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An Emerging Circuit Pharmacology of GABA_A Receptors

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Abstract

In the last 20 years, we have learned a great deal about GABA_A receptor (GABA_AR) subtypes, and which behaviors are regulated or which drug effects are mediated by each subtype. However, the question of where in the brain the GABA_ARs involved in specific drug effects or behaviors are remains largely unanswered. Here we review recent studies taking a circuit pharmacology approach to investigate the functions of GABA_AR subtypes in specific brain circuits controlling fear, anxiety, learning, memory, reward, addiction and stress related behaviors. The findings of the studies highlight the complexity of brain inhibitory systems and the importance of taking a subtype-, circuit-and neuronal population-specific approach to develop future therapeutic strategies using cell type-specific drug delivery.

Keywords

GABA_A receptor; memory; anxiety; addiction; depression; hippocampus

Why study GABA_A receptors in a subtype- and circuit-specific manner?

Inhibitory γ -aminobutyric acid (GABA) neurotransmission in the brain is crucial for the temporally precise activity of **neuronal circuits** (see Glossary) and **synchronized oscillatory activity** of neuronal populations. The diversity and complexity of inhibitory neurotransmission that regulates the activity of neuronal networks is in part accomplished by a large variety of interconnected interneurons (see Box 1). The diversity of the GABAergic interneurons and their activity patterns is matched on the receiving side by a diverse group of GABA receptors. The **ionotropic** GABA_A receptors (GABA_ARs), for instance, are characterized by high levels of structural diversity, as summarized in Box 2. The specific configuration of the receptor affects the channel-gating actions and pharmacokinetic properties, as well as subcellular location and gross expression of the receptor in different brain regions. The subcellular localization of the receptors determines the type and speed of inhibition provided by the receptors to regulate circuit activity. For instance, $\alpha 2$ and $\alpha 3$ subunit-containing GABA_ARs ($\alpha 2$ GABA_ARs and $\alpha 3$ GABA_ARs) are more concentrated at

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synapses than the **extrasynaptic** membrane, while $\alpha 4$ and $\alpha 5$ subunits are more concentrated in perisynaptic and extrasynaptic sites. While the low-affinity synaptic receptors are ideally located to mediate fast **phasic inhibition**, the receptors in extrasynaptic sites respond to low concentrations of ambient GABA and mediate **tonic inhibition**. Moreover, the trafficking of GABA_ARs into different cellular compartments also depends on their subunit composition. For instance, in the CA1 pyramidal cells of the hippocampus, both $\alpha 1$ and $\alpha 2$ subunits have been reported at perisomatic synapses [1], located ideally to control action potential generation. There is some evidence that $\alpha 1$ GABA_ARs may mediate phasic inhibition in distal dendrites [2], where they may play a role in signal integration. $\alpha 2$ subunits are also enriched in synapses at the **axon initial segment** (but see [3] for contradictory findings), where they are ideally located to control action potential conductance and neurotransmitter release. Additionally, subunits are differentially concentrated in specific brain regions (Box 2) and often, in specific cell populations within a region [4]. As a result, the neurocircuitry underlying a specific behavior may be affected disproportionately by GABA_ARs expressed in a specific subunit. Finally, subunit configuration also affects which neuronal oscillations [5, 6] are controlled by a specific group of GABA_ARs, and thus, how a certain subset of GABA_ARs contributes to neuronal synchrony, communication and coordination between neuronal populations.

In summary, while studies using systemic manipulations have implicated certain GABA_AR subtypes in specific behaviors, GABA_ARs modulate these behaviors in a subtype-, circuit- and neuronal population-specific manner, and a full understanding of their roles in brain function is possible only if the study of GABA_ARs is approached with matching complexity. Recent approaches taking advantage of a number of technical advances (see Boxes 3 and 4) facilitated some progress in this direction over the last 10 years. Here we review these studies focusing on the role of GABA_AR subtypes in specific brain regions, circuits and neuronal populations in behaviors related to learning and memory, anxiety and fear, reward and addiction, and stress and depression. However, as will become clear throughout the following sections, **circuit pharmacology** of GABA_ARs is just beginning to flourish and our knowledge of GABA_AR subtype function within any behaviorally-relevant circuit is very fragmentary.

GABA_A Receptors in Anxiety and Fear Circuits

The role of GABA_ARs in the regulation of anxiety and fear responses has been known since **allosteric modulation** of GABA_A receptors was recognized as the mechanism of action for benzodiazepine **anxiolytics** in the 1970's [7]. Later work employing gene-targeted mice demonstrated the anxiolytic and fear-reducing actions of benzodiazepines to be mediated mostly by the $\alpha 2$ GABA_ARs [8-10]. In addition, $\gamma 2$ GABA_ARs [11], $\alpha 1$ GABA_ARs [10, 12], $\alpha 3$ GABA_ARs [13] (but see [14-16] for contradictory findings), and more recently, $\alpha 5$ GABA_ARs [16-18, 4] have been implicated in anxiety, fear and/or response to chronic stress.

There have been a number of attempts to distinguish between the terms “fear” and “anxiety”, with most categorizations defining fear as more physically proximal, more certain, concrete and well-defined than anxiety (see [19] for a review and a proposed

framework). As most studies employ Pavlovian fear-conditioning paradigms to test for fear reactions, fear is also operationalized as a learned construct, while anxiety is innate. Despite these attempts to draw a distinction between the two emotional states, the terms anxiety and fear are still often used interchangeably in the literature. This is partially due to the significant overlap in the neurocircuitry underlying the two states, at least at the macrocircuit level (see below). Brain areas such as the amygdala, the hippocampus and the medial prefrontal cortex (mPFC) are major nodes for the control of both anxiety- and fear-related behaviors (see [20, 21] for recent reviews). Below we discuss each of these areas, our knowledge of inhibitory control of the circuitry within each area through GABA_ARs and how GABA_AR-mediated inhibition in each area regulates anxiety- and fear-related behaviors.

The Amygdala

The amygdala is a medial temporal lobe structure involved in emotional responses and memory and is composed of heterogeneous nuclei. The internal organization of the amygdala is highly complex, with 10 different subnuclei, with projections within and outside of the amygdala. The lateral, basolateral and basomedial nuclei, which together comprise the basolateral complex (BLA), have a structure reminiscent of cortex with excitatory principal neurons and a smaller population of interneurons. In contrast, the central nucleus (CeA), commonly divided into the lateral (CeL) and medial (CeM) central nucleus, is reminiscent of the striatum with primarily GABAergic neurons. Lying between the BLA and CeA are the two intercalated masses of GABAergic neurons ([22], Figure 1). This complex architecture leads to multiple feedforward and feedback loops within the amygdala, confounding the study of information flow. Furthermore, each section of the amygdala hosts diverse groups of genetically-identifiable cell-types that are spread in a salt-and-pepper fashion without anatomical separation [23]. These cell types often respond differentially to fear- and anxiety-related stimuli, where two neighboring cells may have opposite responses to the same stimulus.

All GABA_AR subtypes, with the exception of $\alpha 6$ GABA_ARs, are expressed in varying cell groups and subregions of the amygdala. A simplified representation of the amygdala microcircuitry including some of the known GABA_ARs is depicted in Fig. 1. As shown in Fig. 1B, the pyramidal cells of the basolateral complex are under strong feedforward (via somatostatin positive (SST+) interneurons, as well as lateral intercalated cell masses), as well as feedback (via parvalbumin (PV+) interneurons) inhibitory control. SST+ interneurons form synapses on distal dendrites of pyramidal neurons, where $\alpha 1$ GABA_ARs are expressed in abundance (at least in the lateral nucleus), placing this subunit in an ideal location to mediate cortical suppression of BLA activity. Indeed, it has been reported that $\alpha 1$ GABA_ARs mediate the fear-reducing effects of diazepam during the expression of fear in a conditioned fear task [10], and that the global knockout of $\alpha 1$ GABA_ARs enhances auditory fear conditioning (see Box 5) [12]. Moreover, the enhancement of fear-conditioning is mediated by $\alpha 1$ GABA_ARs not in excitatory neurons, but in GABAergic neurons [12] (most likely PV+ interneurons). Following acquisition of fear conditioning, $\alpha 1$ GABA_AR expression in the BLA is reduced ([24]), possibly enhancing the acquisition of subsequent fear-conditioning experiences. While playing such a substantial role in the acquisition and

expression of conditioned fear, $\alpha 1$ GABA_ARs of the BLA do not seem to be important for anxiety-related behaviors in the elevated plus maze (see Box 5) [25], emphasizing the differences in the brain systems that control the two emotional states.

