Babaev et al. *Experimental & Molecular Medicine* (2018) 50:18
DOI 10.1038/s12276-018-0063-8

Experimental & Molecular Medicine

REVIEW ARTICLE Open Access

# 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 targets[1]. 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 disorders[8], which are among the most common neuropsychiatric diseases, with an estimated lifetime

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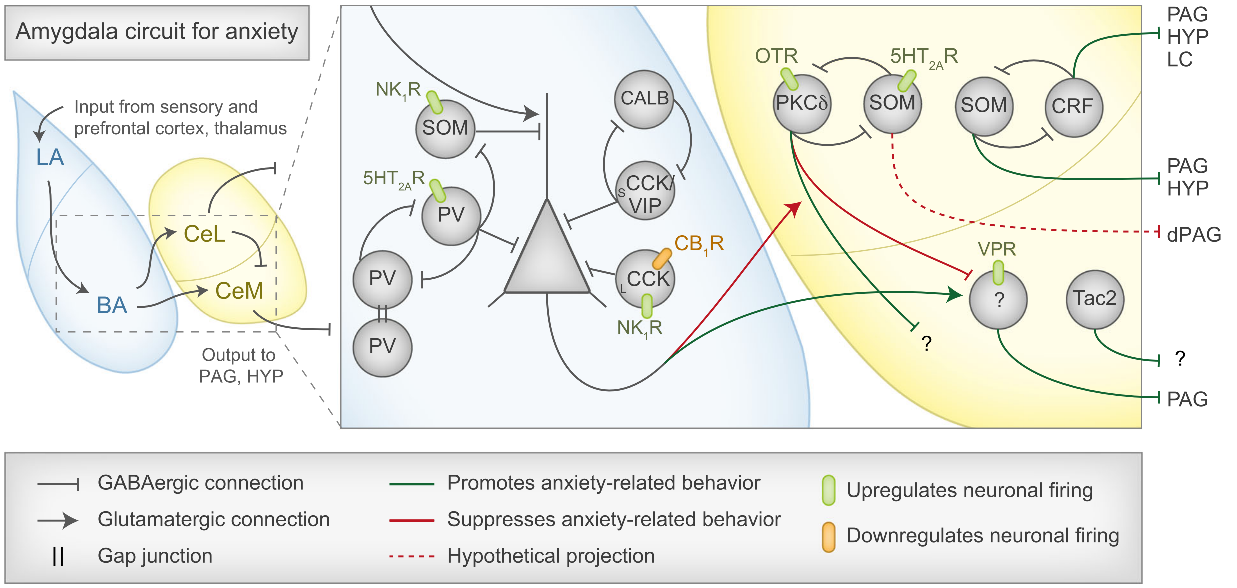

**Fig. 1 Inhibitory circuits of the amygdala that are implicated in the regulation of anxiety-related behavior.** The sensory information from the cortex and thalamus flows in series across the BLA and bifurcates as it enters the CeA. While recent optogenetic studies have begun to clarify the role of individual inhibitory loops of the CeA in anxiety-related behaviors, less is known about the intrinsic circuitry and downstream targets of individual types of neurons in the CeM. For simplicity, all of the afferents to the CeA apart from the BLA afferents are omitted, as well as some of the CeA downstream targets, including the dorsal vagal complex and the hypothalamus (which receives input from the CeM). Abbreviations: BLA basolateral amygdala; CALB calbindin; $_{L}$CCK large cholecystokinin; $_{S}$CCK small cholecystokinin; CeA central amygdala; CeL centrolateral amygdala; CeM centromedial amygdala; CRF corticotropin releasing factor; dPAG dorsal periaqueductal gray; $HT_{R}2A$ serotonin receptor 2 A; HYP, hypothalamus LC, locus coeruleus; $NK1_{R}$ neurokinin 1 receptor; OTR, oxytocin receptor; PAG periaqueductal gray; PV parvalbumin; SOM somatostatin; Tac2 tachykinin 2; VIP vasoactive intestinal peptide; VPR vasopressin receptor

prevalence of more than 28% in adults[9]. 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 disorders[8]. 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 therapy[13].

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 processing[12]. 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 responses[12], 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 BLA[24], 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 anxiety[5], and a subpopulation of inhibitory neurons in the BLA persistently increases its firing during the behavioral manifestations of anxiety[25]. 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 freezing[26]. 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 disorders[9].

