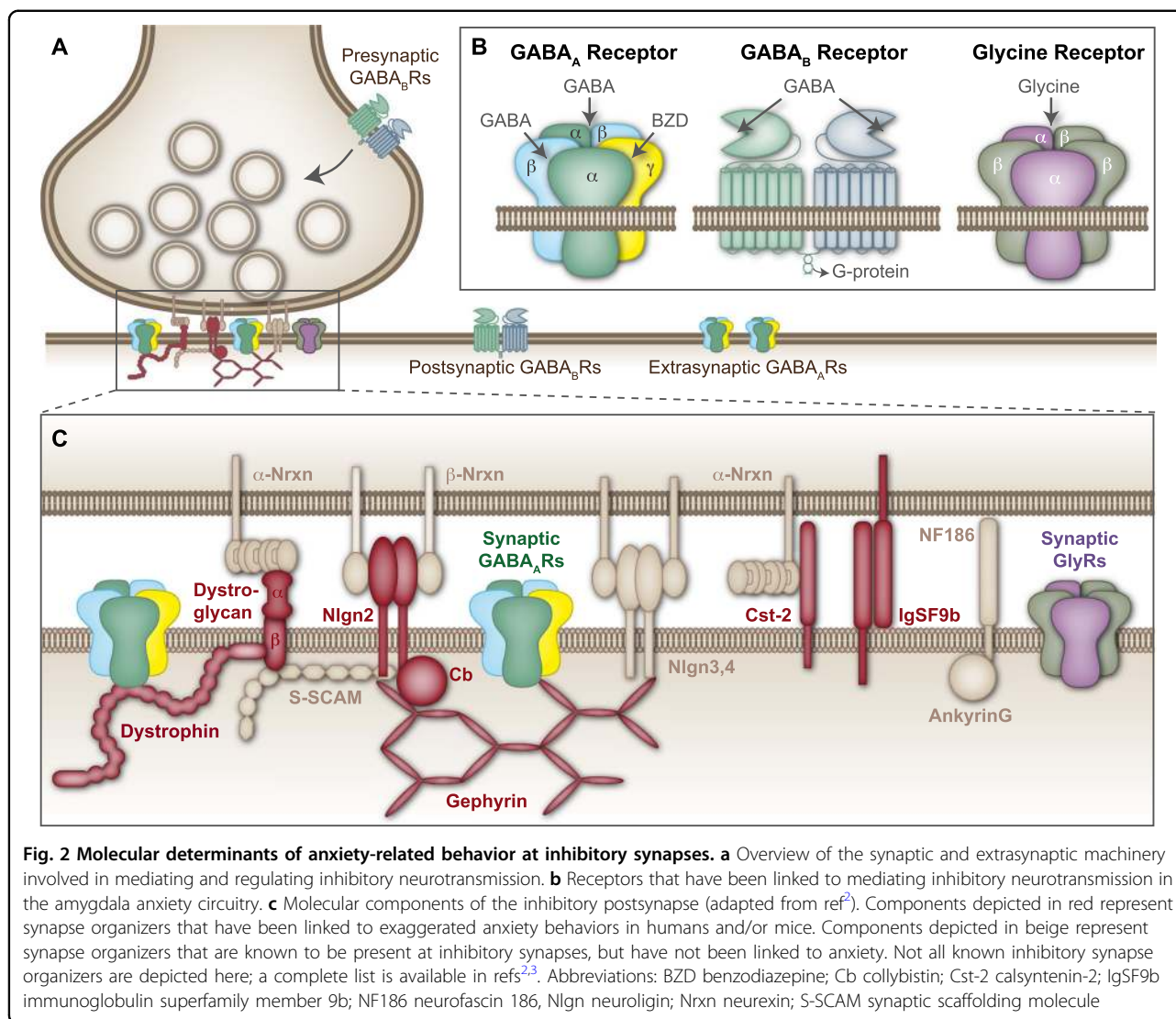


Fig. 2 Molecular determinants of anxiety-related behavior at inhibitory synapses. **a** Overview of the synaptic and extrasynaptic machinery involved in mediating and regulating inhibitory neurotransmission. **b** Receptors that have been linked to mediating inhibitory neurotransmission in the amygdala anxiety circuitry. **c** Molecular components of the inhibitory postsynapse (adapted from ref²). Components depicted in red represent synapse organizers that have been linked to exaggerated anxiety behaviors in humans and/or mice. Components depicted in beige represent synapse organizers that are known to be present at inhibitory synapses, but have not been linked to anxiety. Not all known inhibitory synapse organizers are depicted here; a complete list is available in refs^{2,3}. Abbreviations: BZD benzodiazepine; Cb collybitin; Cst-2 calyntenin-2; IgSF9b immunoglobulin superfamily member 9b; NF186 neurofascin 186; Nlgn neuroligin; Nrnx neurexin; S-SCAM synaptic scaffolding molecule