PV+ interneurons mediating feedback inhibition onto BLA pyramidal neurons form synapses in perisomatic locations and proximal dendrites, where $\alpha 2$ GABA_ARs and $\alpha 3$ GABA_ARs are expressed [26]. Despite this, the $\alpha 3$ GABA_ARs on LA principal neurons are localized preferentially extrasynaptically, mediating tonic inhibition. Recent work shows that PV+ interneurons themselves are under strong tonic inhibitory control through the δ -subunit containing GABA_ARs ($\alpha 1$, which is also expressed strongly on these neurons, might form extrasynaptic assemblies alongside δ to contribute to this tonic inhibition), such that increased activity of δ -containing receptors leads to the disinhibition of principal neurons and increased expression of conditioned fear ([27], Fig. 1B). In addition to PV+ interneurons, δ -containing GABA_ARs are also expressed strongly and exert tonic inhibitory control on intercalated cell masses (Fig. 1C [28]). Thus, δ activation increases excitatory input into the CeA via both the disinhibition of the BLA principal neurons and the suppression of feedforward inhibition via the intercalated cells. The intercalated cell masses also express $\alpha 2$ and $\alpha 3$ GABA_ARs synaptically, which mediates reciprocal inhibition between the two medial cell masses [29]. A recent study shows that the PV+ interneurons also form an inhibitory microcircuit with the local SST+ interneurons, the modulation of which is necessary for the learning of associative fear [30]. However, it is not clear which GABA_ARs are involved in the rapid changes in the activity of this microcircuit during fear learning. The overall effect of increased $\alpha 2$ or $\alpha 3$ GABA_ARs on the BLA-CeA network is also unclear, as these receptors are expressed on both the principal neurons of the BLA, mediating fast phasic [31] and tonic [32] inhibition of the principal neurons respectively, and on the synapses of GABAergic intercalated neurons, exerting opposing effects on CeA excitability.

GABA_ARs are also expressed in the GABAergic neurons of the CeA, having direct control over amygdala output to downstream structures. As neighboring cells in this region can have opposing roles in the control of fear [33, 34], it is essential to delineate the distribution of GABA_ARs on specific cell populations in this area. So far, efforts in this direction have been rather limited, but have further demonstrated how a small receptor population on a single class of cells can disproportionately affect behavior. For instance, a population of GABAergic neurons in the CeL which express protein kinase C-delta (PKC-delta+ neurons) increase their spontaneous activity following fear conditioning [34], and are involved in fear generalization and anxiety (Fig. 1D) [4]. Under baseline conditions where fear generalization or anxiety expression are not warranted, these neurons are under strong tonic inhibitory control. One group reported that selective knockdown of $\alpha 5$ GABA_ARs in this small CeA population leads to increased anxiety-like responses by increasing the spontaneous activity of these neurons [4]. This finding is striking, as $\alpha 5$ GABA_ARs are expressed at negligible levels in the CeA [35, 36], however, they exert strong control over anxiety-related behaviors through controlling the excitability of a cell population that is critical for anxiety expression. Similarly, $\alpha 1$ GABA_ARs, which are expressed at very low levels in the CeA (where fast synaptic inhibition is predominantly mediated via $\alpha 2$ GABA_ARs), were found to be preferentially expressed in a CeM population of

GABAergic neurons that express the corticotrophin releasing factor 1 receptors (CRF1+), mediating tonic inhibition [37]. Considering the role of CRF in CeA in stress and anxiety [38], it is likely that the $\alpha 1$ GABA_ARs in this area modulate stress-induced anxiety responses (Fig. 1E). There is already some evidence from studies using a knockdown of $\alpha 1$ GABA_ARs in CRF+ neurons globally showing that $\alpha 1$ GABA_ARs on CRF+ neurons may play important roles in anxiety-related behaviors and extinction of conditioned fear [39], however, there is no direct evidence to suggest that the CeM $\alpha 1$ GABA_ARs are responsible for these effects is currently lacking. $\alpha 2$ -GABA_ARs are densely expressed in the central amygdala [35, 36, 40], but their precise cellular location and function are still unknown.

The Hippocampus

The hippocampus is a temporal lobe structure with strong connections to the amygdala and the mPFC [20, 21]. In a very simplified view (Fig. 2A), three subregions (CA1, CA3 and dentate gyrus (DG)) with predominantly unidirectional excitatory projections (from DG to CA3 to CA1) comprise the hippocampus. Moreover, a distinction between the ventral (temporal) and dorsal (septal) (anterior and posterior in humans) hippocampus has been drawn based on the functional and gene expression differences between the two areas, with ventral/temporal portions more involved in anxiety and emotional processing, and dorsal/septal portions more involved in learning and memory [41]. $\alpha 1$, $\alpha 2$, $\alpha 3$, $\alpha 4$ and $\alpha 5$ GABA_ARs are all expressed at moderate to high levels in the hippocampus (although at different relative levels in different subregions; [36]), and the importance of the GABA_ARs in this area for the control of fear and anxiety has been long established through **microinfusion studies** using non-selective GABAergic agents (see [42]).

$\alpha 2$ GABA_ARs, which have been consistently implicated in the control of fear and anxiety, are expressed on the dendrites, soma and axon initial segment of principal neurons in all three hippocampal subregions, receiving input from different subtypes of interneuron depending on subcellular location. Selective knockout of $\alpha 2$ GABA_ARs in DG or CA3 principal neurons abolishes the anxiolytic-like effects of systemically administered diazepam [43]. This finding suggests that the $\alpha 2$ GABA_ARs in this intrahippocampal circuit play a pivotal role in the control of anxiety, as the activity of diazepam in all other important nodes in the brain anxiety circuit, such as the amygdala, the extended amygdala and the mPFC, was not sufficient to elicit an anxiolytic-like effect if DG and CA3 were not inhibited through $\alpha 2$ GABA_ARs. Similarly, diazepam's action on the other GABA_AR subtypes in the whole brain, including the CA3 and DG, was not sufficient to provide anxiolysis. More interestingly, the control of fear and anxiety by the $\alpha 2$ GABA_ARs in the hippocampus was characterized by a double dissociation, with separate brain areas contributing to the two functions without overlap (see also Figs. 2B and D). While, as stated above, anxiolytic-like effects of the drug in tests of anxiety, such as the elevated plus maze or the light/dark box (see Box 5), were completely abolished in animals where $\alpha 2$ GABA_ARs were deleted in the CA3 or DG, the deletion of $\alpha 2$ GABA_ARs in CA1 had no effect on diazepam-induced anxiolysis in the same tests. In stark contrast, the fear-reducing effects of diazepam in tests such as fear-potentiated startle or Vogel conflict test (see Box 5) were completely abolished in animals with a deletion of $\alpha 2$ GABA_ARs in CA1. Knocking down $\alpha 2$ GABA_ARs in CA3 or DG had no effect on diazepam-induced reduction of fear (see Figure 3A, Key Figure). In

these experiments, fear was defined as a state caused by a discrete, specific threat, such as a mild electric shock, while anxiety was defined as a situation that increases the possibility of a threat, such as being in an exposed, open, brightly-lit area for a rodent. The fear tests included both learned fear, where the fear reactions to a cue that predicted the threat were measured 24 hours following fear conditioning, and unlearned fear reactions, where a threat and the resulting avoidance response to a threat occur simultaneously. Thus, the anatomical distinctions in the anxiolytic and fear-reducing effects of diazepam reflect differences in the control of the two states rather than the involvement of learning processes. These findings demonstrate the importance of molecular and anatomical specificity in the study of the role of GABA_ARs in behavioral processes: Based on earlier findings where the hippocampus was treated as a unitary structure, it would have been natural to conclude that fear and anxiety are mediated by the same circuit, while these recent experiments [43] clearly demonstrate a well-defined separation at the level of microcircuits.

There is some evidence that extrasynaptic GABA_ARs in the hippocampus may also be involved in anxiety. For instance, the infusion of allopregnanolone, a neurosteroid that acts as a positive allosteric modulator of GABA_ARs, into the dorsal CA1 area was shown to be anxiolytic in an elevated plus maze [44]; although not consistently [45]. While allopregnanolone is not selective for a single GABA_AR subtype, it has high efficacy on α 4GABA_ARs [46, 47]. Moreover, female rats show reduced anxiety-like behavior during diestrus phase compared to estrus. δ GABA_AR expression in the hippocampus is increased during the diestrus compared to estrus phase [48], raising the possibility that this increase in hippocampal δ GABA_AR expression might underlie the apparent anxiolysis. However, DG-selective knockdown of δ GABA_ARs failed to produce any anxiogenic effects [49]. Similarly, DG-selective knockdown of α 5GABA_ARs did not produce any changes in anxiety-related behaviors, casting doubt on the involvement of extrasynaptic GABA_ARs in the DG in anxiety regulation [50].

Other Brain Regions Involved in Control of Fear and Anxiety

There is ample evidence that areas such as the mPFC, septum, thalamus, hypothalamus, periaqueductal grey, raphe nuclei and locus coeruleus are involved in the regulation of anxiety and fear, and the role of the GABA_A receptors in these areas has been demonstrated through microinfusion studies using nonselective GABAergic agents [42, 45, 51]. However, to our knowledge, there are no studies to date that investigate the role of specific GABA_AR subtypes within the circuitry of these areas.

Overall, the number of studies investigating the regulation of anxiety- and fear-related behaviors through GABA_AR subtypes in specific circuits or cell populations is rather limited. For instance, a recent study [52] showed that the selective knockout of γ 2GABA_ARs in SST+ interneurons of the forebrain is anxiolytic. While the study targets a specific subunit in a specific cell population, as γ 2 is widely expressed throughout the brain, and SST+ interneurons are also found in a large number of circuits, the anatomical and molecular specificity of the findings are still rather limited. Another study involving a γ 2GABA_AR knockout focused on cerebellar Purkinje cells, providing more anatomical specificity, and found that baseline anxiety-like behavior or anxiolytic effects of the

neurosteroid pregnanolone were not affected by the knockout, confirming that these cell populations are not involved in anxiety and anxiolysis [53].

We believe that cell-type, circuit and projection-specific study of GABA_AR subtypes in known brain anxiety and fear networks will lead to a better understanding of these behavioral processes, and will provide invaluable translational information for a better understanding of anxiety disorders, which currently are the most common psychiatric disorders with a life-time prevalence of 28% [54].