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 threating 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[a][45] |
| | 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 | PKCδ$^{+}$ | Partial silencing enhances conditioned freezing following fear conditioning |
| | | Optogenetic stimulation reduces avoidance in OF, EPM and LDB[62] |
| | | Optogenetic stimulation reduced the discrimination between CS+ and CS- in fear conditioned animals[63] |
| | | Optogenetic stimulation increases avoidance in EPM and OF[63] |
| | 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[a][19] |
| | | Optogenetic stimulation of CRH$^{+}$ terminals projecting from the CeA to the Locus Coereleus increases avoidance[65] |
| CeC[b] | PKCδ$^{+}$ | 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 valence[30], and inhibitory neurons in the BLA are thought to be as important for encoding stimulus valence as excitatory neurons[29]. 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-threating or even rewarding stimuli[9].

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 interneurons[24] (Fig. 1). Like cortical interneurons[1], 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 targets[24]. While it is widely accepted that inhibition in the BLA must play a critical role in the regulation of anxiety (reviewed in ref[5]), 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 neurons[15] (although see ref[31]), 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 BLA[38]. This response is attenuated by post-weaning social isolation, which leads to anxiety-like behavior in adult rodents[39]. 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 anxiety[40]. 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 animals[45]. 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 EPM[47]. 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 staining[48]. 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 behaviors[49]. However, a subset of $CCK^+/CALB^+$ interneurons are also $NK_{1r}$-positive[49], 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 VIP[50]. $CCK^+$ 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 ref[53]). Moreover, a subset of $CCK^+$ neurons were affected by the anxiogenic lesion of $NK_{1r}{}^+$ interneurons in the BLA[49], 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$^+$ interneurons[50]. 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 response[45].

## 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 stimuli[23]. For example, activation of excitatory CeM-targeting projection neurons in the BLA increases avoidance behavior[16], and activation of CeM projection neurons via vasopressin receptors (VPRs) has been proposed to be a mechanism underlying the anxiogenic effects of vasopressin[59]. 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 behaviors[60].

### *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 Cδ (PKCδ)[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 PKCδ$^+$ 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 PKCδ$^+$ 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]. PKCδ$^+$ 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 PKCδ$^+$ neurons. Additionally, the activity of PKCδ$^+$ neurons predicts the ability to discriminate between neutral and threat-predicting stimuli, and thus, CeL PKCδ$^+$ neurons may contribute to anxiety-related fear generalization[26,63].

PKCδ$^+$ neurons, in turn, are tightly regulated by local inhibitory connections with SOM$^+$ neurons. Optogenetic activation of SOM$^+$ neurons lifts the inhibitory control of PKCδ$^+$ 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 PAG[64]. In addition to inhibiting PKCδ$^+$, SOM$^+$ neurons form mutually inhibitory connections with CeL CRF$^+$ neurons, and during fear conditioning, this network determines the balance between conditioned flight and freezing behaviors[19]. 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) tests[65]. 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 PKCδ$^+$ neurons[66]. 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 humans[67].

## 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 genes[28]. This study demonstrated that optogenetic stimulation of $\mathrm{Tac2}^{+}$ neurons in the CeM elicited immobility-like behavior in naïve mice, in agreement with previous findings that showed that inhibition of $\mathrm{Tac2}^{+}$ 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 freezing[26], but, surprisingly, also promotes appetitive behaviors[28]. Similarly, $\mathrm{PKC\delta}^{+}$ and $\mathrm{SOM}^{+}$ 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 $\mathrm{GABA_A}$ receptor ($\mathrm{GABA_AR}$) 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 component[21] 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 $\mathrm{GABA_AR}$s, $\mathrm{GABA_BR}$s, glycine receptors, and inhibitory synapse organizers in anxiety processing (Fig. 2, Table 2, Table 3).

### $\mathrm{GABA_A}$ Receptors

Fast inhibitory synaptic transmission is primarily mediated by ionotropic $\mathrm{GABA_AR}$s, which are pentameric chloride channels that are composed of various combinations of 19 different subunits (α1-6, β1-3, γ1-3, δ, ε, θ, π, ρ1-3)[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 $\mathrm{GABA_AR}$ 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 $\mathrm{GABA_AR}$ subunits act as targets for