benzodiazepines (see below for details), making it essential from a therapeutic perspective to understand the mechanisms that govern the differential distribution of the many subtypes of GABA_ARs.

γ-subunits

The most abundant GABA_AR subunit in the CNS is the γ2-subunit, which is estimated to be present in at least 90% of all GABA_ARs in the forebrain^{4,83}. The γ2-subunit is highly expressed throughout the amygdala of rodents⁸⁴ and humans⁸⁵, and several lines of evidence support a key role for γ2-GABA_ARs in the anxiety circuitry (see also Table 2). First, benzodiazepines bind to the interface between the α- and γ-subunits, and only γ2-containing GABA_ARs (γ2-GABA_ARs) are sensitive to classical benzodiazepines⁴. Second, auto-antibodies to the γ2-subunit have recently been identified in patients with a range of psychiatric symptoms that include anxiety⁸⁶, indicating

that alterations in γ2-GABA_ARs may contribute to the etiology of anxiety disorders. Third, while homozygous deletion of the γ2-subunit in mice is lethal⁸³, heterozygous γ2-subunit knockout mice or mice with reduced γ2-subunit expression are viable and display increased anxiety behaviors in the EPM, LDB, and FCE paradigms^{83,87}. Deletion of the γ2-subunit from excitatory forebrain neurons early in development (using Emx1-Cre), but not later in postnatal development (using CaMKII-Cre), reproduces these phenotypes⁸⁸, consistent with a developmental origin of anxiety²¹. Interestingly, deletion of the γ2-subunit specifically from PV⁺ or SOM⁺ neurons resulted in a disinhibitory, anxiolytic effect^{47,89}, indicating that γ2-GABA_ARs can have opposing effects on anxiety depending on whether they are expressed in excitatory vs. inhibitory neurons. To which extent these phenotypes are mediated by amygdala-specific functions of γ2-GABA_ARs remains largely unknown.

Table 2 Inhibitory receptors that are linked to the amygdala anxiety circuitry

Protein	Involved in human anxiety	Anxiety phenotype in mouse models	Function in amygdala
$\gamma 1$ -GABA _A R	Unknown	Unknown	Enriched in the CeA, may function at specific synapses in the CeL ^{90,91}
$\gamma 2$ -GABA _A R	BZD binding ^{4,83} ; autoantibodies in patients with anxiety ⁸⁶	Het: Increased anxiety ^{83,87} cKO (Emx1-Cre, developmental): Increased anxiety; cKO (CaMKII-Cre, postnatal): Normal anxiety ⁸⁸ . cKO (PV-Cre or SOM-Cre): Decreased anxiety	Highly expressed throughout the amygdala ^{84,85}
$\alpha 1$ -GABA _A R	Sedative but not anxiolytic effects of BZD ^{4,83}	H/R-KI: No change in the anxiolytic properties of BZD ⁸³ KO, cKO (amygdala): No effect on anxiety ^{83,94} cKO (CRF-Cre): Increased anxiety ⁹⁵	Highly expressed in the BLA, moderately expressed in the CeM, and absent in the CeL ^{84,85,92} Mediates BZD-sensitive IPSCs in the BLA but not the CeA ⁹² .
$\alpha 2$ -GABA _A R	Anxiolytic effects of BZD ^{4,83}	H/R-KI: Abolishes the anxiolytic properties of BZD ⁸³ KO: Increased anxiety, unresponsive to BZDs ^{97,98}	Expressed throughout the BLA and the CeA, particularly prominent in the CeL ^{84,85,92}
$\alpha 3$ -GABA _A R	Anxiolytic effects of BZD? ^{83,101}	H/R-KI: No change in the anxiolytic properties of BZD ⁸³ KO: No anxiety ^{83,101}	Expressed prominently in the BLA and CeA ^{84,85,92} Primarily extrasynaptic in the BLA ¹⁰⁰
$\alpha 5$ -GABA _A R	Anxiolytic effects of BZD? ¹⁰¹	KO: Normal anxiety ⁴ KD in CeL PKC δ^+ neurons: Increased anxiety ⁶³	Expressed at low to moderate levels in the BLA and CeA ^{84,85,92} , extrasynaptic ⁶³
GABA _B R	Anxiolytic effects of agonists ^{77,78}	KO: Increased anxiety ^{77,78}	Expressed throughout the CNS ¹⁰²
β -GlyR	Variants associated with panic disorder ⁷³	Glr ^{b+/spa} mice: Increased anxiety ⁷³	Expressed in the BLA and the CeA ¹⁰³ ; GlyR-mediated currents detected in BLA and CeA ¹⁰⁴