GABA_A Receptors in Learning and Memory Circuits

The **anterograde amnesic** effects of benzodiazepines have been known for a long time [55], suggesting a suppressive role for GABA_ARs in learning and memory. α 5GABA_ARs, which are expressed most intensively in the hippocampus and deep layers of cortex, have been implicated most consistently in memory, with knockdown, knockout, or pharmacological suppression of α 5GABA_ARs leading to improvements in learning and memory [56-61]. There is also evidence suggesting the involvement of α 1GABA_ARs [62, 63], α 2GABA_ARs [50], and α 4/ δ GABA_ARs in learning and memory [64-68].

The Hippocampus

The α 5GABA_ARs are expressed in all three subregions of the hippocampus, with lower expression in the DG, and they mediate tonic, as well as slow phasic inhibition [69, 70]. α 5GABA_ARs contribute to tonic currents, and thus the control of overall network excitability within the hippocampus, particularly when ambient GABA levels are high, as may occur during times of increased neuronal activity, while tonic inhibition through δ GABA_ARs prevails during times of low ambient GABA [71]. This role of α 5GABA_ARs as a brake over hippocampal excitability specifically during times of increased activity may be integral to their role in more complex forms of learning (see below).

As noted in the previous section, the dorsal part of the hippocampus, corresponding to the posterior hippocampus in humans, has been linked to learning and memory. However, the evidence for the involvement of dorsal hippocampal α 5GABA_ARs in memory encoding and consolidation has been sparse [72] and mixed [73, 74], the latter likely due to technical limitations with compound selectivity or insufficient knockdowns.

While the dorsal hippocampus is a reasonable target for learning and memory studies based on earlier work, it is also possible that some of these memory-related effects are mediated by α 5GABA_ARs in the ventral hippocampus. α 5GABA_ARs seem to be more densely expressed in the ventral hippocampus, and ventral hippocampal α 5GABA_ARs play an important role in establishing a high threshold for the induction of long-term potentiation, a physiological measure of synaptic plasticity thought to underlie memory formation [75, 76].

In studies focusing on hippocampal subregions rather than the anatomical division along the septo-temporal axis of the hippocampus, selective knockdown of α 5GABA_ARs in CA3 or DG spanning the dorsal-ventral axis was shown to improve contextual memory in a fear-conditioning task [50]. A reduction of α 5GABA_AR activity increases gamma and ripple

band power in CA3 in hippocampal slices, two hippocampal oscillations known to be involved in memory formation and consolidation which may support context learning [6, 77].

The memory-suppressing effects of $\alpha 5$ GABA_ARs have been demonstrated repeatedly and have led to the development of several negative GABA_AR allosteric modulators with some selectivity for $\alpha 5$ GABA_ARs with the hope that they can be used as cognitive enhancers [40]. However, there is also some evidence supporting a critical role for $\alpha 5$ GABA_ARs in certain memory processes [78, 59]. Supporting this view, knockdown of $\alpha 5$ GABA_ARs specifically in DG granule cells caused consistent impairments in behavioral tasks characterized by high memory interference, such as a context discrimination task with highly similar contexts, reversal learning in a Morris water maze spatial memory task, and extinction of conditioned fear responses (Fig. 3B) [50]. It is possible that during high interference tasks, the spontaneous retrieval and encoding of highly similar information leads to increased activity in the hippocampus, and the tonic inhibitory control of the DG via the $\alpha 5$ GABA_ARs during this heightened activity is essential for integrity of these processes. Another study showed that training in a spatial memory task leads to increased expression of $\alpha 5$ GABA_ARs in the hippocampus [79], which may be an adaptive response to protect the recently encoded memory from interference during the memory consolidation period. The same study also noted a parallel increase in $\alpha 1$ GABA_ARs and of the colocalization of $\alpha 1$ and $\alpha 5$, suggesting the formation of complexes containing both α subunits [79]. Presence and abundance of GABA_A receptors with two different types of α subunits has been described previously [80].

As the knockout of any GABA_AR subtype results in a general shift in excitation / inhibition (E/I) balance, it is possible that the behavioral changes observed above are attributable to a general shift in E/I balance rather than the absence of $\alpha 5$ GABA_ARs in the DG. Importantly, knockdown of synaptic $\alpha 2$ GABA_ARs in the DG granule cells and the resulting reduction in fast phasic inhibition had no effect in high interference tasks [50]. Similarly, knockout of the extrasynaptic anchoring protein radixin leads to a shift in the expression of $\alpha 5$ GABA_ARs from extrasynaptic to synaptic positions, and results in deficits in reversal learning [81], further emphasizing the importance of tonic inhibition through extrasynaptic $\alpha 5$ GABA_ARs in managing memory interference. These findings suggest that extrasynaptic rather than synaptic receptors play an essential role in managing memory interference and enabling behavioral flexibility, at least providing evidence that these processes are not disrupted by any simple shift in E/I balance. However, this still does not fully answer the question of receptor specificity. Given that tonic inhibition in the DG is mediated through both $\alpha 5$ GABA_ARs and $\alpha 4/\delta$ GABA_ARs, a natural question that comes to mind is: would $\alpha 4/\delta$ GABA_ARs of the DG also play a similar role in managing memory interference, or is the specific pharmacology and expression pattern of $\alpha 5$ GABA_ARs required for this process? DG granule cell – selective knockdown δ GABA_ARs has been shown to impair auditory and contextual fear conditioning [49], however, the knockdown mice were unfortunately not evaluated in tasks characterized by high memory interference. There is some evidence that a global knockout of δ GABA_ARs impairs discrimination learning [68], and that the hippocampal δ GABA_ARs may be involved in state-dependency of learning and memory [82], both of which are suggestive of a role for δ GABA_ARs in handling memory interference. Thus, it is possible that tonic inhibitory control of the DG activity, rather than

δ 5GABA_ARs specifically, play an important role in enabling memory control under high interference and behavioral flexibility.

α 1- and α 2GABA_ARs of the hippocampus have also been implicated in learning and memory, although the evidence is limited. Administration of pregnenolone sulfate into the dorsal CA1 area following memory acquisition improves memory in a passive avoidance task [44]. Pregnenolone sulfate is a negative modulator of GABA_ARs including α 1 GABA_ARs [83]. Thus, these findings may suggest a role for CA1 α 1 GABA_ARs in memory consolidation, providing temporal and anatomical specificity to early reports that the amnesic effects of diazepam were abolished in α 1 (H101R) mice, in which diazepam does not bind to α 1GABA_ARs [63]. Selective knockdown of α 2GABA_ARs in CA1 pyramidal neurons improves contextual fear conditioning [43], whereas reduction of α 2GABA_AR activity selectively in the DG improves both object recognition [84] and spatial [50, 43] memory.

While the above studies focused mostly on GABA_ARs in principal neurons of the hippocampus, the GABA_ARs on interneuronal membranes are also likely to play integral roles in learning and memory, as synchronous population activity of hippocampal neurons is regulated by the precise activity patterns of the interneurons [85]. All PV+ interneurons in CA1, CA3 and DG express the α 1 GABA_ARs [86]; Fig. 2). A selective knockout of γ 2GABA_ARs in PV+ interneurons led to a reduction in theta range oscillations in the hippocampus, altered theta-gamma coupling [87], neuronal population activity patterns that have been shown to be important for memory formation, and impaired spatial learning. However, the findings were confounded by other phenotypic changes in the conditional knockout mice, including low body weight and impaired motor performance [88]. Moreover, recent work suggests that long-range GABAergic projections to PV+ interneurons, at least in the DG, might lead to the excitation, rather than hyperpolarization, of these neurons [89]. With this additional information, it is unclear whether the knockout of a GABA_AR subunit would lead to increased or reduced activity of these neurons. Selective knockout of γ 2GABA_ARs on SST+ interneurons of the forebrain, on the other hand, led to a subtler behavioral phenotype with no observed changes in spatial learning, despite increasing both spontaneous and miniature inhibitory postsynaptic currents (IPSCs) in CA1 [52]. Finally, the knockout of δ GABA_ARs in all GABAergic interneurons leads to increases in both tonic and phasic inhibition in CA1 and DG, possibly mediated by different interneuron subtypes [90]. For instance, a large subpopulation of PV+ interneurons in DG express δ subunits, while only a small minority of SST+ interneurons, and none of the calbindin+ or calretinin+ interneurons express the δ subunit [91].

Other Brain Regions Involved in Control of Learning and Memory

The brain circuitry for memory formation and retention clearly extends beyond the hippocampal formation and includes different structures and circuits depending on the type of learning involved. However, most of the brain region- and cell type-specific work investigating the role of GABA_AR subtypes in learning and memory focused on the hippocampus, due to the clear involvement of this brain area in multiple forms of learning and memory. One exception to this is a study showing that the infusion of GABA_AR

antagonist gabazine into the mediodorsal thalamic nucleus facilitates, while δ -preferring agonist gaboxadol impairs fear extinction learning (see Box 5). The effect of gabazine on fear extinction was recapitulated by a selective knockdown of $\alpha 4$ GABA_ARs in the mediodorsal thalamic nucleus [92].

Overall, the findings suggest that GABA_ARs may play a suppressive role in learning and memory in a receptor-, brain-region- and task-specific manner when it comes to the acquisition and retention of relatively simple memories. However, GABA_AR action is necessary, again in a fine-tuned manner, for complex tasks that require adaptive behavioral actions.