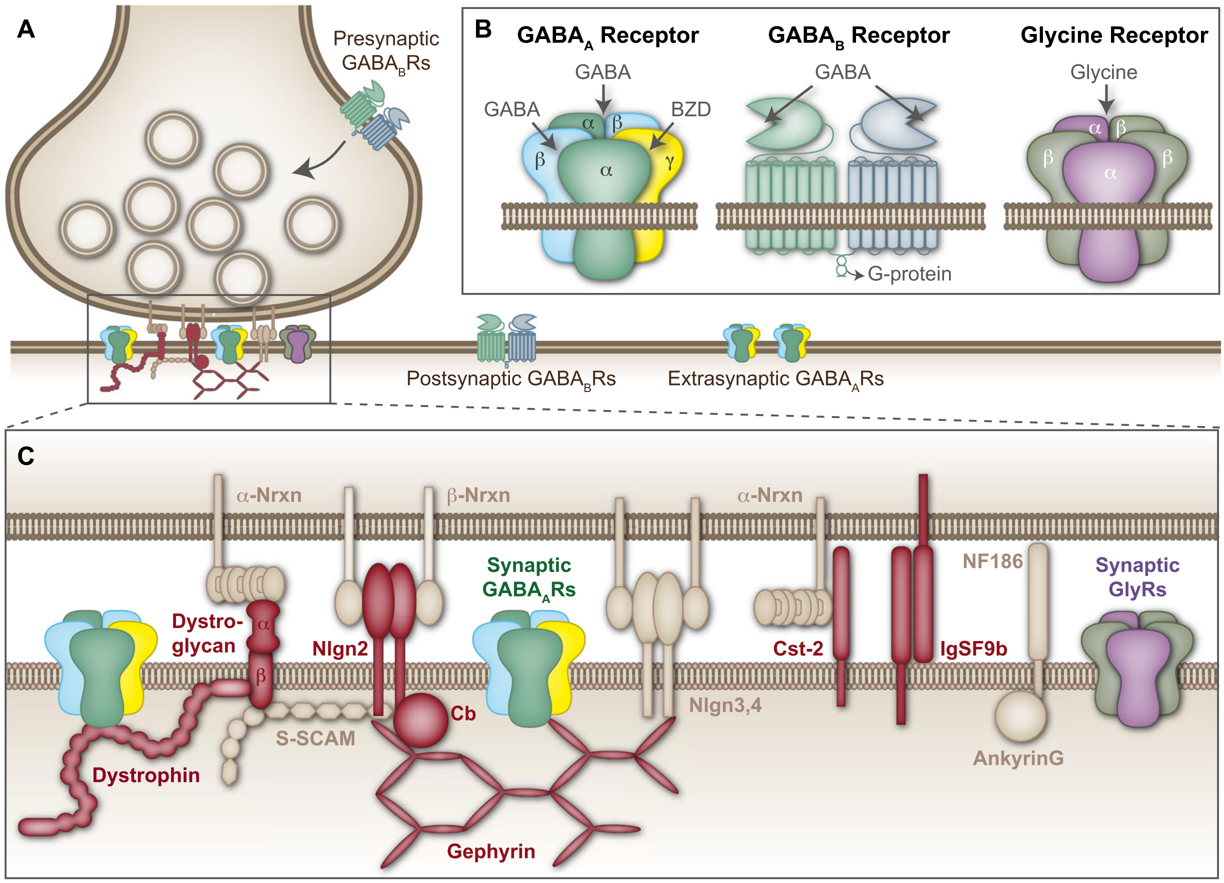

**Fig. 2 Molecular determinants of anxiety-related behavior at inhibitory synapses. a** Overview of the synaptic and extrasynaptic machinery involved in mediating and regulating inhibitory neurotransmission. **b** Receptors that have been linked to mediating inhibitory neurotransmission in the amygdala anxiety circuitry. **c** Molecular components of the inhibitory postsynapse (adapted from ref[2]). 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 collybistin; Cst-2 calsyntenin-2; IgSF9b immunoglobulin superfamily member 9b; NF186 neurofascin 186, Nlgn neuroligin; Nrxn 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 rodents[84] and humans[85], 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 benzodiazepines[4]. Second, auto-antibodies to the γ2-subunit have recently been identified in patients with a range of psychiatric symptoms that include anxiety[86], 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 lethal[83], 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 phenotypes[88], consistent with a developmental origin of anxiety[21]. 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]<br>cKO (Emx1-Cre, developmental): Increased anxiety;<br>cKO (CaMKII-Cre, postnatal): Normal anxiety[88].<br>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]<br>KO, cKO (amygdala): No effect on anxiety[83,94]<br>cKO (CRF-Cre): Increased anxiety[95] | Highly expressed in the BLA, moderately expressed in the CeM, and absent in the CeL[84,85,92]<br>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]<br>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]<br>KO: No anxiety[83,101] | Expressed prominently in the BLA and CeA[84,85,92]<br>Primarily extrasynaptic in the BLA[100] |
| $\alpha$5-GABA$_A$R | Anxiolytic effects of BZD?[101] | KO: Normal anxiety[4]<br>KD in CeL PKC$\delta^+$ 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] |
| $\beta$-GlyR | Variants associated with panic disorder[73] | $Glrb^{+/spa}$ mice: Increased anxiety[73] | Expressed in the BLA and the CeA[103];<br>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$1-GABA$_A$Rs[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 CeA[90], 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 $\gamma$-subunit, virtually all GABA$_A$Rs contain two $\alpha$-subunits, which form the other half of the binding site for benzodiazepines. In the late 1990s, the role of each of the $\alpha$-subunits in mediating the effects of benzodiazepines was investigated in a seminal series of studies using mice that expressed $\alpha$-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$2-GABA$_A$Rs, with a lesser potential contribution from $\alpha$3-GABA$_A$Rs, while $\alpha$1-GABA$_A$Rs specifically mediate the sedative but not anxiolytic effects of benzodiazepines[83]. Here, we summarize what is known about the role of the individual $\alpha$-subunits specifically in the amygdala (see also Table 2).