While the $\gamma 2$ -subunit is dominant, the CeA also contains a striking enrichment of $\gamma 1$ -GABA_ARs^{84,90}. These receptors have been proposed to function specifically at synapses in the CeL that are formed by projections originating in the intercalated nuclei that create feedforward inhibition from the BLA to the CeA⁹⁰, and they confer substantially different physiological and pharmacological properties onto GABAergic transmission at these synapses^{90,91}. Whether these receptors have any relevance to anxiety processing remains to be determined.

α subunits

In addition to the γ -subunit, virtually all GABA_ARs contain two α -subunits, which form the other half of the binding site for benzodiazepines. In the late 1990s, the role of each of the α -subunits in mediating the effects of benzodiazepines was investigated in a seminal series of studies using mice that expressed α -subunit point mutants lacking benzodiazepine sensitivity due to a histidine-to-arginine (H/R) substitution (summarized in ref 83). These studies concluded that the primary anxiolytic effect of benzodiazepines is mediated by $\alpha 2$ -GABA_ARs, with a lesser potential contribution from $\alpha 3$ -GABA_ARs, while $\alpha 1$ -GABA_ARs specifically mediate the sedative but not anxiolytic effects of benzodiazepines⁸³.

Here, we summarize what is known about the role of the individual α -subunits specifically in the amygdala (see also Table 2).

$\alpha 1$ -GABA_ARs are expressed prominently throughout the BLA and, to a lesser extent, the CeM, but are strongly reduced or absent in the CeL, at least in rodents^{84,85,92}. Interestingly, the subunit composition in the BLA appears to shift between $\alpha 1$ - and $\alpha 2$ -GABA_ARs during early postnatal development, indicating that these subunits may play different roles during development⁹³. Functionally, $\alpha 1$ -GABA_ARs contribute substantially to the benzodiazepine-mediated potentiation of IPSCs in the BLA, but not the CeA⁹². Neither constitutive deletion of the $\alpha 1$ -subunit⁸³ nor conditional deletion specifically in the amygdala⁹⁴ had an effect on anxiety behaviors, although the latter reduced benzodiazepine-induced sedative effects. Specific deletion of the $\alpha 1$ -subunit from CRF-expressing neurons in the amygdala, BNST, and paraventricular nucleus resulted in a prominent anxiety behavior during the EPM and OF tests, but this may have been largely due to the role of $\alpha 1$ -GABA_ARs in the BNST⁹⁵. Together with the data from the benzodiazepine-insensitive point mutants described above⁸³, these data indicate that $\alpha 1$ -GABA_ARs in the amygdala may play a relatively small role in anxiety behaviors.

$\alpha 2$ -GABA_ARs are expressed throughout the BLA and CeA, with a particularly prominent expression in the CeL^{84,85,92}. It has been proposed that the majority of the functional GABA_ARs in both the BLA and CeA show a profile consistent with $\alpha 2\beta\gamma 2$ receptors^{90,92}. In the BLA, $\alpha 2$ -GABA_ARs are particularly enriched on the axon initial segment⁹⁶. $\alpha 2$ -subunit KO mice show increased anxiety in the FCE, LDB, and CER paradigms, as well as a reduced anxiolytic response to benzodiazepines^{97,98}, consistent with the notion that $\alpha 2$ -GABA_ARs are the primary mediators of the anxiolytic effects of benzodiazepines⁸³. However, the extent to which these effects are specifically mediated by $\alpha 2$ -GABA_ARs in the amygdala remains largely unknown. Conditional deletion of the $\alpha 2$ -subunit in the hippocampus was recently shown to abolish benzodiazepine-induced anxiolytic effects without altering basal anxiety in an EPM⁹⁹. However, to our knowledge, similar data are not yet available for the amygdala.