GABA_A Receptors in Reward and Addiction Circuits

The mesocorticolimbic dopamine system lies at the heart of the brain reward circuitry. This system originates in the ventral tegmental area (VTA), and includes several areas that receive afferents from the VTA, including the nucleus accumbens (NAc), the prefrontal cortex (PFC), the hippocampus, the amygdala and the bed nucleus of stria terminalis (BNST), lateral hypothalamus and the ventral pallidum. Drugs of abuse exert their addictive actions through increasing dopamine levels in target structures and inducing synaptic plasticity in the VTA. A simplified representation of the VTA – NAc microcircuit, with some of the known GABA_ARs can be seen in Fig. 4.

Certain drugs of abuse, such as benzodiazepines, directly modulate the activity of GABA_ARs. Human genetics studies have noted associations between GABA_AR genes and different aspects of alcoholism [93-98]. More surprisingly, GABA_AR genes (particularly GABRA2 and GABRA4) have also been linked to illicit drug use, to dependence for drugs that have non-GABA-related mechanisms of action and to polydrug use [97, 99-103]. The involvement of GABA_AR genes in the abuse of and dependence on drugs that do not directly affect the GABA_ARs suggest that the GABA_ARs in brain reward circuits may be involved in the development of brain changes that mediate the addictive properties of drugs in general.

An underlying mechanism for the addictive properties of benzodiazepines and $\alpha 1$ -preferring GABAergic compounds has been identified to be VTA **dopaminergic disinhibition** [104]. As the $\alpha 1$ GABA_ARs are expressed on GABAergic neurons that target the dopaminergic neurons in the VTA (see Fig 4B), the activation of these receptors causes **hyperpolarization** of the GABAergic neurons, relieving the inhibitory control over VTA dopamine neurons. On the contrary, $\alpha 3$ and $\alpha 4$ -GABA_ARs are expressed on the dopaminergic neurons of the VTA, and thus, the activation of these receptors should lead to hyperpolarization of the VTA dopaminergic neurons. However, it has been shown in rhesus monkeys that at least under certain circumstances, compounds that do not modulate $\alpha 1$ GABA_ARs are self-administered intravenously, suggesting that they have rewarding properties [105]. Moreover, positive modulation of $\alpha 2$ GABA_ARs is required for the reward-enhancing effects of benzodiazepines [106], and $\alpha 2$ GABA_ARs in the NAc are necessary to maintain midazolam preference in a two-bottle choice drinking test [107]. These findings clearly demonstrate a role for $\alpha 2$ GABA_ARs in benzodiazepine reward and point to the NAc as the possible anatomical locus for this effect. However, it is still perplexing how the inhibition of **medium spiny**

neurons (MSNs) of the NAc through $\alpha 2$ GABA_ARs can lead to rewarding effects. Part of the answer to this question may lie in further divisions within the NAc. It has been demonstrated that the activation of D1+ MSNs leads to positive reinforcement and enhances the rewarding properties of addictive drugs, while the activation of D2+ MSNs may have the opposite effect (see [108] for a contradictory viewpoint). Moreover, infusion of diazepam into the VTA leads to increased activity of D1+ MSNs, but not D2+ MSNs [109]. Unfortunately, the relative abundance of $\alpha 2$ GABA_ARs in the two NAc neuronal populations has not been investigated. Thus, it is not clear whether the activation of NAc $\alpha 2$ GABA_ARs may shift the balance in favor of D1+ MSNs within the NAc and lead to reinforcing effects this way.

Another GABA_AR-modulating drug of abuse, ethanol, at high doses potentiates GABA-gated current in a mostly nonselective manner in terms of the GABA_AR subunits, with the exception of the $\alpha 4/6\beta 3\delta$ GABA_ARs, which seem to be sensitive to low concentrations of ethanol as observed with social drinking [110]. The ethanol modulation of these high-sensitivity receptors may play an important role in the maintenance of ethanol self-administration, as knockdown of $\alpha 4$ GABA_ARs in the NAc shell (but not core) was sufficient to completely abolish self-administration of ethanol in rats [111, 112]. Similarly, ethanol consumption could be increased in a limited access paradigm when the activity of $\alpha 4$ GABA_ARs in the mPFC was potentiated through gaboxadol [113]. δ GABA_ARs in the VTA have also been implicated in the consumption of ethanol, although only under specific circumstances [114]. While emphasizing the importance of the activity of extrasynaptic GABA_ARs in the rewarding effects of ethanol and providing anatomical specificity, these studies do not clarify the overall circuit mechanisms of ethanol reward. For instance, the activation of the $\alpha 4$ GABA_ARs in NAc should have the effect of reducing activity of the MSNs, which should be aversive. Similarly, activating $\alpha 4$ GABA_ARs in mPFC should reduce the excitatory input into VTA dopaminergic neurons, again leading to an overall aversive effect. While $\alpha 4$ GABA_ARs are expressed in both D1+ and D2+ neurons, and have been shown to play opposing roles in the reinforcing properties of other drugs of abuse [115], the relative abundance of the receptor in the two neuronal populations is not clear. Similarly, whether these receptors may be expressed at different levels in prelimbic versus infralimbic subsections of the mPFC, or disproportionately affect different projections from mPFC is not known. Without this information, it is impossible to delineate how the activation of extrasynaptic GABA_ARs leads to an overall reinforcing effect in the brain reward system. Finally, it should be noted that in addition to directly modulating the activity of GABA_ARs, ethanol also increases neurosteroid concentrations, which may also underlie some of its behavioral affects (see [116] for a review). As noted earlier, neurosteroids modulate the activity of GABA_ARs, and thus, ethanol may induce changes in the activity of GABA_ARs via its effect on neurosteroids, in addition to directly binding to GABA_ARs.

Besides playing a role in the reinforcing effects of drugs of abuse, GABA_ARs are also affected by long-term use of drugs. For instance, long-term administration of ethanol reduces the expression of $\alpha 1$ GABA_ARs in the VTA [117], possibly increasing baseline GABAergic inhibition over dopaminergic neurons and requiring higher ethanol concentrations for dopaminergic disinhibition. Such a mechanism might partially underlie accelerated ethanol intake over time in addicted individuals. On the contrary, chronic

nicotine [118] or cocaine [119] increases VTA $\alpha 1$ GABA_AR expression, paralleling behavioral sensitization to the effects of these drugs. A reduction in NAc $\alpha 2$ GABA_ARs accompanies cocaine sensitization through long-term administration [120], while there is some evidence that these same receptors are required for the development of behavioral sensitization to cocaine in the first place [99,121], which may explain both initial sensitization and later acceleration of intake. Withdrawal from cocaine, on the contrary, leads to an increase in the surface expression of $\alpha 2$ GABA_ARs in NAc neurons, possibly making the system even more vulnerable to the effects of cocaine and sensitization [122].

Overall, GABA_ARs in the mesolimbic dopamine system play an integral role in the reinforcing and addictive properties of benzodiazepines, ethanol and other drugs of abuse. While the determination of specific locations of certain GABA_ARs has elucidated some of the underlying mechanisms for the effects of these drugs, further study of GABA_ARs, particularly in different NAc neuronal populations, is required to clarify these mechanisms further.

GABA_A Receptors in Stress and Depression Circuits

Clinical and preclinical studies provide evidence for a GABA hypothesis in depression where decreases in GABA signaling and GABAergic cell types, as well as alterations in receptor subtypes are detected in depressed patients as well as animals exposed to stress (a factor in the development of depression), for a thorough review of these studies see [123]. Conversely, antidepressant treatment or increases in cellular activation (through deep brain stimulation or optogenetic/pharmacological manipulations) reverses these disease and stress-induced changes in GABAergic signaling [123-127]. Genetic manipulation of specific GABA_AR subtypes has helped us to begin to understand the GABAergic circuitry that may be involved in the development of depression. Global heterozygous knockdown of the $\gamma 2$ subunit leads to impaired GABAergic signaling [11], as well as anxiety- and despair-like behavior that is reversed with chronic desipramine (but not fluoxetine) [127] and acute ketamine [126] treatment. More recently it was demonstrated that global knockdown of the $\alpha 2$ subunit also leads to anxiety- and despair-like behavior [9], and that in these mice sensitivity to anxiogenic- and prodepressant-like effects with chronic fluoxetine treatment is increased [128]. Studies in post-partum female mice demonstrated that decreases in the δ subunit lead to increased anxious- and depressive-like behavior as well as maternal neglect, while the δ subunit preferring agonist gaboxadol improved maternal care [129]. Additionally, the neurosteroid allopregnanolone, which acts as a δ GABA_AR preferring positive allosteric modulator, has shown some success in recent clinical trials for the treatment of postpartum depression [130]. Conditional deletion of $\gamma 2$ within the forebrain in early life disrupts GABAergic signaling in a manner similar to that observed with global decreases in $\gamma 2$, and also leads to an anxious and depressive phenotype [131], highlighting the forebrain as a region where inhibitory signaling is particularly sensitive to stress.