$\alpha$1-GABA$_A$Rs 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$2-GABA$_A$Rs during early postnatal development, indicating that these subunits may play different roles during development[93]. Functionally, $\alpha$1-GABA$_A$Rs contribute substantially to the benzodiazepine-mediated potentiation of IPSCs in the BLA, but not the CeA[92]. Neither constitutive deletion of the $\alpha$1-subunit[83] nor conditional deletion specifically in the amygdala[94] 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$1-GABA$_A$Rs in the BNST[95]. Together with the data from the benzodiazepine-insensitive point mutants described above[83], these data indicate that $\alpha$1-GABA$_A$Rs in the amygdala may play a relatively small role in anxiety behaviors.

α2-GABA$_A$Rs 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$_A$Rs in both the BLA and CeA show a profile consistent with α2βxγ2 receptors[90,92]. In the BLA, α2-GABA$_A$Rs are particularly enriched on the axon initial segment[96]. α2-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 α2-GABA$_A$Rs are the primary mediators of the anxiolytic effects of benzodiazepines[83]. However, the extent to which these effects are specifically mediated by α2-GABA$_A$Rs in the amygdala remains largely unknown. Conditional deletion of the α2-subunit in the hippocampus was recently shown to abolish benzodiazepine-induced anxiolytic effects without altering basal anxiety in an EPM[99]. However, to our knowledge, similar data are not yet available for the amygdala.

α3-GABA$_A$Rs are prominently expressed in both the BLA and CeA[84,85,100]. In the BLA, α3-GABA$_A$Rs appear to be primarily localized extrasynaptically, where they play a central role in mediating the tonic inhibition activated by synaptic spillover[100]. The role of α3-GABA$_A$Rs in mediating anxiety behaviors is controversial: while the α3-subunit-specific agonist TP003 induces anxiolytic effects in rodents, the (H/R) α3-subunit point mutation does not alter the anxiolytic effects of benzodiazepines, and constitutive α3-subunit KO mice show no anxiety phenotype[83,101].

α5-GABA$_A$Rs, which also mediate extrasynaptic tonic inhibition[63], 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 α5-subunit in mice results in abnormalities in learning and memory but normal anxiety levels, and the α5-subunit has been primarily studied as a target for cognitive enhancers rather than anxiolytic therapies[4]. More recently, however, it was shown that extrasynaptic α5-GABA$_A$Rs in the CeA exert an anxiolytic effect through tonic inhibition of PKCδ$^+$ neurons in the CeL[63]. 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 α5-GABA$_A$Rs, but not at α2/3-GABA$_A$Rs[101]. Together, these results indicate that the α5-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$_A$Rs, but also has modulatory effects through metabotropic GABA$_B$Rs. GABA$_B$Rs are G$_{i/o}$-protein coupled receptors that consist of two subunits, GABA$_{B(1)}$ and GABA$_{B(2)}$[77,78,102]. GABA$_B$Rs 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$_B$Rs in anxiety processing comes from two avenues[77,78]: (1) GABA$_B$R agonists such as baclofen and GABA$_B$R-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$_B$Rs 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$_A$Rs, glycine receptors (GlyRs) are pentameric ligand-gated chloride channels that are assembled from a family of five subunits, the α1-4 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 CeA[103], and GlyR-mediated currents can be observed in the BLA and CeA[104]. Interestingly, variants in the β-subunit were recently associated with agoraphobia, an anxiety disorder, and mice with reduced β-subunit levels (*Glrb*$^{+/spa}$ mice) showed increased anxiety in an OF test[73]. 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$_A$Rs 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]<br>R215H KI: increased anxiety[111]<br>cKO (PFC): decreased anxiety[113]<br>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 gepyhrin, $GABA_A$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 epilepsy[107]. 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 phenotype[79]. Gephyrin is expressed throughout the brain, including in both the BLA and CeA in humans[108] and rodents[109]. The role of gephyrin in clustering $GABA_A$Rs has not been studied specifically in the amygdala, but in other brain regions, gephyrin plays a critical role in binding to γ2- or α2-containing $GABA_A$Rs[105,106], which mediate the anxiolytic responses of benzodiazepines as described above[83].