$\alpha 3$ -GABA_ARs are prominently expressed in both the BLA and CeA^{84,85,100}. In the BLA, $\alpha 3$ -GABA_ARs appear to be primarily localized extrasynaptically, where they play a central role in mediating the tonic inhibition activated by synaptic spillover¹⁰⁰. The role of $\alpha 3$ -GABA_ARs in mediating anxiety behaviors is controversial: while the $\alpha 3$ -subunit-specific agonist TP003 induces anxiolytic effects in rodents, the (H/R) $\alpha 3$ -subunit point mutation does not alter the anxiolytic effects of benzodiazepines, and constitutive $\alpha 3$ -subunit KO mice show no anxiety phenotype^{83,101}.

$\alpha 5$ -GABA_ARs, which also mediate extrasynaptic tonic inhibition⁶³, are expressed at low to moderate levels throughout the BLA and CeA^{84,85}, in contrast to their high expression levels in the hippocampus. Accordingly, deletion of the $\alpha 5$ -subunit in mice results in abnormalities in learning and memory but normal anxiety levels, and the $\alpha 5$ -subunit has been primarily studied as a target for cognitive enhancers rather than anxiolytic therapies⁴. More recently, however, it was shown that extrasynaptic $\alpha 5$ -GABA_ARs in the CeA exert an anxiolytic effect through tonic inhibition of PKC δ ⁺ neurons in the CeL⁶³. Moreover, in a recent study using the benzodiazepine-sensitive point mutants of the α -subunits, the predominant anxiolytic effects of diazepam in the EPM and LDB resulted from the actions of diazepam at $\alpha 5$ -GABA_ARs, but not at $\alpha 2/3$ -GABA_ARs¹⁰¹. Together, these results indicate that the $\alpha 5$ -subunit may play a more important role in the anxiety circuitry than previously appreciated.

GABA_B Receptors

GABA not only mediates fast inhibitory neurotransmission through its effects at GABA_ARs, but also has modulatory effects through metabotropic GABA_BRs. GABA_BRs are G_{i/o}-protein coupled receptors that consist

of two subunits, GABA_{B(1)} and GABA_{B(2)}^{77,78,102}. GABA_BRs are expressed almost universally throughout the CNS, and they inhibit neuronal activity through both presynaptic (inhibition of neurotransmitter release) and postsynaptic mechanisms (activation of inwardly rectifying potassium channels, resulting in membrane hyperpolarization)¹⁰². Evidence for a role of GABA_BRs in anxiety processing comes from two avenues^{77,78}: (1) GABA_BR agonists such as baclofen and GABA_BR-positive allosteric modulators have anxiolytic effects in both humans and rats; and (2) KO mice for both the GABA_{B(1)} and GABA_{B(2)} receptor subunits display prominent anxiety-like behaviors^{77,78}. However, the specific mechanisms by which GABA_BRs modulate amygdala anxiety circuits remain largely unexplored and are likely to be highly complex.

Glycine Receptors

A second inhibitory neurotransmitter in the mammalian CNS is the amino acid glycine. Like GABA_ARs, glycine receptors (GlyRs) are pentameric ligand-gated chloride channels that are assembled from a family of five subunits, the $\alpha 1-4$ and β subunits^{103,104}. Glycinergic transmission is well documented in the spinal cord, retina, and brainstem, but its role in the forebrain has received substantially less attention^{103,104}. Nevertheless, GlyRs are expressed throughout the forebrain, including in both the BLA and CeA¹⁰³, and GlyR-mediated currents can be observed in the BLA and CeA¹⁰⁴. Interestingly, variants in the β -subunit were recently associated with agoraphobia, an anxiety disorder, and mice with reduced β -subunit levels (*Glr β* ^{+/*spa*} mice) showed increased anxiety in an OF test⁷³. Further exploration of the role of GlyR-mediated inhibition in the amygdala anxiety circuits is therefore warranted.

Inhibitory synapse organizers

In addition to the receptors that directly mediate inhibitory synaptic transmission, all inhibitory synapses contain a number of postsynaptic and transsynaptic scaffolding proteins that are essential in organizing their structure and function^{2,3}. Intriguingly, mutations in several of these molecules have been linked to psychiatric disorders, including anxiety disorders and other comorbid conditions. Here, we summarize what is known about the function of these molecules specifically in the amygdala and/or in anxiety behaviors (see also Fig 2 and Table 3).