Distinct GABAergic cell types within the forebrain and in particular the prefrontal cortex and hippocampus make up an intricate circuitry, serving to modulate cellular activity through synapses on excitatory projection neurons as well as other interneurons. GABAergic interneurons can be subdivided by their cellular morphology, projection patterns, as well as

the expression of different proteins. As noted earlier, PV+ and SST+ interneurons form relatively non-overlapping interneuron populations [132, 133]. Decreases in SST expression are detected in the dorsolateral prefrontal cortex and subgenual anterior cingulate cortex of depressed patients as well as the prefrontal cortex of mice exposed to chronic unpredictable stress, while changes in PV are less consistent [134-136]. Manipulation of SST firing through designer receptor exclusively activated by a designer drug (DREADD) technology, demonstrated that acute inhibition of this GABAergic cell type has an anxiogenic- and prodepressant-like effect while chronic inhibition has an anxiolytic- and antidepressant-like effect [137]. More recently, conditional deletion of $\gamma 2$ from SST+ interneurons in the forebrain was shown to disinhibit this interneuron population, in turn inhibiting excitatory pyramidal cells within CA1 of the hippocampus and layer 2/3 of the cingulate cortex and having anxiolytic- and antidepressant-like effects [52]. While these studies are much more targeted than the global gene deletion studies discussed earlier, the $\gamma 2$ subunit is found in 90% of GABA_ARs. Thus, narrowing the scope to identify the specific GABA_A α subunits involved would provide greater circuit specificity. Additionally, pharmacological compounds with α subtype selectivity are a promising potential therapeutic tool as they lack some of the side effects associated with nonselective GABAergic compounds such as sedation and tolerance.

There is clinical evidence for the antidepressant effects of subtype selective GABA_A compounds as eszopiclone, a positive GABA_A modulator with postulated selectivity for $\alpha 2/\alpha 3$ GABA_ARs [138], has a synergistic effect when given in combination with fluoxetine in patients with depression and insomnia [139]. Interestingly, both positive and negative allosteric modulation of $\alpha 5$ GABA_ARs produces rapid antidepressant-like effects and/or reductions in stress-related behaviors in rodents exposed to chronic stress [17, 18, 140, 141]. Although it is not entirely clear how positive and negative modulation of the same receptor subtype could have antidepressant-like effects, it should be noted that the effects of the negative allosteric modulators are observed both 1 hour and 24 hours following administration. Considering the relatively short half-life of the drugs used (0.5 hours) [142], by the 24-hour time point, the drug is no longer in the system, and the anti-stress and antidepressant-like effects are likely due to a different mechanism than the direct repression of the activity of $\alpha 5$ GABA_ARs (although it is possible that a long-term reduction in the activity of the receptor may be induced by the drug). Experiments with positive allosteric modulators, on the other hand, were conducted shortly after drug administration [18], and the effects observed are likely to be the result of an increase in the activity of $\alpha 5$ GABA_ARs.

The potential $\alpha 5$ -mediated circuitry involved in stress responsiveness can begin to be teased apart based on the localized expression pattern of $\alpha 5$ GABA_ARs in the olfactory bulb, cortex, hippocampus, and granule cells within the cerebellum [36]. The $\alpha 5$ positive allosteric modulator SH-053-2'-F-R-CH₃ increased $\alpha 5$ transcript expression in the prelimbic cortex and hippocampus of female mice exposed to chronic stress [18]. Furthermore, the antidepressant-like effects of the $\alpha 5$ negative allosteric modulator L-655,708 are blocked with an intrahippocampal infusion of lidocaine [140]. We believe that cell-type selective gene deletion or chemogenetic manipulation studies could help to further characterize the specific $\alpha 5$ -mediated circuitry within the prefrontal cortex and hippocampus involved in

depression, and potentially help to better understand how pharmacological manipulation of this receptor subtype could be used to treat depression.

Concluding Remarks and Future Directions

High structural diversity of GABA_ARs, combined with their differential expression in brain circuits and subcellular compartments leads to highly complex and precise inhibitory control of brain circuits. While our knowledge on the circuit-specific functions of GABA_ARs is limited (see Outstanding Questions), there is evidence suggesting that the same GABA_AR subtype can be expressed in different neuronal populations and different subcellular locations in different brain areas, modulating population excitability and synchronization in different ways. Thus, a full understanding of the role of GABA_ARs in the brain is possible only through a subtype-, circuit- and neuronal population-specific study of their functions. In recent years, studies taking such focused approaches have revealed that the behavioral functions of GABA_ARs do indeed vary vastly depending on the circuits and neurons they are expressed in, and a specific GABA_AR subtype expressed at low levels at a critical point in a circuit can have a disproportionately large effect on behavioral outcomes. As an example, due to their expression almost exclusively in the hippocampus and the cortex, α 5GABA_ARs have mostly been studied in relation to their effects on cognition. However, a recent study showed that α 5GABA_ARs in a small neuronal population in the central amygdala can regulate anxiety behaviors [4]. Subsequent studies have shown that indeed, systemic α 5 manipulations can affect anxiety-related behavior [16]. Thus, circuit-specific understanding of GABA_AR function can identify new targets for pharmacological therapies, even when the drugs are administered systemically. Moreover, recent developments in drug delivery technologies are likely to make neuronal population-specific delivery of drugs viable in the near future. A potentially powerful novel experimental technique for neuronal cell-type-specific drug delivery is DART (drugs acutely restricted by tethering). A protein called HaloTag is expressed in defined neuronal cells (e.g. using a cell-type-specific promoter) and captures and tethers drugs to the cell surface over seconds to minutes, producing an approximately 100-fold enrichment of the drug on the surface of the HaloTag-expressing cells. Extremely low doses of the compound which have no effect on their own in control animals are administered systemically and only on the surface of the HaloTag-expressing neurons is the compound concentration high enough to elicit an effect [143]. Rectification of circuit dysfunction could be achieved through such targeted delivery of GABA_AR subtype-selective agents without the side-effects of global GABA_AR modulation. Thus, the recently emerging studies of the circuit pharmacology of GABA_ARs are essential both from a fundamental point of understanding brain function, and from a translational point of developing effective pharmacological therapies for circuit dysfunction in cognitive and neuro-psychiatric disorders, e.g. α 5 positive allosteric modulators may be useful to attenuate hippocampal hyperactivity in order to improve cognitive function in schizophrenia, age-related cognitive dysfunction and mild cognitive impairment due to Alzheimer's disease.

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Glossary

Allosteric modulation

receptor activity change via a modulatory site distinct from the orthosteric site at which the neurotransmitter binds. Can be positive or negative

α1-sparing compounds

GABA_AR modulators which positively modulate GABA_ARs containing the α2, α3 and α5 subunits, but not GABA_ARs containing the α1 subunit

Amygdala

brain structure containing nuclei deep and medially within the temporal lobes. Involved in the processing of emotions

Anterograde amnesia

inability to create new memories after an amnesia-causing event

Anxiolytics

agents that inhibit anxiety and fear

Axon initial segment (AIS)

neuron segment located between the axon hillock and the myelin sheath with a high density of voltage-gated ion channels. Putative initiation site for action potentials

Circuit Pharmacology

novel term describing how specific GABA_A receptor subtypes in circuit- and cell-type-specific locations mediate pharmacological actions, e.g., behavioral phenotypes

Dendritic projections

nerve extensions from the cell body of a neuron to other neural cells from which the dendrites forms excitatory or inhibitory synapses

Dopaminergic disinhibition

dopaminergic neurons in the ventral tegmental area are inhibited by GABAergic interneurons. If these GABAergic interneurons are also inhibited, this leads to disinhibition (activation) of dopaminergic neurons

Extrasynaptic

extrasynaptic neurotransmission occurs outside of the synaptic terminals, activating receptors that are also outside of the synaptic terminals

Gephyrin

protein component of the postsynaptic protein network of many but not all inhibitory synapses. For example, $\alpha 2$ -containing GABA_ARs but not $\alpha 5$ -containing GABA_ARs are colocalized with gephyrin

Hyperpolarization

a change in membrane potential rendering a cell more negative

Ionotropic receptors

ligand-gated ion channels

IPSCs

inhibitory postsynaptic currents. Spontaneous IPSCs (sIPSCs) are driven by intrinsic properties of presynaptic cell activity, whereas mini IPSCs (mIPSCs) are recorded in the presence of tetrodotoxin which blocks action potential formation and propagation, and can be used to determine the releasable neurotransmitter pool size

Medium spiny neurons

MSNs are GABAergic projection neurons in the nucleus accumbens and striatum. They are positive for D1 or D2 dopamine receptors

Microinfusion studies

microinfusion of compounds into defined brain regions to study the role of receptors

Neuronal circuits

interconnected neurons wired together

Phasic inhibition

short-lasting form of neuron inhibition which is generated by the activation of synaptic GABA_ARs following action potentials in a presynaptic neuron

Postsynaptic pentameric complexes

GABA_A receptors are complexes containing five subunits, which are typically located in the membrane of the postsynaptic neuron

Radixin

cytoskeletal actin-binding protein of the ezrin/radixin/moesin (ERM)-family which –through membrane association and phosphorylation-dependent conformational change, is thought to be RhoA GTP- and Rho-kinase (ROCK) dependent – anchors $\alpha 5$ -GABA_ARs at extrasynaptic sites

shRNA

short hairpin RNA, an artificial RNA molecule used to silence gene expression by RNA interference

Synchronized oscillatory activity

rhythmic or repetitive neural activity

Tonic inhibition

sustained inhibition of neurons, e.g., by activation of extrasynaptic GABA_ARs

Box 1**Interneuron diversity**

Neuronal networks are formed by glutamatergic excitatory projection neurons and local GABAergic interneurons that gate signal flow and determine network dynamics. The GABAergic interneurons, which are far outnumbered by excitatory projection neurons, are essential for sculpting network responses. GABAergic interneurons form functional classes based on morphological types, targeting bias, anatomical properties, biophysical properties, synaptic properties and in vivo patterns of activity. For example, in the neocortex, approximately 40% of interneurons are positive for parvalbumin (PV), 30% positive for somatostatin (SST), and 30% for the ionotropic serotonin receptor 5HT3a (5HT3aR). The latter group can be subdivided into neurons that are positive for vasoactive intestinal polypeptides (VIP, approximately 40% of 5HT3aR-positive neurons) and non-VIP neurons. Morphologically, PV-positive neurons are basket cells targeting the soma or Chandelier neurons targeting the axon initial segments of excitatory neurons. PV-positive cells are fast-spiking and show little or no spike frequency adaptation. SST-positive neurons preferentially target dendrites of excitatory neurons. They are low threshold spiking cells with low frequency firing and spike frequency adaptation.