### *Neuroligin-2 (Nlgn2)*

Nlgn2 is an inhibitory synapse-specific member of the Neuroligin (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 autism[72], in addition to Nlgn2 mutations previously associated with schizophrenia[2]. 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 mice[74], 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 $GABA_A$Rα1, as well as a reduced mIPSC frequency, and this reduction in inhibition is accompanied by an overactivation of BA principal neurons under anxiogenic conditions[74]. In contrast, in the CeM, Nlgn2 deletion has only very minor consequences for synaptic inhibition[74], indicating that Nlgn2 may play different roles at inhibitory synapses excitatory and inhibitory neurons. Interestingly, overexpression of Nlgn2 in mice also induces an anxiety phenotype[112], while local deletion of Nlgn2 specifically in the PFC of adult mice has an anxiolytic effect[113], 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 Nlgn2[110]. Human variants in Cb have been associated with anxiety, as well as with epilepsy and intellectual disability[71], and deletion of Cb in mice results in a severe anxiety phenotype[76]. Cb is expressed in the BLA, where its deletion results in a prominent loss of gephyrin and $GABA_AR\gamma2$ clusters without altering GlyRs or VIAAT puncta[76]. 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 $GABA_AR\alpha2$ subunits (at least when transfected into heterologous HEK cells)[116], which in turn may play a particularly important role in anxiety[83].

### *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 $GABA_AR\alpha2$ 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 conditioning[82], and reduced locomotion and increased anxiety in an OF[81], but not an EPM paradigm[80]. Dystroglycan, the transmembrane complex of the DGC, is expressed in mouse amygdala at low levels[117]. Its function in the amygdala has not been studied, although in other brain regions, deletion of dystroglycan impairs the function of GABAergic synapses[2]. 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 CeA[118]. 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 transmission[119]. The anxiety phenotype of Cst-2 KO mice is not straightforward, with one study showing no anxiety phenotype in both OF and EPM[119], and another study reporting increased anxiety-like behavior in the OF, but reduced anxiety-like behavior in the EPM[120].

### *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 acquisition[121], likely through a disruption of the synaptic plasticity in the BLA-PFC pathway[122]. 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 schizophrenia[2]. 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 circuity (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 disorders[20]. Indeed, $GABA_AR$-targeting benzodiazepines were long considered to be a primary treatment for anxiety disorders, and they are still extensively used in the clinic[10]. 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 brain[6]. Identification of the α2- and α3-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 α2- or α3-subunits showing a reduced dependence liability and sedation compared to benzodiazepines. Although several potential anxiolytic compounds targeting α2 and $\alpha3$-$GABA_AR$ 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 ref[4]), 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 rodents[20] 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 phytomedicines[126].

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 populations[127]. 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 Disease[128]. 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.

**Acknowledgements**

The authors are grateful to Dr. Nils Brose for his continuous advice and support of their research in the Department of Molecular Neurobiology, which is funded by the Deutsche Forschungsgesellschaft, the European Commission, and the Bundesministerium für Bildung und Forschung. D.K-B. was the recipient of a NARSAD Young Investigator Grant (Brain & Behavior Research Foundation). O.B. was a student of the Göttingen Graduate School of Neurosciences and Molecular Biosciences (GGNB), and was funded by a Ph.D. fellowship from the Minerva Foundation. C.P.C. was a student of the Neurasmus Master program and was supported by an Erasmus Mundus scholarship (European Commission). The authors thank Dr. Hugo Cruces-Solis and Heba Ali for the valuable feedback and discussions on this manuscript.

**Conflict of interest**

The authors declare that they have no conflict of interest.

Received: 23 January 2018 Accepted: 25 January 2018.

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