Gephyrin

Gephyrin is the central postsynaptic scaffolding protein at inhibitory synapses, and it plays a key role in the clustering of GABA_ARs and GlyRs, as well as in numerous intracellular signaling pathways^{105,106}. Gephyrin

Table 3 Inhibitory synapse organizers that are linked to the amygdala anxiety circuitry

Protein	Involved in human anxiety	Anxiety phenotype in mouse models	Function in amygdala
Gephyrin	Unknown	cKO (CaMKII): Increased anxiety ⁷⁹	Expressed throughout the amygdala ^{108,109}
Nlgn2	Genetic variant associated with anxiety ⁷²	KO: increased anxiety ^{74,75} R215H KI: increased anxiety ¹¹¹ cKO (PFC): decreased anxiety ¹¹³ Overexpression: increased anxiety ¹¹²	Expressed in the BLA and to a lesser extent in the CeA ⁷⁴ ; decreased mIPSCs in the BA, no effect in the CeA; decreased perisomatic GABA _A Rs in the BA ⁷⁴
Nlgn3	Unknown	KO: normal anxiety ¹¹⁵	Unknown
Nlgn4	Unknown	KO: normal anxiety ¹¹⁴	Unknown
Cb	Genetic variants associated with anxiety ⁷¹	KO: increased anxiety ⁷⁶	Expressed in the BLA; decreased gephyrin, GABA _A R levels in the BLA ⁷⁶
Dystrophin	Increased anxiety in DMD	KO: complex anxiety phenotype ^{80,81}	Expressed in the BLA but not the CeA; decreased GABA _A Rs and mIPSCs in the BLA ^{80,82}
Dystro-glycan	Unknown	Unknown	Expressed at low levels in the amygdala ¹¹⁷ .
Cst-2	Unknown	KO: complex anxiety phenotype ^{119,120}	Highly expressed in the BLA, weakly expressed in the CeA ¹¹⁸
NF186	Unknown	cKD (amygdala): impaired fear extinction, but normal anxiety ^{121,122}	Localized to the axon initial segment in the BLA; reduced mIPSCs in amygdala-specific KD ^{121,122}
IgSF9b	Variants associated with depression ²	KO and cKD (CeA): decreased anxiety (Babaev and Krueger-Burg, unpublished data)	Expressed throughout the BLA and the CeA (Babaev and Krueger-Burg, unpublished data)

mutations have not been directly linked to anxiety disorders in humans, but are associated with autism, schizophrenia, and epilepsy¹⁰⁷. Consistent with the central role of gephyrin in regulating synaptic inhibition, constitutive gephyrin KO mice die shortly after birth, but conditional deletion specifically in excitatory neurons of the forebrain using a CaMKII-Cre driver line results in an increased anxiety phenotype⁷⁹. Gephyrin is expressed throughout the brain, including in both the BLA and CeA in humans¹⁰⁸ and rodents¹⁰⁹. The role of gephyrin in clustering GABA_ARs has not been studied specifically in the amygdala, but in other brain regions, gephyrin plays a critical role in binding to γ 2- or α 2-containing GABA_ARs^{105,106}, which mediate the anxiolytic responses of benzodiazepines as described above⁸³.

Neuroigin-2 (Nlgn2)

Nlgn2 is an inhibitory synapse-specific member of the Neuroigin (Nlgn) family of synaptic adhesion molecules, which regulate synaptic structure and function through interactions with their presynaptic Neurexin (Nrxn) binding partners^{2,3,110}. A nonsense variant in Nlgn2 was recently identified in a patient with severe anxiety and autism⁷², in addition to Nlgn2 mutations previously associated with schizophrenia². In mice, both the deletion of Nlgn2 and a schizophrenia-associated Nlgn2 mutation, R215H, result in severe anxiety phenotypes^{74,75,111}. Nlgn2