In addition to the markers already mentioned, other markers such as the calcium-binding proteins calbindin and calretinin, but also reelin, cholecystokinin and neuropeptide Y, are used to define interneurons. These markers are not expressed in non-overlapping populations of interneurons and thus do not describe discrete interneuron populations. They are, however, useful for defining subpopulations of interneurons.

As noted in the main text, there is some evidence that specific interneuron subtypes may form synapses with specific GABA_AR subtypes in the postsynaptic membrane. However, recent studies have yielded contradictory results [3], and the question of input specificity remains unanswered.

Interneurons can be involved in feedforward inhibition, feedback inhibition and disinhibition. In feedforward inhibition, there is an excitatory input both onto the principal cell and the interneuron, and an inhibitory input from the interneuron to the principal cell. The interneuron gets excited in response to the excitatory input and “forwards” an inhibitory response to the principal cell. In feedback inhibition, the principal neurons and the inhibitory neurons form a circuit loop, where the excited principal neurons send excitatory projections to the local interneurons, which in turn send an inhibitory “feedback” signal to the principal neurons. In disinhibition, one interneuron inhibits another interneuron, thus preventing it from inhibiting a principal cell.

For recent reviews on interneurons see [144] for cortical interneurons and for hippocampal interneurons see [145].

Box 2**Distribution and functions of GABA_A receptor subtypes**

GABA_ARs are typically postsynaptic pentameric complexes, made from a subunit repertoire of at least 19 subunits (α 1-6, β 1-3, γ 1-3, δ , ϵ , θ , π , ρ 1-3) which form a central channel with permeability for chloride ions. GABA-mediated chloride influx results in hyperpolarization of the postsynaptic neuron. Most GABA_ARs contain α , β and γ subunits, including the most abundant α 1 β 2 γ 2 receptor, which makes up approximately 60% of all GABA_ARs in the brain. GABA_ARs containing the α 1, α 2, α 3 or α 5 subunit (α 1GABA_AR, α 2GABA_AR, α 3GABA_AR or α 5GABA_AR) are modulated by classical benzodiazepines, those containing α 4 (α 4GABA_AR) and α 6 (α 6GABA_AR) are not. The expression of the α 6 subunit is largely limited to the cerebellum. The functions of GABA_AR subtypes, as defined by the presence of a specific α subunit, have been elucidated by the following methods: 1) Studies of gene knockout mice, 2) studies of gene knock-in mice, e.g. with point mutations in α subunits rendering the respective GABA_ARs insensitive to modulation by benzodiazepines, alcohol or volatile general anesthetics, 3) pharmacological studies using subtype-selective positive or negative allosteric modulators, and 4) using short hairpin RNA (shRNA) approaches. The big caveat with studying knockout mice lies in compensatory upregulations of other GABA_AR subunit genes, which may obscure the functions of the knocked-out gene. Pharmacological studies with subtype-selective compounds have the major limitation that none of the known compounds is truly specific for any one subtype, making it difficult if not impossible to link effects of such compounds to individual receptor subtypes with absolute certainty. Despite this shortcoming as tools for basic science, for therapeutic purposes, relative selectivity rather than absolute specificity is often sufficient, and thus, these compounds represent a promising therapeutic venue. See Table I for a summary of the general distribution and abundance of α 1, α 2, α 3, α 4, and α 5 subunits in the mouse brain, as well as typical subcellular locations and known functions of each subunit [146-148]. With this knowledge, new drugs may be developed which selectively maintain some but not other effects of classical benzodiazepines, e.g., anxiolysis without sedation, or have pharmacological effects that are not observed with classical benzodiazepines, e.g., antihyperalgesia (i.e. reduction of an increased sensitivity to pain). As noted above, current subtype-selective compounds lack absolute specificity for any one GABA_AR subtype, which is a shortcoming for their use as tools for basic science. However, for therapeutic purposes, relative selectivity rather than absolute specificity is often sufficient, and thus, these compounds represent a promising therapeutic venue.

Box 3**Tools for studying GABA_A receptor subtypes in defined circuits**

In order to identify the circuits or neuronal populations in which a GABA_AR subtype mediates a pharmacological action or a physiological function, i.e., for “Circuit Pharmacology”, the following methods are of interest:.

1. **Stereotaxic injection of a pharmacological compound** into a specific brain region. Stereotaxic injections of pharmacological compounds have major limitations: they are not specific for any cell type and diffusion of the compound makes it difficult to be sure where exactly it is acting.
2. **Conditional mutagenesis** to achieve complete or partial reduction of the expression of a GABA_AR subtype. Cre recombinase can be expressed either from a mouse transgene or from a viral vector in an animal in which the gene to be knocked down (or parts thereof) have been flanked by loxP sites. Cre-loxP-mediated recombination then leads to the excision of the loxP-flanked gene. In either case, and with a much wider range of promoters available as mouse transgenes, it may be possible to limit the knockout / knockdown to a specific cell type by expressing the cre under a promoter expressed only in the cell type of interest. Moreover, stereotaxic injection of a viral vector carrying cre under the control of a specific promoter can further limit the knockout / knockdown anatomically to the injection area. Similarly, a viral vector expressing an **shRNA** construct specific for the GABA_AR subunit to be deleted that requires cre-loxP-mediated recombination for its expression can be stereotaxically injected into mice expressing a Cre transgene in a cell type of interest, so that only in the cell type of interest the shRNA is expressed and the protein of interest knocked down. While providing cell-type and anatomical specificity, conditional mutagenesis techniques are not without drawbacks: Conditional knockouts of GABA_AR receptor subunits can cause a general shift in excitation/inhibition balance of the brain region of interest, and/or affect other parameters, such as neurogenesis [149]. For stereotaxic injection of viral vectors, diffusion beyond the target area can be difficult to control, especially for small targets. Thus, it should be noted that the findings from studies reviewed in this manuscript may at times reflect changes in these extraneous variables, in addition to changes stemming from the lack of a GABA_AR subtype.

In addition to optogenetically controlling GABA_ARs (see Box 4), it is also possible to address circuit-relevant functions of GABA_ARs from inside the cell by targeting anchoring proteins, e.g. **radixin**, which anchors α 5GABA_ARs to extrasynaptic sites (see main text) [150], and the synapse-specific tetraspanin LHFPL4, a regulator of postsynaptic clustering. It has been shown recently that knockout of LHFPL4 results in a cell-type-specific reduction in GABA_AR and gephyrin clusters and accumulation of large intracellular gephyrin aggregates in vivo; GABA_ARs are still trafficked to the

neuronal cell surface, but no longer localized at synapses, resulting in a profound loss of fast IPSCs [151].

Box 4**Circuit pharmacology in the era of chemogenetics and optogenetics**

Conditional mutagenesis in mice (Box 3) has been used for more than two decades, while chemogenetics and optogenetics are more recent developments. In these applications, constructs expressing engineered activating or inhibitory G protein coupled receptors that are modulated by a ligand (chemogenetics) or constructs expressing engineered activating or inhibitory ion channels that are controlled by light (optogenetics) are typically expressed in a conditional manner and in a specific brain area, providing both cell type and anatomical specificity. Optogenetic applications have the additional advantage of temporal specificity through the control of neuronal activity using light. Through the examination of artificial activation or inhibition of specific cell-types, brain areas and projections, these techniques have contributed to our understanding of brain circuits and their functions. However, these techniques are not designed to identify signal transduction pathways or proteins such as specific GABA_AR subtypes that are naturally involved in modulation of the activity of specific cells. Moreover, activation or inhibition patterns with chemogenetic and optogenetic approaches may or may not correspond to physiologically relevant patterns of activity regulation that a protein of interest, e.g. a specific GABA_AR subtype, is involved in. In summary, techniques such as conditional mutagenesis still have utility in answering questions such as the pharmacological functions of defined gene products in defined circuit locations (i.e. questions related to “circuit pharmacology”), which cannot be answered with the newer techniques. These approaches should complement rather than substitute each other.