is expressed both in the BLA and (to a lesser extent) CeA of mice⁷⁴, but interestingly appears to play very different roles in these two structures. In the BA, deletion of Nlgn2 results in a prominent reduction in perisomatic, but not dendritic clusters of gephyrin and GABA_AR α 1, as well as a reduced mIPSC frequency, and this reduction in inhibition is accompanied by an overactivation of BA principal neurons under anxiogenic conditions⁷⁴. In contrast, in the CeM, Nlgn2 deletion has only very minor consequences for synaptic inhibition⁷⁴, indicating that Nlgn2 may play different roles at inhibitory synapses excitatory and inhibitory neurons. Interestingly, overexpression of Nlgn2 in mice also induces an anxiety phenotype¹¹², while local deletion of Nlgn2 specifically in the PFC of adult mice has an anxiolytic effect¹¹³, indicating that Nlgn2 may also have a complex role in the anxiety circuitry outside of the amygdala. Unlike Nlgn2, two members of the Nlgn family that are found at inhibitory synapses, Nlgn3 and Nlgn4, are not known to affect the anxiety behaviors of either humans or mice^{2,114,115}, indicating that Nlgn2 may play a distinct and unique role in the anxiety circuitry.

Collybistin (Cb)

Cb is a guanine exchange factor (GEF) that regulates inhibitory synapse function through interactions with gephyrin and Nlgn2¹¹⁰. Human variants in Cb have been associated with anxiety, as well as with epilepsy and

intellectual disability⁷¹, and deletion of Cb in mice results in a severe anxiety phenotype⁷⁶. Cb is expressed in the BLA, where its deletion results in a prominent loss of gephyrin and GABA_AR α 2 clusters without altering GlyRs or VIAAT puncta⁷⁶. While inhibitory synaptic transmission was not assessed in the amygdala, mIPSCs in the hippocampus were reduced in both frequency and amplitude. Cb may play a particularly important role in the clustering of GABA_AR α 2 subunits (at least when transfected into heterologous HEK cells)¹¹⁶, which in turn may play a particularly important role in anxiety⁸³.

Dystrophin glycoprotein complex (DGC)

The DGC, which links the cytoskeleton to the extracellular matrix, is best known for its role at the neuromuscular junction and its involvement in Duchenne muscular dystrophy (DMD)^{80,82}. More recently, however, it has also been shown to play an important role in the formation of inhibitory synapses in the forebrain^{2,80}. Dystrophin, the intracellular component of the DGC, is expressed in the BLA, but not the CeA in mice^{80,82}, and dystrophin KO mice (mdx mice, a mouse model of DMD) show reduced clusters of GABA_AR α 2 and altered inhibitory synaptic transmission in the BLA. Behaviorally, these mice are characterized by increased defensive behaviors in response to restraint stress^{80,81}, impaired cued fear conditioning⁸², and reduced locomotion and increased anxiety in an OF⁸¹, but not an EPM paradigm⁸⁰. Dystroglycan, the transmembrane complex of the DGC, is expressed in mouse amygdala at low levels¹¹⁷. Its function in the amygdala has not been studied, although in other brain regions, deletion of dystroglycan impairs the function of GABAergic synapses². Given that DMD is associated with psychiatric phenotypes including anxiety, in addition to muscular dystrophy, it is conceivable that impaired inhibitory synaptic transmission may contribute to these symptoms.

Calsyntenin-2 (Cst-2)

Cst-2 is an inhibitory synapse-specific member of the Cadherin superfamily of cell adhesion proteins^{118,119}. In mice, Cst-2 is highly expressed in the BLA and weakly in the CeA¹¹⁸. The consequences of Cst-2 deletion in the amygdala have not been assessed, but in the hippocampus, Cst-2 deletion specifically reduces inhibitory, but not excitatory synaptic transmission¹¹⁹. The anxiety phenotype of Cst-2 KO mice is not straightforward, with one study showing no anxiety phenotype in both OF and EPM¹¹⁹, and another study reporting increased anxiety-like behavior in the OF, but reduced anxiety-like behavior in the EPM¹²⁰.

Neurofascin

Neurofascin is a cell adhesion molecule that (among other functions) localizes to the axon initial segment of neurons, where it regulates the postsynaptic structure of inhibitory inputs originating from PV⁺ chandelier cells^{121,122}. Recent studies showed that Neurofascin knockdown specifically in the BLA of rats results in a reduction in mIPSC amplitude, as well as an impairment in fear extinction but not anxiety or fear acquisition¹²¹, likely through a disruption of the synaptic plasticity in the BLA-PFC pathway¹²². Whether Neurofascin contributes to anxiety processing in other contexts is currently unknown.

Immunoglobulin superfamily member 9b (IgSF9b)

IgSF9b is a recently identified cell adhesion molecule at inhibitory synapses that has been associated with major depression and the affective symptoms of schizophrenia². In mice, IgSF9b is expressed in both the BA and CeA, and deletion of IgSF9b results in increased inhibitory synaptic transmission in the CeM (Babaev and Krueger-Burg, unpublished data). Intriguingly, IgSF9b deletion has a prominent anxiolytic effect in Nlgn2 KO mice, pinpointing IgSF9b as a key regulator of the anxiety circuitry (Babaev and Krueger-Burg, unpublished data).

Therapies targeting amygdala inhibitory neurons and synapses

The central role of the amygdala inhibitory network in the modulation of anxiety responses makes it an ideal target for the treatment of anxiety disorders²⁰. Indeed, GABA_AR-targeting benzodiazepines were long considered to be a primary treatment for anxiety disorders, and they are still extensively used in the clinic¹⁰. However, they are often associated with dependence and side effects (such as sedation, ataxia, fatigue), which can be attributed, at least to a great extent, to the non-specific modulation of GABA_AR throughout the brain⁶. Identification of the α 2- and α 3-subunits as the benzodiazepine-sensitive subunits of GABA_AR has opened a new door for the development of more efficient drugs^{4,83}, with behavioral studies using partial agonists of the α 2- or α 3-subunits showing a reduced dependence liability and sedation compared to benzodiazepines. Although several potential anxiolytic compounds targeting α 2 and α 3-GABA_AR have been developed in recent years, only a few have reached clinical trials, such as TPA023, MRK-409, and ocinaplon. Unfortunately, most trials had to be terminated due to preclinical toxicity or failure to provide an anxiolytic effect devoid of sedation (as reviewed in ref⁴), leaving room for improvement in this line of research.

Apart from the direct pharmacologic modulation of GABA_AR, alternative therapeutic strategies aimed at increasing GABAergic neurotransmission are being

explored for the treatment of anxiety disorders. They include, for example: (1) Targeting the neurosteroid system, which modulates GABA_AR activity. The anxiolytic effect of this approach has been confirmed by direct administration of neurosteroids in rodents²⁰ and administration of compounds that enhance neurosteroid synthesis, such as XBD173 and etifoxine^{20,123}, in humans. (2) Targeting GABA_B receptors, which are also involved in the modulation of anxiety. Positive allosteric modulation of the GABA_B receptor had an anxiolytic effect in rodent anxiety models (with compounds CGP7930 and GS39783)^{77,78} and has been approved for clinical testing for the first time (ADX71441)¹²⁴. (3) Enhancing GABA through blockade of GABA transaminase (e.g., with vigabatrin) or inhibition of GABA transporters (e.g., with tiagabine)¹²⁵. (4) Modulating the GABAergic system with phytomedicines¹²⁶.

Still, with our ever increasing understanding of the great anatomical and molecular complexity of the amygdala, it is becoming clear that even more specific treatments for anxiety disorders can be achieved through local manipulations of specific inhibitory neuronal populations¹²⁷. Techniques such as Cre/lox recombination, optogenetics and chemogenetics, which enable the dissection of complex brain circuits at the level of molecularly distinct neurons, have been extensively used in basic research to investigate the contribution of different interneurons in the amygdala to emotional behaviors^{7,12}. Although the use of AAVs still represents a major challenge for the translation of these and other techniques into the clinic, recent results indicate that gene therapy is becoming a viable option for the treatment of brain disorders, with successful clinical trials including the treatment of macular degeneration and Parkinson's Disease¹²⁸. The use of AAVs for the treatment of anxiety disorders has the potential to provide greater efficacy with fewer side effects. However, much still needs to be done to identify the neuronal circuits that underlie anxiety and identify common biological features that could be used to target these specific neuronal populations.

Conclusion

While the importance of inhibition in the processing of anxiety information in the amygdala is universally acknowledged, it is striking how few studies have directly investigated the function of individual inhibitory neuronal subtypes, receptors, or synapse organizers specifically within this behavioral circuit. Nonetheless, there is a growing awareness that this specificity is essential for the development of more effective treatments with fewer side effects. With the advent of increasingly sophisticated tools to dissect behaviorally relevant neuronal circuits and synapses^{16,18,19,28,29,45,52,127}, the stage is set for future

studies to generate a substantially more detailed map of the role of inhibition in the amygdala anxiety circuitry.

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Conflict of interest

The authors declare that they have no conflict of interest.

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