An interesting recent development are photoswitch ligands and complementary genetically modified GABA_AR subunits, which allow the generation of light-sensitive GABA_ARs, with the light providing 40-80% antagonism at different recombinant $\alpha(x)\beta2\gamma2S$ ($x=\alpha1-\alpha6$) receptors and an $\alpha1(T124C)$ knock-in mouse with “photoswitch-ready” version of a GABA_AR $\alpha1$ subunit [152]. It could be demonstrated that IPSC photoswitching occurs in cerebellar molecular layer interneurons which express the $\alpha1$ subunit, but not in cerebellar Golgi cells which do not express the $\alpha1$ subunit. Moreover, while a non-subtype-selective blockade of all GABA_ARs with picrotoxin is known to dampen γ oscillations [153], photo-antagonizing specifically $\alpha1$ GABA_ARs resulted in an increase in γ power [152]. It was hypothesized that since inhibitory synapses between PV + interneurons are highly enriched in $\alpha1$ GABA_ARs [154], these results support a crucial role of inhibition-inhibition (I-I) in γ rhythmogenesis. What is, to our knowledge, currently lacking is a demonstration that photo-antagonizing specific GABA_AR subtypes results in a change in behaviors such as cognitive tasks and anxietylike behaviors.

Box 5**Behavioral tests to detect anxiety-related and fear-related behaviors****Elevated plus maze test**

a test used to measure anxiolytic (or anxiogenic) drug action in rodents. The plus-shaped instrument is elevated from the floor and has two open arms, and two closed arms with walls. Due to their native aversion of open, exposed spaces, rodents prefer to spend most of their time in the closed arms, and increased time spent in the open arms indicates anxiolysis.

Light/dark box test

a test used to measure anxiolytic (or anxiogenic) drug action in rodents. Due to their native aversion of brightly lit spaces, rodents prefer to stay in the dark compartment of a two-compartment box, where the other compartment is brightly lit. Increased time spent exploring the light compartment indicates anxiolysis.

Fear conditioning

a behavioral paradigm in which an initially neutral stimulus (conditioned stimulus (CS), e.g., a tone) is paired repeatedly with an aversive stimulus (unconditioned stimulus (US), e.g., a foot shock). Over time, the CS starts eliciting the same response (e.g., freezing) as the US, as it becomes a cue predicting the US. Common measures of conditioned fear are freezing behavior in the presence of the CS, or fear-potentiation of acoustic startle responses.

Fear-potentiated startle

Rodents will exhibit startle responses when exposed to loud noises, and this startle response is enhanced by co-presentation of a CS to which the animal was previously fear-conditioned (fear-potentiated startle).

Fear extinction

a decline in conditioned fear responses following non-reinforced presentation of a feared conditioned stimulus. For instance, after fear conditioning to a tone, if the tone is presented repeatedly in the absence of foot shock, the freezing responses will become smaller over time. This is not simply “forgetting”, but learning a new relationship (i.e., tone: safe).

Vogel conflict test

a conflict-based test in which water-deprived rodents can obtain water at the expense of receiving a mild electric shock delivered to the tongue. Classical benzodiazepines reduce conflict (i.e., increase drinking) behavior in the Vogel conflict test. While it is frequently referred to as measuring “anxiolytic-like” properties of drugs, in contrast to tests of unconditioned anxiety (e.g., the elevated plus maze or the light/dark box) it involves a specific aversive stimulus (like fear conditioning) and thus may be considered a test of fear-related behavior.

For detailed protocols, see [43, 50].

Outstanding Questions

- How are different GABAAR subtypes distributed on different cell populations in structures such as the amygdala, the hippocampus, the ventral tegmental area and the nucleus accumbens?
- Is there input-specificity in terms of which interneurons form synapses with which GABAAR subtypes in the postsynaptic membrane? Interneuron populations are characterized by exceptional diversity in terms of their anatomical structure, chemical identity, intrinsic activity, location (e.g., which layers of the cortex or the hippocampus) and which neurons (and subcellular compartments of the neurons) they form synapses. This diversity has implications for circuit dynamics. The question of whether different classes of interneurons may preferentially form synapses with specific GABAAR subtypes in the postsynaptic membrane has been tackled, e.g., in the CA1 region of the hippocampus, with some initial clues, but the answer remains largely unknown.
- Are changes in GABAAR expression in human psychiatric disorders specific to certain foci in certain circuits, and thus, could targeted delivery methods be used in the future to deliver subtype-selective compounds to these critical points to provide maximal symptom relief with minimal side-effects? There is already some evidence of changes in the expression of GABAAR subunits specifically in certain brain regions in different disorders, and technologies providing drug delivery targeted to specific neuronal populations are emerging.

Highlights

Both receptor subtype and circuit location determine the physiological and pharmacological functions of GABA_A receptor (GABA_AR) subtypes.

α2 GABA_ARs in distinct hippocampal microcircuits are required for the anxiety-reducing and the fear-reducing actions of systemically administered diazepam.

α5 GABA_ARs in PCKδ⁺ neurons of the central amygdala have been linked to modulation of anxiety.

α5 GABA_ARs in dentate gyrus granule cells are required for learning and memory tasks involving memory interference. Positive allosteric modulators of α5 might be useful to attenuate interference related cognitive symptoms and hippocampal hyperactivity in some psychiatric disorders.

In the mesolimbic dopamine system, both α1 GABA_ARs in GABAergic ventral tegmental area interneurons and α2 GABA_ARs in medium spiny neurons in the nucleus accumbens are required for full reinforcing effects of benzodiazepines.

Figure 1. Simplified depiction of intra-amygdalar circuitry with selected GABA_ARs

The basic composition of the amygdala is shown in **A**. The lateral (LA) and basal (BA) nuclei together form the basolateral amygdala (BLA, not shown), and have a cortex-like structure with a large number of excitatory projection neurons regulated by a smaller number of GABAergic interneurons. The central amygdala, on the other hand, consisting of centrolateral (CeL) and centromedial (CeM) amygdala has a striatum-like structure with GABAergic neurons serving both as principal and interneurons. Along the lateral and medial sides of the basolateral complex lie GABAergic intercalated masses. Boxes B-E provide highly simplified views highlighting a few of the known GABA_AR subtypes involved in this complex inhibitory system. In these boxes, the GABA_A receptor subunits $\alpha 1$, $\alpha 2$, $\alpha 3$, $\alpha 4$, $\alpha 5$ and α are shown, indicating GABA_A receptor complexes which contain the respective (and additional) subunits (referred to as $\alpha 1$ GABA_ARs, $\alpha 2$ GABA_ARs, $\alpha 3$ GABA_ARs, $\alpha 4$ GABA_ARs, $\alpha 5$ GABA_ARs, and α GABA_ARs).

B. Shown here is an excitatory projection neuron in the LA receiving inhibitory control from PV+ and SST+ interneurons as well as a nearby GABAergic intercalated cells. PV+ interneurons of the LA form synapses on the perisomatic region (defined by the cell body, axon initial segment, and proximal dendrites) of principal cells (shown here synapsing on the cell body). PV+ interneurons have been shown to be under strong tonic inhibition and to express $\alpha 1$ and δ subunits, but it is not clear if these subunits are expressed in the same receptor complexes. The δ subunit on these interneurons has been linked to conditioned fear. SST+ interneurons form synapses on distal dendrites of pyramidal neurons, where $\alpha 1$ subunits are highly expressed (at least in the LA).

C. The medial intercalated cells, which are GABAergic neurons that provide an inhibitory interface between input and output nuclei and modulate amygdala output, e.g., as shown here for GABAergic neurons in CeL, express $\alpha 2$ GABA_ARs and $\alpha 3$ GABA_ARs synaptically,

which mediates reciprocal inhibition between the two medial cell masses. Tonic inhibitory control of the intercalated cell masses is provided by δ -(likely $\alpha 4\delta$ -) containing GABA_ARs.

D. In the central amygdala PKC δ + neurons express $\alpha 5$ GABA_ARs, which have been shown to reduce anxiety and fear generalization. **E.** In the centromedial amygdala (CeM), CRF-neurons express δ GABA_ARs mediating tonic inhibition, and these neurons inhibit CRF+ neurons, which project to the bed nucleus of the stria terminalis (not shown) and express $\alpha 1$ GABA_ARs, although at very low levels, which may be involved in modulation of anxiety-related behaviors.

Abbreviations: BA: basal amygdala, CeL: centrolateral amygdala, CeM: centromedial amygdala, CRF: corticotropin releasing factor, Hipp: hippocampus, LA: lateral amygdala, NAc: nucleus accumbens, PAG: periaqueductal gray, PKC δ : protein kinase C delta, PV: parvalbumin, SST: somatostatin, VTA: ventral tegmental area.

Figure 2. Simplified depiction of intrahippocampal circuitry with selected GABA_ARs

The basic composition of the hippocampus is shown in **A**, where unidirectional excitatory neurons project through the trisynaptic circuit from the dentate gyrus (DG, blue color) to the Cornu Ammonis subfield 3 (CA3, red) to Cornu Ammonis subfield 1 (CA1, yellow). Not shown here are that the DG receives an input from the entorhinal cortex (EC) and the monosynaptic pathway from EC to CA1. GABAergic inhibition of the hippocampal microcircuitry is highly complex. $\alpha 1$, $\alpha 2$, $\alpha 3$, $\alpha 4$ and $\alpha 5$ GABA_ARs are all expressed at moderate to high levels in the hippocampus in varying subcellular locations.

B-D depict only a small subset of these with some known (shown in black) and some hypothesized (shown in gray) GABA_AR expression patterns. Principal neurons are shown as grey circles (DG granule cells) or triangles (CA1 or CA3 pyramidal neurons), GABAergic interneurons as red circles. In addition to omitting a large variety of interneurons, the figure is also missing the excitatory mossy cells of the DG, several projections outside of the conventional trisynaptic circuitry, as well as long-projecting GABA neurons for simplification.

B. $\alpha 2$ GABA_ARs are found at high levels in all regions of the hippocampus including the DG (depicted on the dendrites, soma, and axon initial segment of the principal neuron), where their presence has been implicated in reduction of anxiety. $\alpha 4\delta$, which is also expressed strongly in the DG (shown here on the soma and dendrites of the principal neuron) but less in CA3 and CA1, keeps the DG granule cells under strong tonic inhibitory control together with $\alpha 5$ GABA_ARs, which are present at low levels in the DG. By mediating tonic inhibition, $\alpha 5$ GABA_ARs on DG granule cells are involved in memory processes related to memory interference. Various interneuron subtypes help maintain inhibitory control of the DG. $\alpha 1$ GABA_ARs are expressed strongly on PV+ basket cells that synapse on the somatic and perisomatic regions of the principal neurons in the hippocampus (depicted in **B-D**). Although typically synaptic on excitatory neurons, $\alpha 1$ on these PV+ basket cells is expressed extrasynaptically, and it has been shown to form receptor complexes together with

δ in the DG. This strong $\alpha 1$ expression keeps the PV+ basket cells under strong inhibition by other interneurons in the area, forming an inhibitory microcircuit.

C. Like in the DG, $\alpha 2$ GABA_ARs are expressed at high levels in CA3 (depicted on the soma and axon initial segment of an excitatory pyramidal cell), where their presence on principal neurons has been implicated in reduction of anxiety. Input-specific expression of $\alpha 1$ and $\alpha 2$ subunits (receiving input from PV+ and CCK+ basket cells, respectively) has previously been suggested in both CA3 and CA1 (depicted in **C** and **D**), but recently questioned (see text).

D. In CA1 $\alpha 2$ GABA_ARs on excitatory cells (depicted on the soma and axon initial segment) are involved in fear reduction. As mentioned above, input-specific expression of $\alpha 1$ and $\alpha 2$ subunits (receiving input from PV+ and CCK+ basket cells, respectively) has been suggested in both CA3 and CA1. $\alpha 1$ is also expressed to a smaller extent on bistratified cells (PV/SST).

Abbreviations: CCK: cholecystokinin, DG: dentate gyrus, EC: entorhinal cortex, Hipp: hippocampus, NAc: nucleus accumbens, PV: parvalbumin, SST: somatostatin, SUB: subiculum, VTA: ventral tegmental area.

(A)

	Diazepam effects	
Control	Anxiolytic in elevated plus maze and light/dark box	Fear-reducing in fear-potentiated startle and Vogel conflict test
$\alpha 2$CA1KO	Anxiolytic in elevated plus maze and light/dark box	No effect
$\alpha 2$CA3KO	No effect	Fear-reducing in fear-potentiated startle and Vogel conflict test
$\alpha 2$DGKO	No effect	Fear-reducing in fear-potentiated startle and Vogel conflict test

(B)

Behavioral task	Level of memory interference	$\alpha 5$ DGKO performance compared to controls
Spatial memory		
Water maze	Low	Normal
Water maze reversal learning	High	Worse
Associative fear memory		
Contextual fear conditioning	Low	Better
CFC extinction learning	High	Worse
CFC discrimination learning	High	Worse
Auditory fear conditioning	Low	Normal
AFC extinction learning	High	Worse
Object recognition memory		
Novel object recognition test	Low	Normal

Figure 3. Differential roles of $\alpha 2$ and $\alpha 5$ GABA_ARs of intrahippocampal circuits in emotional and cognitive processes

A The micrographs in the left column are false-color immunohistochemical images (yellow - orange - red - blue, from high to low intensity) of coronal brain sections taken from a representative animal of each genotype. Control indicates $\alpha 2$ floxed control mice, $\alpha 2$ CA1KO indicates mice with an $\alpha 2$ knockdown in CA1 pyramidal neurons, $\alpha 2$ CA3KO indicates mice with an $\alpha 2$ knockdown in CA3 pyramidal neurons, and $\alpha 2$ DGKO indicates mice with an $\alpha 2$ knockdown in DG granule cells. The middle and right columns highlight the behavioral tests that were performed with these mice to determine anxiolytic and fear-reducing effects of diazepam and present the results of systemically applied diazepam in these mice, showing that distinct hippocampal microcircuits mediate the anxiolytic and the fear-reducing actions of diazepam. Specifically, while $\alpha 2$ GABA_ARs in CA1 pyramidal neurons are required for the fear-reducing effects of diazepam, $\alpha 2$ GABA_ARs in CA3 pyramidal neurons and $\alpha 2$ GABA_ARs in DG granule cells are required for the anxiolytic actions of diazepam. The micrographs are reproduced from reference [43].

B. Here we summarize behavioral results obtained in $\alpha 5$ DGKO mice, i.e., mice with a knockdown of the $\alpha 5$ subunit in DG granule cells. In the left column, the behavioral tasks performed are listed, in the middle column we indicate the level of memory interference that each of these tests incurs, and in the right column the performance of the $\alpha 5$ DGKO mice is

compared to that of $\alpha 5$ floxed controls. Memory interference occurs when there is high similarity between several sets of material that are being encoded simultaneously or between what has been previously learned and what is currently encoded, e.g., in reversal learning, extinction learning and discrimination learning. While the $\alpha 5$ DGKO mice perform similar to controls or even better than controls in tasks with low memory interference, they consistently perform worse in tasks with high memory interference, indicating a rigid behavioral phenotype. The DG has been shown to be important for a process that serves to disambiguate and isolate the information encoded for the different materials, and the findings summarized here suggest that tonic inhibition mediated by $\alpha 5$ GABA_ARs in DG granule cells are essential for this process.

CFC: Contextual fear conditioning, AFC: Auditory fear conditioning, $\alpha 2$ CA1KO: Specific knockdown of $\alpha 2$ in CA1 pyramidal neurons $\alpha 2$ CA3KO: Specific knockdown of $\alpha 2$ in CA3 pyramidal neurons, $\alpha 2$ DGKO: Specific knockdown of $\alpha 2$ in dentate gyrus granule cells.

Figure 4. Simplified depiction of ventral tegmental area – nucleus accumbens circuitry with selected GABA_ARs

Multiple types of interneurons exist in both the nucleus accumbens (NAc) and the ventral tegmental area (VTA), only a small subset of which are included in the figure. GABA_ARs that have been demonstrated at the protein level (black) or only at the mRNA level (gray, $\alpha 4$ GABA_ARs on dopaminergic neurons in the VTA) are indicated.

A. The GABAergic MSNs in the NAc are either D1+ or D2+ (not shown). While $\alpha 2$ and $\alpha 4$ GABA_ARs are expressed in both D1 and D2 MSNs in the NAc, it is not clear if the relative abundance of each subunit on one population versus the other may be different.

B. Within the VTA, $\alpha 3$ and likely $\alpha 4$ GABA_ARs are expressed on the dopaminergic neurons. In contrast, $\alpha 1$ GABA_ARs are expressed on GABAergic neurons that target the dopaminergic neurons in the VTA, providing inhibitory control over the dopaminergic neurons. The $\alpha 1$ GABA_ARs on GABAergic neurons have been shown to disinhibit the dopaminergic neurons, resulting in synaptic strengthening of glutamatergic afferents to the dopaminergic neurons (not shown), increased preference for midazolam and benzodiazepine enhancement of brain stimulation reward (“benzodiazepine reward processes”). The $\alpha 2$ GABA_ARs on GABAergic MSNs in the NAc are required for benzodiazepine enhancement of brain stimulation reward and midazolam preference (“benzodiazepine reward enhancement”). Abbreviations: D1: dopamine receptor 1, D2: dopamine receptor 2, Hipp: hippocampus, mPFC: medial prefrontal cortex, MSN: medium spiny neuron, NAc: nucleus accumbens, VTA: ventral tegmental area.

Table I

^a. Relative abundance, distribution, and function of selected GABA_AR α subtypes

		$\alpha 1$	$\alpha 2$	$\alpha 3$	$\alpha 4$	$\alpha 5$
Abundance		60%	15-20%	10-15%	< 5%	< 5%
Synaptic	++	++	++	++	+	+
Extrasynaptic	+	-	+	+	++	++
Functions	Sedation			Sensorimotor gating		Anxiolysis
	Annesia			Suppression of thalamic oscillations		Response to stress
	Anticonvulsant			Myorelaxation		Sensorimotor gating
	Dependence liability			Cognition in SCZ		Cognitive impairment (low interference tasks)
	Premature visual cortical plasticity			Antihyperalgesia		Cognitive improvement (high interference tasks)

^aThe immunohistochemical pictures are a courtesy of Jean-Marc Fritschy, University of Zurich, and were published previously [155] and are used with permission.

The pictures are false color-coded with intensities decreasing in the following order: white, yellow, red, blue. ++, the majority of receptors are in the specified location; +, the minority of receptors are in the specified location; -, there are no receptors in the specified location. Receptors containing $\alpha 4$ subunits predominantly carry a δ subunit and are extrasynaptic. There is also evidence for receptors containing the $\alpha 4$ and $\gamma 2$ subunits, which are synaptic. Throughout the text, we assume $\alpha 4$ GABA_ARs are of the former type unless otherwise specified in the studies. The $\alpha 6$ subunit which is not shown here is largely restricted to the cerebellum and not modulated by classical benzodiazepines. There is also evidence for GABA_AR complexes containing two different α subunits [156], the functional significance of which is not entirely clear, but is beginning to be addressed [79]).